0013-7227/96/$03.00/0
Endocrinology

Vol. 137, No. 12
Printed in U.S.A.

In Vivo Effects of Insulin-Like Growth Factor-I on the Development of Sensory Pathways: Analysis of the Primary Somatic Sensory Cortex (S1) of Transgenic Mice*

GABRIEL GUTIÉRREZ-OSPINA†, ALI SUHA CALIKOGLU, PING YE, AND A. JOSEPH D'ERCOLE

Department of Pediatrics, Division of Endocrinology, University of North Carolina, Chapel Hill, North Carolina 27599-7220

ABSTRACT

In the rodent brain, insulin-like growth factor I (IGF-I) messenger RNA is transiently expressed in sensory projection neurons during periods of synaptogenesis and neuronal growth. Transgenic (Tg) mice with brain IGF-I overexpression and ectopic brain expression of IGF-binding protein-1 (IGFBP-1), an inhibitor of IGF-I actions, show changes in brain size and myelination. We used these mouse models to evaluate *in vivo* IGF-I effects on sensory pathway development by conducting anatomical studies in the S1 barrel field. Brain size, cortical area, and barrel field dimensions were increased in IGF-I and reduced in IGFBP-1 Tg mice compared with those in wild-type (wt) mice. The brain and cerebral cortex of Tg mice with the highest transgene expression were the most altered in size. Cortex and barrel field size changes were not precisely proportional, because in some Tg mice barrels were relatively more affected than the cortex, whereas in others the opposite was observed. Brain IGF-I overexpression increased the average number of neurons per barrel, neuronal cell body cross-sectional area, and barrel neuropil volume, whereas brain expression of IGFBP-1 reduced each. Neuronal density was greatly reduced in IGF-I Tg mice and increased in IGFBP-1 Tg mice. No differences in body weight, whisker pad and follicle areas, and whisker pad innervation density were found among Tg and wt mice. These observations indicate that IGF-I enhances neuronal growth in developing sensory pathways and support the concept that modified availability of local trophic factors, such as IGF-I, changes brain, neocortical, and S1 relative dimensions by altering neuronal survival and neuropil elaboration. Study of the S1 cortex provides an excellent model to probe the *in vivo* mechanisms of IGF actions. (*Endocrinology* **137:** 5484–5492, 1996)

INCREASING evidence suggests that insulin-like growth factor I (IGF-I) plays an important role in central nervous system development (1). For example, *in situ* hybridization studies have detected IGF-I messenger RNA (mRNA) in the subventricular zone, hippocampus, retina, and cerebellum at developmental times when glial and neuronal precursors undergo cell division (2–6). Transient IGF-I mRNA expression occurs in the developing retina, hippocampus, cerebellum, cerebral cortex, and several sensory thalamic and brain stem nuclei during periods of neuronal growth and synaptogenesis (2, 4). In the olfactory bulb and hypothalamus, structures that remain plastic into adulthood, IGF-I mRNA expression persists throughout life (2, 4). Furthermore, recent studies in transgenic (Tg) mice show that IGF-I regulates brain growth. Tg mice overexpressing IGF-I have increased brain weight and size compared with their wild type (wt) littermates (7–11). Conversely, mice carrying IGF-I genes disrupted by homologous recombination (12), and those with brain ectopic expression of IGF-binding protein-1 (IGFBP-1) (10, 11, 13), an inhibitor of IGF-I actions, have smaller brains than their wt counterparts. Changes in brain size in these Tg mouse lines are due in part to the effects of IGF-I on oligodendrocyte survival and function (9–12), but there is also evidence of alterations in neuron number (7, 12, 13).

The transient early postnatal expression of IGF-I in sensory projection neurons suggests that IGF-I may be especially important to neuronal growth and synaptogenesis in developing sensory systems (2). To directly evaluate the *in vivo* effects of IGF-I on sensory system development, we used IGF-I and IGFBP-1 Tg mice as models to determine whether altered IGF-I availability influences the growth of neurons and their circuits in the somatic sensory cortex (S1). Specifically, we focused our analysis on the posterior medial barrel subfield (PMBSF) (14), a region in cerebral cortex layer IV that is a portion of the trigeminal thalamo-cortical pathway (TThC). In rodents, the TThC is responsible for transmitting tactile information from facial whiskers to the cerebral cortex. Each of the 34 PMBSF barrels is the representation of a single whisker in the facial whisker pad. These 3-dimensional barrel structures are composed of a cell dense wall surrounding a hollow that is predominately composed of neural connections among axon terminal fields from the ventroposteromedial thalamic nucleus neurons and cortical layer IV dendrites. For this reason, barrel dimensions correlate with changes in the number of neurons and the complexity and number of their connections (14, 15).

Received June 6, 1996.
Address all correspondence and requests for reprints to: Dr. A. Joseph D'Ercole, Department of Pediatrics, Division of Endocrinology, CB#7220, University of North Carolina, Chapel Hill, North Carolina 27599-7220. E-mail: AJD.PEDSLAN@mhs.unc.edu.
* This work was supported by NIH Grant HD-08299.
† Fellow of the National Council of Science and Technology, Mexico.

We chose the PMBSF for study because 1) IGF-I is highly expressed by thalamic and cortical cells of the TThC, with a peak at the time of PMBSF formation (2), a finding that suggests a critical role for IGF-I in its development; and 2) because of its sharp anatomical definition with classical histological techniques, the PMBSF permits and facilitates quantitative analysis of neuron growth and thus provides a near-optimal system to evaluate the effects of IGF-I on central nervous system neuron growth. We asked whether IGF-I availability modifies the size, structure, and composition of PMBSF barrels. Our observations indicate that IGF-I stimulates increases in cortical and PMBSF barrel size and their relative dimensions. In the PMBSF, IGF-I increases neuron number and the portion of barrel volume occupied by neuropil, probably by enhancing neuron survival and stimulating the growth of thalamic and cortical neuronal connections, respectively. Each of these changes proceeded with no alterations in body and sensory periphery influences.

Materials and Methods

Mice and experimental design

Studies were conducted in adult (~90 days of age) heterozygous IGF-I (11) and IGFBP-1 (13) Tg mice carrying IGF-I and IGFBP-1 human transgenes driven by the metallothionein-1 promoter and their wt littermates. All Tg mice studied were from the third to ninth generations and had been maintained by breeding to nontransgenic mice of the C57/B6 strain. Mice were kept in temperature- and light-controlled rooms, fed standard laboratory chow *ad libitum*, and given free access to water containing 25 mM $ZnSO_4$. IGF-I and IGFBP-1 transgene brain expression in these Tg mice has been previously characterized (11, 13), and the effects of their expression on brain size, total brain weight, and myelination have been extensively discussed and documented (7–11, 13). Although both transgenes are widely expressed in the brain, regional variations in weight result from differences in the abundance of IGF-I and IGFBP-1 mRNAs expressed in different areas as well as differences in transgene expression among mouse lines (11). The expression of these transgenes in the brain begins after birth and peaks between postnatal days 21–30, following the developmental pattern of expression of endogenous methallothionein-1 (11, 13).

