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Serotonin 1B receptors in the developing somatosensory and visual cortices are located on thalamocortical axons

(barrels/somatotopy/radioligand binding/^{125}I-labeled-cyanopindolol)

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ABSTRACT **Serotonin (5-HT)-immunoreactive axons are densely distributed in the primary visual and somatosensory cortices of rats, mice, and hamsters for the first 2 weeks of life, and a recent study from this laboratory has demonstrated that 5-HT$_{1B}$ receptors assume a pattern that exactly matches that of the serotoninergic axons. The differential distribution of these receptors is also transient. In the present study, we combined receptor binding autoradiography with neurochemical ablation of 5-HT axons or electrolytic lesions of the dorsal thalamus in an effort to determine the neural elements upon which the 5-HT$_{1B}$ receptors were located. Subcutaneous injections of the toxin 5,7-dihydroxytryptamine, made on the day of birth, totally eliminated the dense and patterned 5-HT innervation of the somatosensory and striate cortices of rats killed on postnatal day 8 but had no qualitative effect upon the distribution or density of 5-HT$_{1B}$ receptors in either of these cortical regions in animals killed at the same age. Conversely, electrolytic lesions of the dorsal thalamus made on postnatal day 6 resulted in a complete loss of the dense and patterned distribution of 5-HT$_{1B}$ receptors in rats killed on postnatal day 8. These results indicate that thalamocortical axons transiently express 5-HT$_{1B}$ receptors.**

Thalamic axons and lamina IV neurons in the cortex of perinatal rodents are arrayed in clusters that correspond to the body surface (1–3). The development of this precise, anatomically demonstrable somatotopic representation depends upon peripheral information (4) that is almost certainly conveyed to the cortex by thalamocortical fibers. For about the first 2 weeks of life, the serotoninergic innervation of the cerebral cortex has a distribution closely matching that of the somatosensory and visual thalamocortical afferents (5–8) and there is recent evidence that depletion of serotonin (5-HT) from the developing somatosensory cortex may delay the maturation of the somatotopic pattern of thalamocortical afferents and lamina IV neurons (9, 10).

We do not know the mechanisms by which 5-HT might influence the development of either thalamocortical axons or cortical cells. One approach toward answering this question is determining the types and locations of the 5-HT receptors in the cortex during development. A recent study from this laboratory has demonstrated that one class of 5-HT receptors, 5-HT$_{1B}$, is transiently arrayed in the somatosensory and visual cortices in a way that closely matches the distribution of specific thalamic afferents and the transient 5-HT innervation of these cortical fields (11). In the adult brain, 5-HT$_{1B}$ receptors have been shown to be autoreceptors that regulate 5-HT release from serotoninergic axons (12, 13) and heteroreceptors that are likely to be located on retinal axons, spinal primary afferents, and other non-5-HT axons (14–16). These results raised the possibility that the 5-HT$_{1B}$ receptors in the developing visual and somatosensory cortices might be located on thalamocortical and/or 5-HT axons. The present study combined radioligand binding and lesion techniques to differentiate these possibilities.

Table 1. Animals used in the different experiments comprising this report

Manipulation	Age	*n*	Assay
Normal	P-8	5	Immunocytochemistry
		3	Autoradiography
Normal	>P-60	5	Immunocytochemistry
		3	Autoradiography
5,7-DHT injection at birth	P-8	7	Immunocytochemistry (left hemisphere) Autoradiography (right hemisphere)
Thalamotomy on P-6	P-8	3	Autoradiography

P-, postnatal day; 5,7-DHT, 5,7-dihydroxytryptamine.

MATERIALS AND METHODS

Four different groups of rats contributed data to these experiments (Table 1). The first and second groups were normal perinatal (P-8) and adult (>P-60) rats that were used in either immunocytochemical or ligand binding studies to obtain normative data for comparison with those from experimental animals. The third group of rats sustained subcutaneous injections of the 5-HT neurotoxin 5,7-DHT (2 mg in a total volume of 50 μl) on the day of birth and was killed on P-8. The fourth group sustained electrolytic lesions of the dorsal thalamus on P-6 and was killed on P-8. The thalamic lesions in this study were made using methods that have been described in detail by Chiaia *et al.* (17).