To rule out effects secondary to insertional mutations, initial studies were carried out in multiple transgenic mouse lines (L): 26, 32, 43, 50, and 52 IGF-I Tg mice, and B and C IGFBP-1 Tg mice. Because the greatest differences in PMBSF and barrel area were found in 26L IGF-I and BL IGFBP-1 Tg mice (see Table 1), further studies were pursued only in mice of these lines and their wt littermates. Wt littermates of these lines were used as controls for all experiments. In addition, to evaluate whether altered IGF-I overexpression was directly related to changes in brain morphology, 52L IGF-I Tg mice were cross-bred with BL IGFBP-1 Tg mice (IGF-I/IGFBP-1 cross Tg mice).

Although barrel-like structures have been described in the thalamus (*i.e.* barreloids) and brain stem (*i.e.* barrelets) (16–18), we restricted our analyses to PMBSF cortical barrels because they can be readily identified in mature animals after histochemical and cresyl violet staining. This is not true for barrelets and barreloids, even in optimal sections, whose boundaries tend to blur as postnatal development progresses. Anatomical measurements were performed blindly. All mouse protocols were approved by an institution review committee at the University of North Carolina-Chapel Hill.

Measurements of cortex, PMBSF, and barrel cross-sectional areas and barrel volume

To determine the cross-sectional area of the cortex, the PMBSF and its constituent barrels, as well as barrel volume, anesthetized mice were perfused with normal saline followed by 10% glycerol. The brains were removed and weighed, and the cerebral cortex, including the olfactory bulb, was dissected, flattened, and frozen, as described by Riddle *et al.* (19). Tangential sections (30 μm) throughout the cortex were cut in a cryostat. Sections were dried on the slides at 37 C for 30 min; fixed in 10% formalin, pH 7.6, at 4 C for 1 min; and rinsed in distilled water. They were then stained for the activity of the oxidative enzyme, succinic dehydrogenase (SDH), by reacting them with 0.05 M sodium succinate and 0.55 mM nitro blue tetrazolium in 0.05 M phosphate buffer, pH 7.6. The slides were rinsed again in distilled water and coverslipped with Aqua-Mount (Lerner Laboratories, Pittsburgh, PA). Cortical and PMBSF two-dimensional maps were then traced at final magnifications of ×3 and ×40, respectively, using a camera lucida. Completed maps were digitized, and cortical, PMBSF, and barrel cross-sectional areas were measured using the Image-Pro imaging analysis system (Media Cybernetics, Silver Spring, MD), based upon methods described by Riddle *et al.* (19). Total barrel area was determined by adding cross-sectional areas of PMBSF barrels.

To rule out the possibility that altered or compromised oxidative metabolism, *i.e.* altered SDH activity, accounted for the differences in barrel area between wt and Tg mice, sets of sequential tangential sections of the cortex were cut and stained alternatively with cresyl violet and SDH histochemistry. Barrel boundaries defined by both cresyl violet and SDH staining coincided precisely in both wt and Tg mice (see also Ref. 19 for a similar observation in rats); thus, changes in barrel area revealed by SDH histochemistry in Tg mice demonstrate modifications in barrel dimensions and do not simply reflect changes in metabolism.

The series of SDH-stained cortical tangential sections obtained from 26L IGF-I and BL IGFBP-1 Tg mice and their wt type littermates also were used to estimate the average PMBSF barrel height (layer IV thickness) and volume. The height of each of the 34 PMBSF barrels was determined in each cerebral cortical hemisphere of 6 animals/group by multiplying the number of sections in which each barrel appeared by the thickness of these sections. Barrel volume was obtained by multiplying the barrel cross-sectional area by the section thickness and then totaling the volumes of each section.

Possible effects of modified IGF-I availability on other modules of neuronal circuitry were evaluated in the olfactory bulb. The average cross-sectional area of olfactory glomeruli (n = 1000/group) was mea-

TABLE 1. Body and brain weight, and brain/body ratios in IGF-I, IGFBP-1, and IGF-I/IGFBP-1 (52L/BL Cross) Tg mice and their wild-type (wt) littermates

Line	BW (g)	Brain wt (g)	Brain/body ratio
Normal (n = 6)	33.18 ± 4.09	0.46 ± 0.010	0.013 ± 0.002
26L IGF-I (n = 6)	32.71 ± 2.34	0.88 ± 0.010[a] (91% ↑)	0.026 ± 0.004[a]
50L IGF-I (n = 6)	35.60 ± 6.04	0.63 ± 0.020[a] (37% ↑)	0.017 ± 0.003
52L IGF-I (n = 7)	36.92 ± 3.37	0.68 ± 0.010[a] (48% ↑)	0.018 ± 0.003[b]
32L IGF-I (n = 7)	36.14 ± 2.05	0.72 ± 0.010[a] (56% ↑)	0.019 ± 0.001[c]
43L IGF-I (n = 8)	38.59 ± 6.46	0.56 ± 0.002[a] (22% ↑)	0.014 ± 0.003
IGF-I/IGFBP-1 (n = 6)	32.32 ± 3.74	0.53 ± 0.020[a] (13% ↑)	0.016 ± 0.001
CL IGFBP-1 (n = 5)	36.25 ± 3.28	0.36 ± 0.003[a] (22% ↓)	0.009 ± 0.001[c]
BL IGFBP-1 (n = 6)	32.43 ± 1.50	0.35 ± 0.010[a] (24% ↓)	0.010 ± 0.001[c]

Values are the mean ± SEM.
[a] $P < 0.0001$ *vs.* wt mice.
[b] $P < 0.05$ *vs.* wt mice.
[c] $P < 0.01$ *vs.* wt mice.

sured in camera lucida drawings from the same series of SDH-stained tangential sections previously used to determine PMBSF and barrel areas.

Estimations of size, number, and density of neurons in PMBSF barrels

Neuron cross-sectional area and number were estimated in PMBSF barrels using cresyl violet-stained, cryostat tangential sections (50 μm) after paraformaldehyde brain fixation. The mean neuronal area in PMBSF barrels was estimated using previously described methods (15, 20). Single sections with clearly visualized barrel boundaries were selected from both cerebral cortical hemispheres of each of three mice per group, and about 50 neurons that had a clearly visible nucleus, nucleolus, and cytoplasm were traced from the hollow of each of the 34 barrels in a hemisphere using a camera lucida at a final magnification of ×1500. A total of 9000 neuron tracings in wt and Tg mice were collected and digitized (3000/mouse). The mean cross-sectional area was determined using the Image-Pro system. This sampling method is unlikely to introduce a bias because the mean neuronal area changes little as a function of layer IV depth, and no regional differences in neuronal size within barrels have been reported (15).

To estimate neuronal number in PMBSF barrels, neurons were counted in each of the 34 PMBSF barrels in single sections previously used for determining neuron area (15, 20). Barrel boundaries were traced, and neuron profiles were counted in barrel sides and hollows from each hemisphere using a camera lucida at a final magnification of ×450. The average neuron number in each PMBSF barrel was then estimated with the formula: barrel neuron number = number of neurons in each barrel/single section × barrel height (microns)/50 (microns; section thickness). The PMBSF barrel neuron density was estimated using the equation: Neuron density/mm^3 = PMBSF barrel neuron number/barrel volume.

Measurements of whisker pads and whisker follicle areas and number of axons in the infraorbital nerve

Whisker pads from the same 26L IGF-I, BL IGFBP-1 Tg, and wt mice used to determine PMBSF and barrel areas were carefully dissected and postfixed in 10% formalin for 7 days and then transferred to the same fixative containing 20% sucrose until they sank. The pads were then flattened between two microscope slides separated by 2 mm, frozen in 4-methylbutane, and cut tangentially (50 μm) in a cryostat. Whisker pad two-dimensional maps were drawn from sequential sections using a camera lucida (final magnification, ×14). Areas of whisker pads (n = 12/group) and follicles represented in the PMBSF (n = 408/group) were determined following a protocol similar to that used for measuring PMBSF and barrels areas.

To count the number of axons in the infraorbital nerve, 26L IGF-I, BL IGFBP-1 Tg, and wt mice (n = 6/group) were perfused with 3% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4; 0.1 M). The brain and skin were carefully removed (with exception of the whisker pads), and the remaining head, including the infraorbital nerve, was postfixed in the same fixative for 7 days. The samples were transferred to a 0.25-M EDTA, pH 7.4, solution for 3 days at 37 C to decalcify the skull bones and thereby facilitate infraorbital nerve dissections. Infraorbital nerves were thoroughly washed in phosphate buffer and embedded in glycol methacrylate following the supplier's protocol (Energy Beam Science, Agawam, MA). Richardson's blue-stained transverse sections (5 μm) were used to count the number of myelinated axons in the infraorbital nerve, as described by Sikich *et al.* (21).

Validation of methods

Brain shrinkage or swelling after perfusion were evaluated according to the method described by Riddle *et al.* (19). Because paraformaldehyde and glycerol perfusion altered brain size to the same extent in wt and Tg mice, no corrections for brain shrinkage or swelling were introduced in the data. Validation of the methods used in the present study comes from the finding that values obtained for each of the morphological parameters determined in our wt mice are fully comparable to values reported previously in mice (15, 20–22).

Data analysis

Means for all groups were obtained for each morphological parameter measured, and statistical significance was assessed by ANOVA and later confirmed using Student's *t* test (StatView II). For ease of comparison, some differences among groups are reported as the percent change; statistical analyses of these comparisons, however, were performed with the measured parameters rather than with percent change calculations. For some analyses, simple regressions were used.

Results

General observations

Overall brain size and cortical and barrel field cross-sectional areas differed among wt and Tg mice and among different lines of Tg mice (Table 1). Barrel number, shape, and anatomical definition, however, were comparable in Tg and wt mice (Fig. 1). No significant differences in body weight were observed between wt and Tg mice (Table 1). This is consistent with the relatively low levels of peripheral expression of both IGF-I and IGFBP-1 transgenes in these Tg mouse lines (11, 13).

Brain weights and brain/body ratios

Consistent with previous observations (7, 8, 10, 11, 13), IGF-I-overexpressing Tg mice showed significant increases in total brain weight, whereas IGFBP-1 Tg mice had reduced brain weights compared with wt mice (Table 1). In IGF-I Tg mice, brain weight increases ranged from 22% to 91% in 43L and 26L Tg mice, respectively. In CL and BL IGFBP-1 Tg mice, brain weight was decreased 22% and 24%, respectively. Cross-breeding 52L IGF-I Tg mice with BL IGFBP-1 Tg mice (IGF-I/IGFBP-1 cross) produced animals with 13% heavier adult brains than those in wt mice. IGF-I/IGFBP-1 cross Tg mice had 28% ($P < 0.0001$) smaller brains than native 52L IGF-I Tg mice, but 34% ($P < 0.0001$) larger brains than native BL IGFBP-1 TG mice, indicating that IGFBP-1 attenuates the effects of the IGF-I transgene on brain growth. Furthermore, the degree of transgene expression in brain corresponded to brain size, as assessed by Northern blot analysis (data not shown). The rank order of transgene IGF-I mRNA abundance in IGF-I Tg mice (26L > 32L >52L > 50L > 43L) was the same as that of brain weight (Table 1), whereas the BL IGFBP-1 Tg mice expressed more IGFBP-1 transgene and had smaller brains than CL IGFBP-1 Tg mice. Taken together, these findings indicate that the changes in brain size in IGF-I and IGFBP-1 Tg mice depend upon the amount of IGF-I available, and suggest that limitations in IGF-I availability may impose a developmental constraint on brain, cortical, and barrel growth (see below).

Brain weight/body weight ratios were increased in most IGF-I Tg mouse lines and in IGF-I/IGFBP-1 cross Tg mice, and were decreased in IGFBP-1 Tg mice compared with those in wt mice (Table 1). These differences in brain weight/body weight ratios are due to changes in brain weight with no alterations in body weight. Changes in brain size in IGF-I and IGFBP-1 Tg mice, therefore, cannot be attributable to modifications of body weight and/or body dimensions.

Cortical area, total barrel area, and barrel/cortex ratios

Cortical area was increased from 29% in 43L to 81% in 26L IGF-I Tg mice compared with that in wt mice (Table 2). In