Animals were killed by rapid decapitation and brains were removed. Both cortices were quickly removed and one or both were "flat" frozen on dry ice by applying pressure to the pial surface with a glass microscope slide. All of these specimens were stored at −70°C until needed. Frozen tissue was mounted onto a cryostat chuck and 15-μm sections were cut parallel to the pial surface or in the coronal plane. Sections were thaw mounted onto 0.5% gel-coated slides and stored at −20°C until used. In some normal animals and rats that received 5,7-DHT injections, one cortex was postfixed for 2–4 days, flattened on the stage of a freezing microtome, and cut into 50-μm sections to demonstrate the distribution of 5-HT axons or, in the case of the 5,7-DHT-treated animals, to demonstrate the extent to which these fibers were depleted.

The thalami of the rats that sustained electrolytic lesions were postfixed for 3–6 days, cut into 50-μm coronal sections

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Abbreviations: 5-HT, serotonin; P-, postnatal day; CYP, cyanopindolol; 5,7-DHT, 5,7-dihydroxytryptamine.

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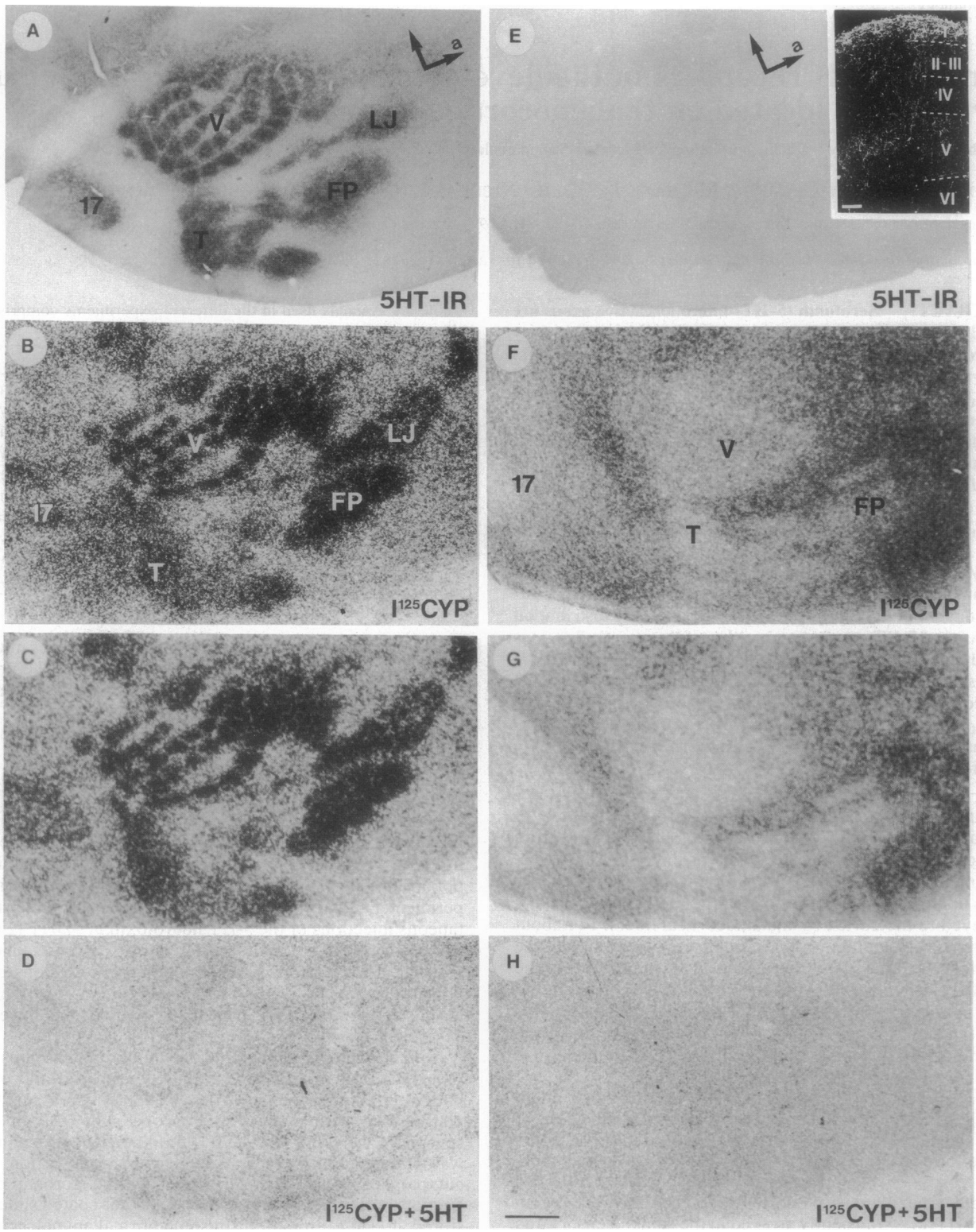