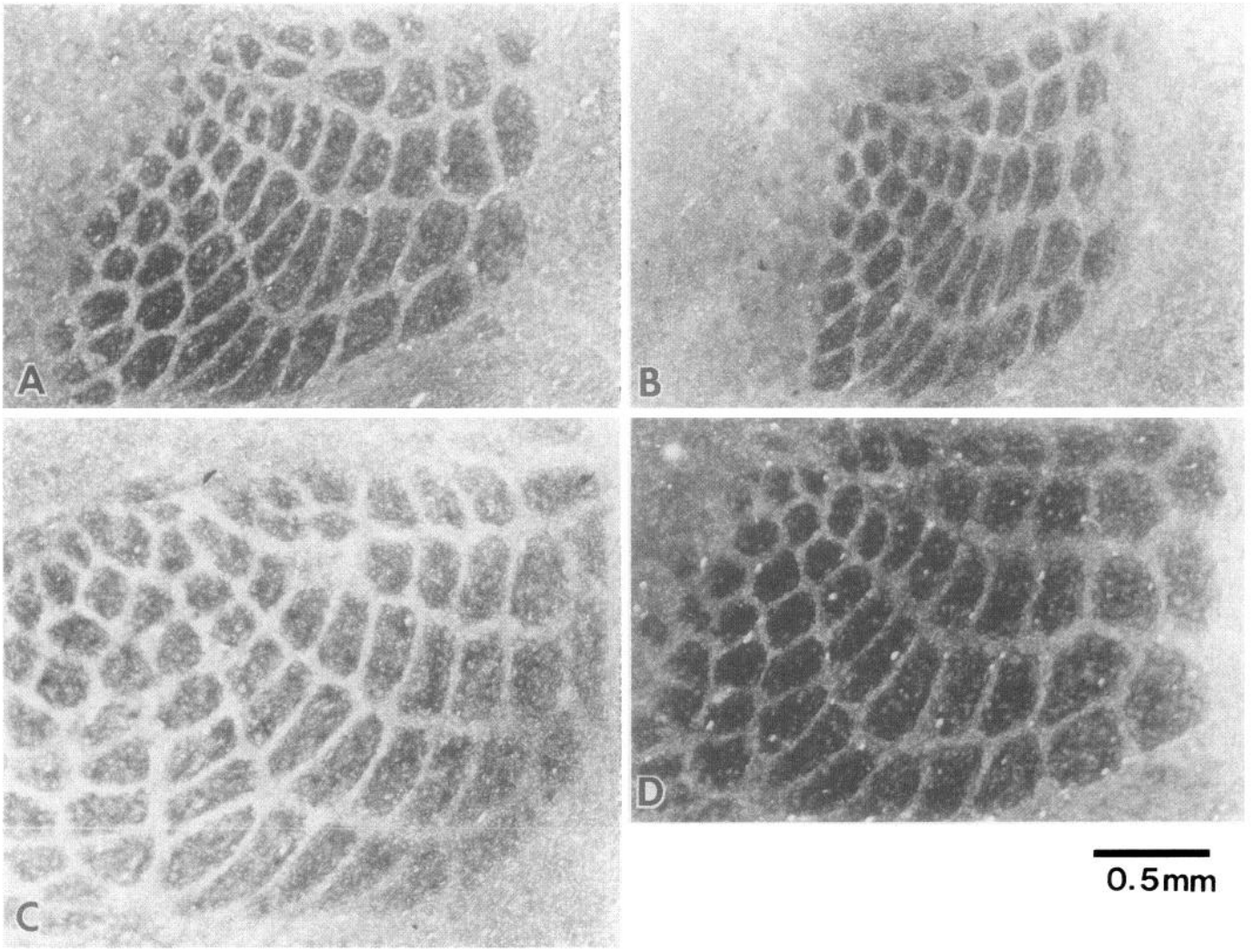

FIG. 1. The PMBSF in wt and Tg mice. Photomicrographs from SDH-stained tangential sections throughout layer IV of S1 in wt mice (a) and in BL IGFBP-1 (b), 26L IGF-I (c), and IGF-I/IGFBP-1 cross Tg mice (d). The number and shape of PMBSF barrels are comparable in wt and Tg mice.

TABLE 2. Total barrel and cortical areas, and barrel/cortex ratios in IGF-I, IGFBP-1, and IGF-I/IGFBP-1 (52L/BL Cross) Tg mice and their wild-type (wt) littermates

Line	Sum of barrel areas (mm^2)	Cortex area (mm^2)	Barrel/cortex ratio
Normal (n = 6)	1.44 ± 0.05	51 ± 1	0.028 ± 0.006
26L IGF-I (n = 6)	2.42 ± 0.10[a] (68% ↑)	92 ± 4[a] (81% ↑)	0.026 ± 0.002
50L IGF-I (n = 6)	2.19 ± 0.09[a] (52% ↑)	73 ± 2[a] (43% ↑)	0.030 ± 0.004
52L IGF-I (n = 7)	2.18 ± 0.01[a] (51% ↑)	75 ± 1[a] (47% ↑)	0.029 ± 0.001
32L IGF-I (n = 7)	2.03 ± 0.09[a] (41% ↑)	83 ± 2[a] (64% ↑)	0.024 ± 0.004[b]
43L IGF-I (n = 8)	1.91 ± 0.07[a] (33% ↑)	65 ± 1[a] (29% ↑)	0.029 ± 0.006[c]
IGF-I/IGFBP-1 (n = 6)	1.83 ± 0.06[a] (27% ↑)	66 ± 1[a] (30% ↑)	0.028 ± 0.006
CL IGFBP-1 (n = 5)	1.26 ± 0.02[b] (12% ↓)	45 ± 1[b] (10% ↓)	0.027 ± 0.002
BL IGFBP-1 (n = 6)	1.09 ± 0.03[a] (24% ↓)	41 ± 1[a] (29% ↓)	0.026 ± 0.003

Values are the mean ± SEM.
[a] $P < 0.0001$ *vs.* wt mice.
[b] $P < 0.01$ *vs.* wt mice.
[c] $P < 0.05$ *vs.* wt mice.

contrast, cortical area was decreased in CL and BL IGFBP-1 mice by 22% and 24%, respectively. In IGF-I/IGFBP-1 cross Tg mice, cortical area was increased 13% ($P < 0.0001$) and 37% ($P < = 0.0001$) above values for cortical area in wt and native IGFBP-1 Tg mice, respectively, but was decreased 13% ($P < 0.0001$) compared to those in native IGF-I 52L Tg mice. Relative cortical area (for the IGF-I Tg mice, 26L > 32L > 52L > 50L >43L; for the IGFBP-1 Tg mice, BL < CL) corre-

sponded to the degree of transgene expression. Comparisons of percent changes in brain weight and cortical area among Tg mice, however, indicate that cortical area variations are not precisely proportional to changes in total brain weight. For instance, in 26L IGF-I Tg mice, brain weight increased more (91%) than cortical area (81%), whereas in 32L IGF-I Tg mice, cortical area increased more (64%) than brain weight (56%; Tables 1 and 2). Ectopic expression of IGFBP-1 also affected total brain weight and cortical area somewhat differently. In CL IGFBP-1 Tg mice, brain weight was 22% decreased, whereas cortical area was only decreased by 10%. In BL IGFBP-1 Tg mice, both dimensions were more similarly reduced (24% and 19%, respectively). The differential effects of IGF-I and IGFBP-1 expression on brain and cortical dimensions also were seen in IGF-I/IGFBP-1 cross Tg mice. In these mice, brain weight was only increased by 13%, whereas cortical area was increased by 30% compared to those in wt mice. Brain weight and cortical relative dimensions, thus, were altered independently from each other to some extent, probably due to regional variation in IGF-I availability (11, 13).

Total barrel area was increased from 33% in 43L to 68% in 26L IGF-I Tg mice compared with that in wt mice (Table 2). In IGFBP-1 Tg mice, total barrel area was decreased 12% in mice from the CL and 24% in those from the BL. In IGF-I/IGFBP-1 cross Tg mice, total barrel area was increased 27% ($P < 0.0001$) and 40% ($P < 0.0001$) compared with those in normal and native BL IGFBP-1 Tg mice, respectively, and was decreased 16% ($P < 0.0001$) compared with that in native 52L IGF-I Tg mice. Comparisons of percent changes in cortical and total barrel areas among Tg mice indicate that these changes are not proportional. Indeed, in the IGF-I Tg mouse lines 50L, 52L, and 43L and in both lines of IGFBP-1 Tg mice, barrels were relatively more affected than the cortex, whereas the opposite was true for 26L and 32L IGF-I and IGF-I/IGFBP-1 cross Tg mice. This differential response by the cortex and barrels to the expression of both transgenes led to small, but significant, variations in barrel/cortex ratios among Tg mouse lines (Table 2). These ratios indicate that larger brains do not always have correspondingly larger barrels (compare, for instance, 50L, 52L, and 32L in Table 1). The changes in total barrel area in our Tg mice, thus, do not arise simply as a consequence of changes in cortical area. These observations suggest that cortical and S1 barrel field relative dimensions may change to some extent independently after variations in the availability of neurotrophic factors, such as IGF-I.

PMBSF and barrel cross-sectional area

PMBSF and barrel cross-sectional areas were increased in all five lines of IGF-I Tg mice compared to those in wt littermates (Table 3). The percent increase in PMBSF area, however, varied from 25% in 43L Tg mice to 60% in 26L Tg mice, and that in barrel area ranged from 27% to 67% in these same Tg mouse lines. Barrel cross-sectional area, thus, increased 2–12% more than PMBSF area in IGF-I Tg mice, indicating that barrels are relatively more affected by IGF-I overexpression than the PMBSF as a whole. This is consistent with the finding that barrels grow relatively more than S1 and the cortical mantle during postnatal brain development in rats (19).

PMBSF and barrel cross-sectional areas were similarly affected by IGFBP-1 expression; both were decreased by 13% in CL and by 25% in BL IGFBP-1 Tg mice compared with those in wt mice (Table 3). In IGF-I/IGFBP-1 cross Tg mice, barrel cross-sectional area was increased by 14% ($P < 0.0001$) and 51% ($P < 0.0001$) compared with those in wt and native IGFBP-1 Tg mice, respectively. IGF-I/IGFBP-1 cross Tg mice, however, had reduced PMBSF and barrel areas compared to native 52L IGF-I Tg mice. Finally, PMBSF and barrel cross-sectional areas were highly correlated in all lines of Tg mice (r = 0.55; $P = 0.006$ for IGF-I Tg mice and r = 0.97; $P < 0.0001$ for IGFBP-1 Tg mice). In contrast, neither PMBSF nor barrel area was correlated with cortical area in IGF-I (26L: r = 0.41; $P = 0.72$; 32L: r = 0.17; $P = 0.68$; 43L: r = 0.53; $P = 0.47$; 50L: r = 0.64; $P = 0.35$; 52L: r = 0.16; $P = 0.83$) and IGFBP-1 (BL: r = 0.86; $P = 0.6$; CL: r = 0.10: $P = 0.86$). Changes in the dimensions of the barrel field in IGF-I, IGFBP-1, and IGF-I/IGFBP-1 cross Tg mice, therefore, do not result from uniform proportional expansions or reductions of the cortex, because heavier brains containing the largest cortical mantles did not always have the largest somatic representations. These observations also strongly argue against the changes in PMBSF and barrel size being simply a result of differential flattening due to histological processing or a consequence of differences in the plane of sectioning.

TABLE 3. Cross-sectional area of the PMBSF and its constituent barrels and interbarrel area in the primary somatic sensory cortex of IGF-I, IGFBP-1, and IGF-I/IGFBP-1 (52L/BL Cross) Tg mice and their wild-type (wt) littermates

Line	Average barrel area (mm^2)	PMBSF area (mm^2)	Interbarrel area (mm^2)
Normal (n = 6)	0.049 ± 0.004	1.80 ± 0.07	0.29 ± 0.02
26L IGF-I (n = 6)	0.082 ± 0.007[a] (67% ↑)	2.89 ± 0.01[a] (60% ↑)	0.47 ± 0.04[a] (62% ↑)
50L IGF-I (n = 6)	0.077 ± 0.008[a] (56% ↑)	2.61 ± 0.09[a] (45% ↑)	0.42 ± 0.01[b] (45% ↑)
52L IGF-I (n ± 7)	0.074 ± 0.006[a] (50% ↑)	2.50 ± 0.06[a] (38% ↑)	0.35 ± 0.02[c] (21% ↑)
32L IGF-I (n = 7)	0.067 ± 0.004[a] (36% ↑)	2.41 ± 0.05[a] (34% ↑)	0.38 ± 0.02[c] (31% ↑)
43L IGF-I (n = 8)	0.063 ± 0.004[a] (27% ↑)	2.26 ± 0.05[a] (25% ↑)	0.39 ± 0.04[c] (35% ↑)
IGF-I/IGFBP-1 (n = 6)	0.056 ± 0.004[a] (14% ↑)	2.26 ± 0.01[a] (25% ↑)	0.28 ± 0.08 (4% ↓)
CL IGFBP-1 (n = 5)	0.043 ± 0.004[c] (13% ↑)	1.56 ± 0.03[a] (13% ↓)	0.30 ± 0.01 (4% ↑)
BL IGFBP-1 (n = 6)	0.037 ± 0.004[a] (25% ↓)	1.32 ± 0.04[a] (26% ↓)	0.25 ± 0.02 (14% ↓)

Values are the mean ± SEM.
[a] $P < 0.0001$ *vs.* wt mice.
[b] $P < 0.01$ *vs.* wt mice.
[c] $P < 0.05$ *vs.* wt mice.

Barrel height and volume in the PMBSF

Because the greatest differences in PMBSF and barrel areas were found in 26L IGF-I and BL IGFBP-1 Tg mice when compared with wt mice, detailed evaluation of the *in vivo* effects of IGF-I on barrel structure and development were pursued in these Tg mouse lines. As with PMBSF and barrel cross-sectional area, barrel height and barrel volume were, respectively, increased 18% and 98% in 26L IGF-I Tg mice and decreased 20% and 39% in BL IGFBP-1 Tg mice, compared to those in wt littermates (Fig. 2).

Cross-sectional area, number, and density of neurons in PMBSF barrels

The cross-sectional area of barrel neuron cell bodies was increased 33% in IGF-I/26L Tg mice and decreased 10% in BL IGFBP-1 Tg mice compared with those in wt animals (Table 4; photomicrographs demonstrating this difference are shown in Fig. 3). The average neuron number in each barrel (counted in single barrel sections), however, did not vary significantly among wt and Tg mice (Table 4; see also Fig. 3). Because barrel volumes differed among the groups of mice, the average number of neurons in each barrel was increased 24% in 26L IGF-I Tg mice and decreased 15% in BL IGFBP-1 Tg mice compared to that in wt mice (Table 4). The increases in neuron number in IGF-I Tg mice and the decreases in IGFBP-1 Tg mice are consistent with changes in DNA content measured in brains of IGF-I and IGFBP-1 Tg mice (9, 11, 13). Despite the increment in the number of neurons per barrel, neuronal density was decreased 39% in PMBSF barrels of 26L IGF-I Tg mice (Table 4; see also Fig. 3). The opposite was true for BL IGFBP-1 Tg mice. Although barrel neuron number was decreased, barrel neuronal density was increased 39% compared with that in wt mice. Similar increases in cortical neuronal density have been reported in IGF-I knock-out mice (12), a mouse model that, like IGFBP-1 Tg mice, reflects decreased IGF-I availability. Modifications in IGF-I availability, therefore, led to changes in neuron size, number, and density in Tg mice.