FIG. 1. Serotonin immunoreactivity (IR) and ^{125}I-CYP binding in the cortices of a P-8 and an adult rat. (*A*) Section through the flattened cortex of a P-8 animal. Dense immunoreactivity marks area 17 (17) and the granular portion of the primary somatosensory cortex (S-I). In the latter region, it matches the somatotopic representation of the body surface. V, representation of the vibrissae; T, trunk; FP, forepaw; LJ, lower jaw. The arrows point toward anterior (a) and lateral. (*E*) Disappearance of the differential distribution of 5-HT immunoreactivity in the cortex of an adult rat. (*Inset*) Dark-field photomicrograph of a coronal section that shows the laminar patterned 5-HT innervation of S-I that remains in the adult animal. (*B*) Ligand binding in a section through the flattened cortex of a P-8 animal. Note the dense and somatotopically organized labeling in the primary somatosensory cortex and the heavy binding in area 17. (*C*) Computer-enhanced version of the autoradiogram shown in *B*. This enhancement was carried out to increase the contrast between regions of high and low binding. (*F*) Binding of ^{125}I-CYP in a section through the flattened cortex of an adult rat. Note the *reduced* density of ligand binding in area 17 and the primary somatosensory cortex. (*G*) Computer-enhanced version of the autoradiogram shown in *F*. (*H* and *I*) Autoradiograms made from sections through the cortices of a P-8 and adult rat, respectively. In these, binding of ^{125}I-CYP to 5-HT$_{1B}$ receptors has been blocked by the addition of 20 μM 5-HT. (Bar = 500 μm; that for the *Inset* = 100 μm.)

on a freezing microtome, and stained with cresyl violet to determine the extent of the damage.

Autoradiographic assay for 5-HT_{1B} receptors followed the protocol of Manaker and Verderame (18). Frozen sections were brought to room temperature and preincubated in ice-cold buffer (50 mM Tris·HCl/2.5 mM $MgCl_2$/10 μM pargyline/30 μM isoproterenol, pH 7.4) for 10 min. They were then incubated in the same buffer containing 100 pM ^{125}I-labeled cyanopindolol (^{125}I-CYP) at room temperature for 1 hr. Preliminary studies verified that 30 μM isoproterenol effectively blocked all ^{125}I-CYP binding to β-adrenergic receptors. Specific 5-HT binding was blocked by the addition of 20 μM 5-HT. After incubation, sections were washed twice in cold buffer, dipped in cold distilled water to remove the buffer salts, and dried with a cool air stream. Dried slides were then apposed to LKB Ultrofilm for 48–72 hr and the exposed film was developed in D-19 for 5 min.

Immunocytochemistry for 5-HT followed the protocol used by Rhoades *et al.* (7) and Bennett-Clarke *et al.* (8). The immunocytochemical methods used to process these sections are fully described in those two papers.