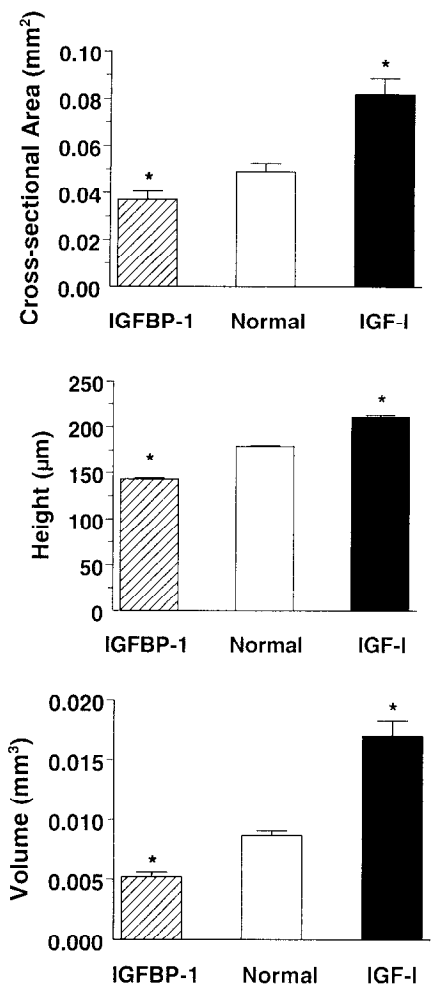

FIG. 2. Cross-sectional area (*top panel*), height (*middle panel*), and volume (*bottom panel*) of PMBSF barrels in 26L IGF-I and BL IGFBP-1 Tg mice and their wt littermates. n = 3 for cross-sectional areas and volumes, and n = 6 for heights. Data are the mean ± SEM. *, $P < 0.001$.

Area of olfactory glomeruli

The area of olfactory glomeruli was increased 10% in 26L IGF-I Tg mice compared to that in wt littermates (mean ± SEM, 0.0070 ± 0.0011 and 0.0064 ± 0.0017 mm^2, respectively; $P < 0.05$). The area of these modules in BL IGFBP-1 Tg mice (0.0064 ± 0.0010 mm^2), however, did not differ from that of olfactory glomeruli in wt animals. Because S1 barrels were relatively more affected than olfactory glomeruli, it is apparent that IGF-I exerts a degree of regional specificity. Support for this conclusion comes from the finding that IGF-I treatment stimulates the growth of the parietal cortex, but not that of the olfactory bulb when these regions are implanted into the eye's anterior chamber in rats (23, 24). In addition, our observations for the olfactory bulb argue against the changes in PMBSF and barrel dimensions in Tg mice being the result of differential flattening or differences in the cutting angle, because barrel and olfactory glomeruli areas were both determined from the same series of flattened tangential sections.

Areas of whisker pads and follicles, and the number of infraorbital nerve myelinated axons

The area of the whisker pads, the cross-sectional area of whisker follicles, and the number of myelinated axons in the infraorbital nerve were not statistically different among 26L IGF-I and BL IGFBP-1 Tg mice and their wt littermates (Table 5). Changes in the relative size of the PMBSF and its constituent barrels, thus, are independent of changes in the sensory periphery.

Discussion

Our data indicate that IGF-I overexpression in Tg mice increases brain, cortical, and PMBSF barrel dimensions, whereas ectopic brain expression of IGFBP-1, an inhibitor of IGF-I actions, has opposite effects. Tg mice with the highest IGF-I or IGFBP-1 transgene expression had the most affected brains and cortical mantles. Furthermore, mice carrying both IGF-I and IGFBP-1 transgenes had brains, cortical mantles, and barrel fields intermediate in size to those of native IGF-I

TABLE 4. Cross-sectional area, number, and density of neurons in PMBSF barrels of 26L IGF-I and BL IGFBP-1 Tg mice and their wild-type (wt) littermates

Line	PMBSF barrel neuron			
	Cross-sectional area (μm^2)	No./single 50 μm section	Total no.	Density ($10^5/mm^3$
Control (n = 3)	68.13 ± 12.28	444 ± 29	1589 ± 101	1.8 ± 0.41
26L IGF-I (n = 3)	90.42 ± 13.41[a] (33% ↑)	466 ± 20	1966 ± 84[a] (24% ↑)	1.1 ± 0.08[a] (39% ↓)
BL IGFBP-1 (n = 3)	61.41 ± 10.58[b] (10% ↓)	470 ± 30	1344 ± 86[b] (15% ↓)	2.5 ± 0.43[a] (39% ↑)

Values are the mean ± SEM.
[a] $P < 0.0001$ *vs.* wt mice.
[b] $P < 0.05$ *vs.* wt mice.

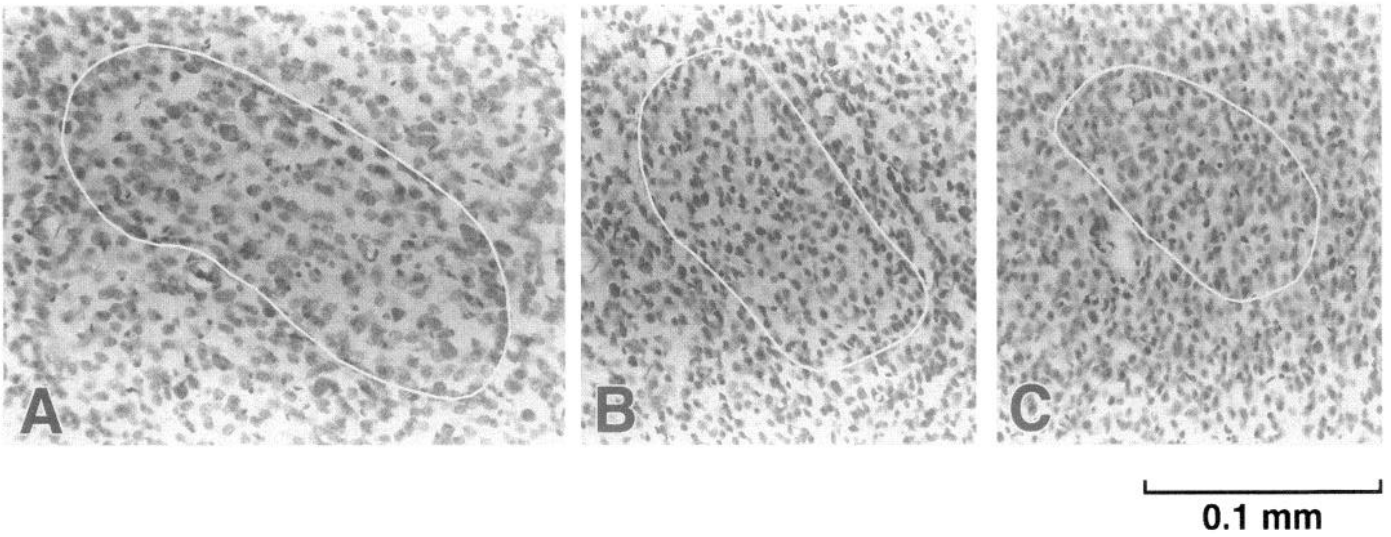

FIG. 3. Photomicrographs of cresyl violet-stained sections from 26L IGF-I (A) and BL IGFBP-1 (C) Tg mice and from a wt littermate (B). The *white lines* in each photomicrograph outline individual barrels. The differences in barrel cross-sectional area, neuron cell body size, and neuron density can be seen.

TABLE 5. Whisker pad and follicle area and number of trigeminal nerve myelinated axons in 26L IGF-I BL IGFBP-1 Tg mice and their wild-type (wt) littermates

Line	Whisker pad area (mm^2)	Whisker follicle area (mm^2)	No. of axons
Normal (n = 6)	19.02 ± 1.03	0.246 ± 0.009	12,820 ± 1,800
26 L IGF-I (n = 6)	18.91 ± 0.67	0.239 ± 0.008	12,240 ± 2,100
BL IGFBP-1 (n = 6)	19.91 ± 0.69	0.261 ± 0.001	13,125 ± 1,975

Values are the mean ± SEM.

and IGFBP-1 Tg mice, indicating that IGFBP-1 attenuates the effects of IGF-I overexpression. These results demonstrate that altered IGF-I availability is responsible for the changes in brain, cortical, and PMBSF barrel dimensions in these Tg mice and confirm the importance of IGF-I in regulating overall brain and cortical growth. Our observations in S1 are consistent with findings showing that developing projection neurons of the somatosensory system exhibit IGF-I expression during periods of neuronal growth and synaptogenesis (2, 6). Together, these data strongly suggest that IGF-I modulates neuronal growth in the developing somatosensory pathway, and that limitations of its availability impose a developmental constraint to sensory neuron growth. Furthermore, our findings show that the PMBSF provides an excellent model to precisely delineate IGF-I actions and mechanisms in the promotion of neuron growth.

Changes in cortical and barrel field dimensions were not proportional. In some Tg mice, the cortex was more affected than the barrels, whereas the opposite occurred in other Tg mice. A likely explanation for this finding is that regional variations in IGF-I availability, resulting from differential expression of the transgenes, lead to variations in cortical and PMBSF barrel dimensions. We have shown that the expression of these transgenes varies among brain regions, and that those regions with the greatest transgene expression exhibit the greatest alterations in growth (11). The size of the PMBSF, however, precludes direct assessment of transgene mRNA abundance. It, nonetheless, seems reasonable to assume that regional variations in the availability of IGF-I influence the relative dimensions of the neocortex and PMBSF barrels. The differential spatio-temporal distributions of IGF-I receptors and IGFBPs and regional differences in the onset of transgene expression also could contribute to variations in regional cortical growth.

Unlike barrel dimensions, barrel shape and number were not affected by modifications in IGF-I availability, suggesting

that IGF-I is not involved in barrel formation. This is not surprising, because IGF-I mRNA expression is first detectable in the thalamo-cortical pathway on about postnatal day 5 in rats (2), after the time when most of the PMBSF has already been formed in S1 (14). Furthermore, we found the PMBSF to be normally formed, but extremely small, in IGF-I knock-out mice (unpublished data). However, it remains possible that IGF-I has an influence on the time when thalamic afferents arrive in the developing cortex. Taken together, our observations suggest that barrel formation and growth are independent processes.

Some of the cellular processes by which modifications in IGF-I availability alter brain size have been assessed. It has been shown that myelin production and content are increased in IGF-I Tg mice and decreased in IGFBP-1 Tg (9, 11) and IGF-I knock-out mice (12). The present observations in the barrel field of IGF-I and IGFBP-1 Tg mice indicate that modifications of IGF-I availability also change the number and size of neurons and the volume of cortical layer IV occupied by neuropil. Barrels in IGF-I Tg mice had larger cells and contained more neurons than those in wt mice. Barrel neuronal density, however, was reduced, and barrel volume was increased, indicating an increase in the barrel volume devoted to neuropil. In contrast, barrels in IGFBP-1 Tg mice had smaller cells and contained fewer neurons than those in wt mice. Barrel neuronal density was increased, whereas barrel volume was decreased, indicating a diminution of neuropil volume (25). Changes in neuron number and neuropil volume are concordant with the total DNA content previously described in the brains of our Tg mice (7, 13). Thus, modifications in neuron number and size and in neuropil volume account for changes in PMBSF barrel size, and probably for those in overall brain and cortical dimensions, in IGF-I and IGFBP-1 Tg mice.

The mechanisms by which modifications in IGF-I availability alter layer IV neuron number are not known. The number of neuron precursors, the rate of precursor proliferation, the length of the proliferation phase, and/or neuronal survival may influence neuron number (26–29). *In vitro* and *in vivo* studies have shown that IGF-I can enhance neuron precursor proliferation (1) and neuron survival (1, 30, 31). Our data do not directly distinguish among these possibilities. However, we believe that modifications in IGF-I availability predominantly affect the survival of cortical and thalamic neurons. A number of observations support this contention. In the mouse, cortical layer IV neurons are generated at the end of gestation (32), and cell death occurs between embryonic day 19 and postnatal day (PN) 10 in the thalamus and S1, peaking on PN0 in the thalamus and PN7 in the neocortex (33–35). The transgenes in our Tg mice are first expressed after birth (11, 13), at a time when cell death occurs but after the generation of cortical layer IV neurons. Brain weights in these Tg mice do not differ from those in their wt littermates at birth (11, 13), suggesting that the transgenes have not yet had an impact on brain development. Taken together, it appears that the changes in barrel cytoarchitecture in Tg mice result from the effects of IGF-I on neuronal survival. It has been shown that the number of cortical cells correlates with that of thalamic afferents (36) and, thus, with thalamic neuron number. Because the number of thalamic afferents correlates with area dimensions in the neocortex (27, 37–40), the promotion of neuron survival probably occurs both in the neocortex and at subcortical levels.