RESULTS AND DISCUSSION

In rats killed on P-8, 5-HT-immunoreactive axons (Fig. 1*A*) and 5-HT_{1B} receptors (Fig. 1 *B* and *C*) are densely distributed in the primary somatosensory and visual cortices and their pattern in the somatosensory cortex closely matches that of the thalamic afferents and cortical barrels that distinguish this region. In adult rats, the dense and patterned distribution of 5-HT axons is lost (Fig. 1*E*) and the density of 5-HT_{1B} receptors in both of these cortical fields is lower than that in the surrounding cortex (Fig. 1 *F* and *G*). Specific binding of ^{125}I-CYP in the cortices of the perinatal and adult rats was completely blocked by addition of 20 μM 5-HT to the assay (Fig. 1 *D* and *H*).

Subcutaneous injection of 5,7-DHT on the day of birth resulted in a virtually complete loss of the dense and patterned 5-HT innervation of the visual and somatosensory cortices (Fig. 2*A*), but a small number of relatively coarse fibers remained (Fig. 2*B*). Such lesions had no qualitative effect upon the distribution or density of 5-HT_{1B} receptors in either the visual or somatosensory cortex (Fig. 2*C*). This result was obtained in every rat in which successful 5,7-DHT injections and radioligand binding were accomplished (n = 7) and it indicates that most, if not all, of the 5-HT_{1B} receptors in the developing cortex are not located on serotoninergic axons. It shows further that the density of these receptors is not rapidly down-regulated after loss of most of the 5-HT innervation of the cortex.

In contrast, ablation of the dorsal thalamus on P-6 resulted in a complete loss of the dense and patterned distribution of 5-HT_{1B} receptors in animals killed on P-8. Data from one of the animals that showed this result are depicted in Fig. 3. The thalamic lesion (Fig. 3*A*) destroyed all of the ventrobasal complex and lateral geniculate nucleus. In the cortex ipsilateral to the lesion (Fig. 3*C*), there was no patterning of 5-HT_{1B} receptors. In the cortex contralateral to the lesion (Fig. 3*B*), the normal pattern could still be seen. This result was obtained in all rats (n = 4) in which successful thalamic lesions and autoradiography were accomplished. In cases where lesions missed the ventrobasal complex and lateral geniculate nucleus, there was no significant change in the cortical distribution of 5-HT_{1B} receptors.

The results of this study indicate that the dense and patterned 5-HT_{1B} receptors that are present for a short period in the developing visual and somatosensory cortices are located on thalamocortical axons. This result, when coupled with the finding that 5-HT-immunoreactive axons are also transiently patterned in a similar manner, suggests strongly that 5-HT may have an important influence upon growing thalamocortical afferents.

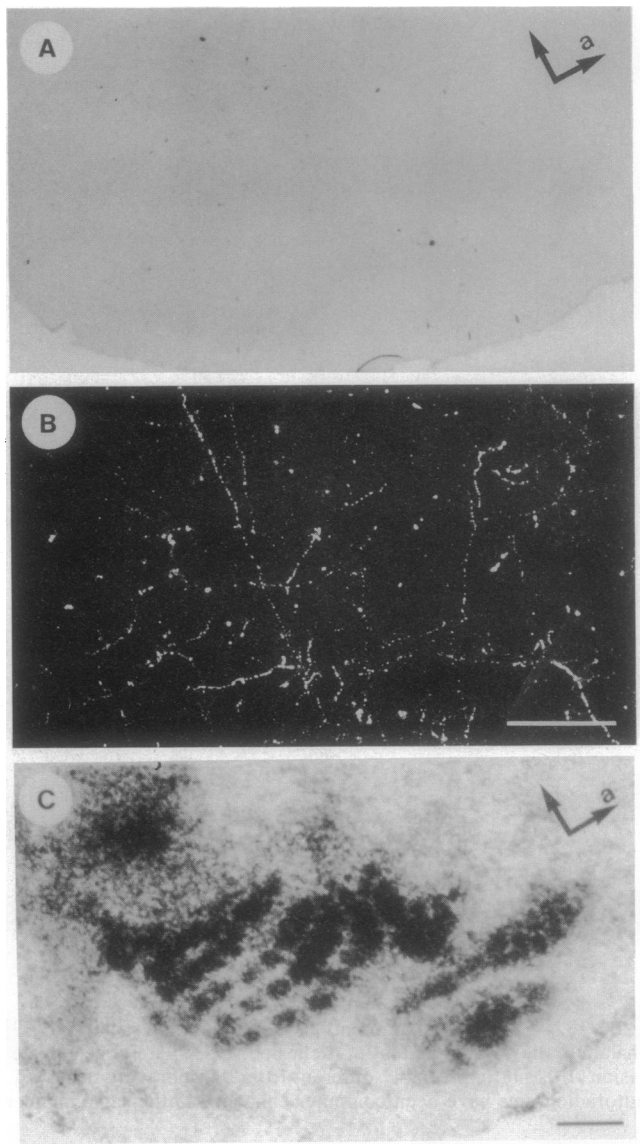

FIG. 2. Lack of effect of neonatal administration of 5,7-DHT upon patterning of 5-HT_{1B} receptors in the cortex of a P-8 rat. (*A*) Bright-field photomicrograph of a section through the flattened cortex of a P-8 rat that received an injection of 5,7-DHT on the day of birth. The section has been processed for 5-HT immunocytochemistry. Note the lack of the pattern normally observed in the cortex at this age. (*B*) Higher-power dark-field photomicrograph showing that a small number of 5-HT-positive fibers do remain in the cortex after neonatal treatment with 5,7-DHT. (*C*) Pattern of ^{125}I-CYP binding in the cortex of the animal that provided the data shown in *A* and *B*. It has been mirror-reversed for easier comparison with *A* and computer-enhanced in the same manner as Fig. 1 *C* and *G*. Note the patterned distribution of 5-HT_{1B} receptors. (*A* and *C*, bar = 500 μm; that for *B* = 100 μm.) The arrows in *A* and *C* point toward anterior (a) and lateral.

The suggestion that 5-HT plays a role in neural development is not new and there is substantial evidence from a number of systems that this amine has profound influences upon several aspects of neuronal differentiation. Lauder and Krebs (19) have provided considerable data suggesting that altered 5-HT levels may affect neurogenesis. It has also been demonstrated that increasing concentrations of 5-HT can influence the differentiation of neurons maintained in culture (20–23). In particular, Chubakov *et al.* (24) have shown that low (20 μM) concentrations of 5-HT stimulate neuronal

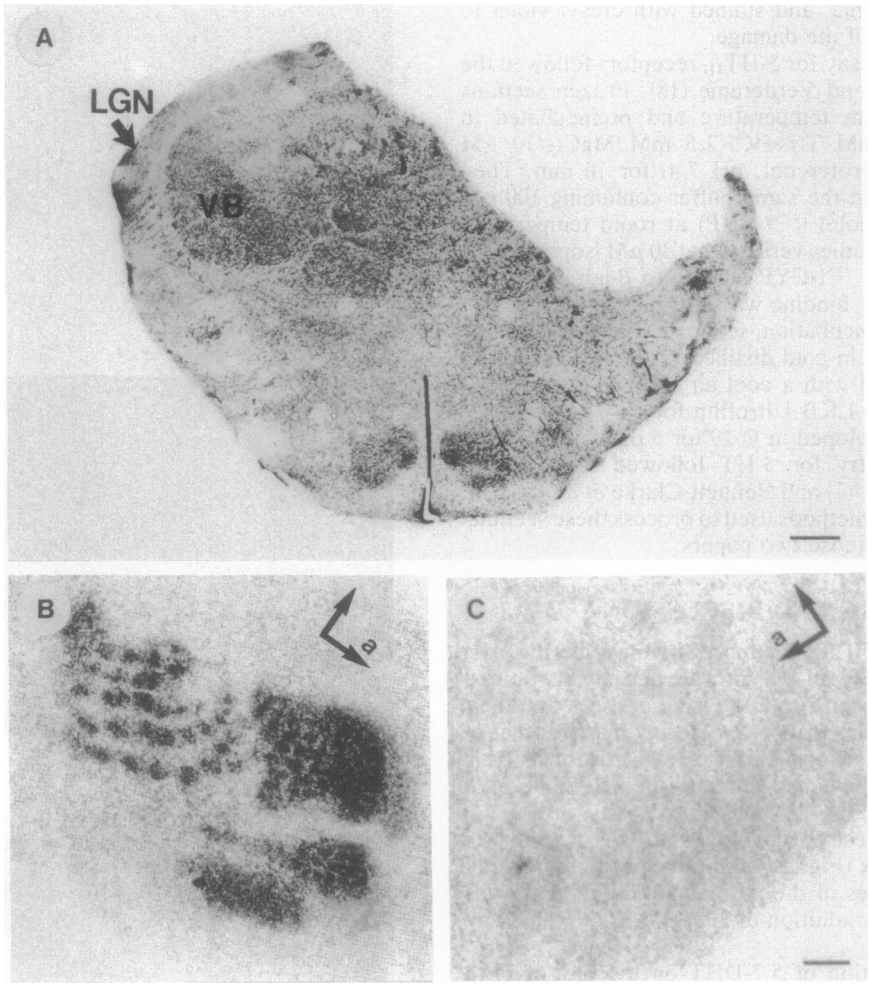

FIG. 3. Effect of thalamotomy on P-6 upon the distribution of ^{125}I-CYP binding in the cortex on P-8. (*A*) Nissl-stained section through the thalamus showing the extent of the lesion. LGN, lateral geniculate nucleus. (*B*) Pattern of ^{125}I-CYP binding in the cortex *contralateral* to the lesion. It is not appreciably different from that normally observed on P-8. (*C*) Loss of the pattern in the cortex ipsilateral to the lesion. The autoradiograms have been computer-enhanced in the same manner as those in Figs. 1 *C* and *G* and 2*C*. (Bars = 500 μm.)

differentiation, neuropil formation, and synaptogenesis in cultures of neonatal rat visual cortex. The possibility that 5-HT may play a role in the development of corticopetal projections is particularly attractive in view of the work of Kater and colleagues (25–28). They demonstrated that addition of 5-HT to the culture medium surrounding actively growing neurons from *Helisoma* caused an immediate cessation of neurite elongation. Importantly, 5-HT consistently arrested neurite elongation for identified neurons B19, P1, and P5 but not for cells B4 and B5 (28). More recently, Goldberg and Kater (29) showed that depletion of 5-HT in developing *Helisoma in vivo* by administration of 5,7-DHT also altered the development of B-19.

The potential significance of 5-HT$_{1B}$ receptors in cortical development is not at all understood. The fact that these receptors are negatively coupled to adenylate cyclase (30) might implicate them in mediating a modulatory effect of 5-HT upon thalamocortical axonal development. As noted above, two studies have already indicated that depletion of 5-HT from the developing cortex delays, but does not prevent, the formation of somatotopically organized aggregates of thalamocortical axon arbors and granule cells in lamina IV (9, 10). Interpretation of these results is somewhat limited by the fact that 5-HT was depleted by systemic injection of *p*-chloroamphetamine and this may have substantial effects upon many aspects of neural and nonneural development (31, 32). The observation that thalamocortical afferents transiently express a specific 5-HT receptor raises the possibility that the role of 5-HT in the development of the somatotopically organized aggregation of axons and cells in the primary somatosensory cortex can be assayed by blockade of this receptor rather than depletion of 5-HT. Although there are not a large number of specific 5-HT$_{1B}$ agonists or antagonists, Neale *et al.* (33) have reported that the fused arylpiperazine CGS 12066 binds with high affinity to the 5-HT$_{1B}$ site and with much lower affinities to the 5-HT$_{1A}$ and 5-HT$_2$ sites. Placement of implants impregnated with this compound in the developing cortex might permit determination of the role that the transiently expressed 5-HT$_{1B}$ receptors play in thalamocortical development.

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