Peripheral influences have been implicated in determining various structural features of the barrel field (14, 41–44). Barrel size and cell number appear proportional to the whiskers' sensory innervation density (14, 45), which, in turn, depends on whisker size (14). We investigated whether S1 structural changes in Tg mice occurred in response to modifications in body dimensions or peripheral innervation density. No differences in body weight, whisker follicle and pad sizes, or the innervation density to whisker pads were found among Tg and wt mice. These observations indicate that when there are changes in local trophic interactions, structural changes in the brain, cortex, and barrel field can proceed independently from body and sensory periphery influences. This conclusion is strengthened by the recent findings that certain mutant mice lack barrels despite having an intact sensory periphery [a naturally occurring mutant mouse described by Welker *et al.* (46) and monoamine oxidase A knock-out mice (47)]. In the latter knock-out mice, barrel formation is profoundly altered by changes in serotonin availability, suggesting that this amine plays a trophic role in the developing S1 (47). Furthermore, a disproportion between the number of nerves innervating whiskers and barrel size has been noted after reducing whisker innervation density following prenatal treatment with antinerve growth actor antibodies (21), after selective whisker ablation (42), and in mice with supernumerary whiskers (14).

Elimination of redundant connections by means of neuronal competition for neurotrophic factors is thought to play a major role in establishing brain neuronal circuitry (48, 49). Agmon *et al.* (39, 40) have recently shown, however, that thalamic terminal arbors in the barrel cortex are progressively elaborated with high topological precision, as opposed to being eliminated from an initially exuberant pattern as occurs in the visual system (37, 38, 50). Riddle *et al.* (19) documented that barrel neuropil is progressively and selectively constructed as postnatal development proceeds. This evidence suggests that progressive, and not regressive, developmental processes shape neuronal connections in the barrel cortex. Our observations that barrels remain discrete anatomical units despite their striking change in size and that neuropil volume is greatly modified in IGF-I and IGFBP-1 Tg mice suggest that selective elaboration of barrel neuropil is regulated by IGF-I. Together these observations indicate that rules governing the development of thalamo-cortical connections are different in distinct cortical areas. Although redundant connections are pruned in the visual cortex by means of neuronal competition for neurotrophic factors, the barrel cortex seems to selectively elaborate neuronal connections in a fashion dependent upon the amount of trophic factors available.

Acknowledgment

The authors are grateful to Luisa E. Brighton for technical assistance.

References

1. **Ishii DN** 1993 Neurobiology of insulin and insulin-like growth factors. In: Loughlin SE, Fallon JH (eds) Neurotrophic Factors. Academic Press, New York, pp 415–442
2. **Bondy CA** 1991 Transient IGF-I gene expression during the maturation of functionally related central projection neurons. J Neurosci 11:3442–3455
3. **Lee W-H, Javedan S, Bondy CA** 1992 Coordinate expression of insulin-like growth factor system components by neurons and neuroglia during retinal and cerebellar development. J Neurosci 12:4737–4744
4. **Bartlett WP, Li X-S, Williams M, Benkovic S** 1991 Localization of insulin-like growth factor-1 mRNA in murine central nervous system during postnatal development. Dev Biol 147:239–250
5. **Bartlett WP, Li X-S, Williams M** 1992 Expression of IGF-I mRNA in the murine subventricular zone during postnatal development. Mol Brain Res 12:285–291
6. **Bondy C, Lee W-H** 1993 Correlation between insulin-like growth factor (IGF)-binding protein 5 and IGF-I gene expression during brain development. J Neurosci 13:5092–5104
7. **Behringer RR, Lewin TM, Quaife CJ, Palmiter RD, Brinster RL, D'Ercole AJ** 1990 Expression of insulin-like growth factor I stimulates normal somatic growth in growth hormone-deficient transgenic mice. Endocrinology 127:1033–1040
8. **Mathews LS, Hammer RE, Behringer RR, D'Ercole AJ, Bell GI, Brinster RL, Palmiter RD** 1988 Growth enhancement of transgenic mice expressing human insulin-like growth factor I. Endocrinology 123:2827–2833
9. **Carson MJ, Behringer RR, Brinster RL, McMorris FA** 1993 Insulin-like growth factor I increases brain growth and central nervous system myelination in transgenic mice. Neuron 10:729–740
10. **Ye P, Carson J, D'Ercole AJ** 1995 Insulin-like growth factor-I influences the initiation of myelination: studies of the anterior commissure of transgenic mice. Neurosci Lett 201:235–238
11. **Ye P, Carson J, D'Ercole AJ** 1995 *In vivo* actions of insulin-like growth factor-I (IGF-I) on brain myelination: studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice. J Neurosci 15:7344–7356
12. **Beck KD, Powell-Braxton L, Widmer H-R, Valverde J, Hefti F** 1995 Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal and striatal parvalbumin-containing neurons. Neuron 14:717–730
13. **D'Ercole AJ, Dai Z, Xing Y, Boney C, Wilkie MB, Lauder JM, Han VK, Clemmons DR** 1994 Brain growth retardation due to the expression of human insulin-like growth factor binding protein-1 (IGFBP-1) in transgenic mice: an *in vivo* model for analysis of IGF function in the brain. Dev Brain Res 82:213–222
14. **Welker E, Van der Loos H** 1986 Quantitative correlation between barrel-field size and the sensory innervation of the whiskerpad: a comparative study in six strains of mice bred for different patterns of mystacial vibrissae. J Neurosci 6:3355–3373
15. **Pasternak JF, Woolsey TA** 1982 The number, size and spatial distribution of neurons in lamina IV of the mouse SmI neocortex. J Comp Neurol 160:291–306
16. **Belford GR, Killackey J** 1979 The development of vibrissae representation in subcortical trigeminal centers of the neonatal rat. J Comp Neurol 188:63–74
17. **Durham D, Woolsey TA** 1984 Effects of neonatal whisker lesions on mouse central trigeminal pathways. J Comp Neurol 223:424–447
18. **Ma PM** 1991 The barrelettes-architectonic vibrissal representations in brain stem trigeminal complex of the mouse. I. Normal structural organization. J Comp Neurol 309:161–199
19. **Riddle D, Richards A, Zsuppan F, Purves D** 1992 Growth of the rat somatic sensory cortex and its constituent parts during postnatal development. J Neurosci 12:3509–3524
20. **Curcio CA, Coleman PD** 1982 Stability of neuron number in cortical barrels of aging mice. J Comp Neurol 212:158–172
21. **Sikich L, Woolsey TA, Johnson EMJ** 1986 Effect of a uniform partial denervation of the periphery on the peripheral and central vibrissal system in guinea pigs. J Neurosci 6:1227–1240
22. **Shuz A, Palm G** 1996 Density of neurons and synapses in the cerebral cortex of the mouse. J Comp Neurol 286:442–455
23. **Giacobini MMJ, Olson L, Hoffer BJ, Sara VR** 1990 Truncated IGF-1 exerts trophic effects on fetal brain tissue grafts. Exp Neurol 108:33–37
24. **Giacobini MMJ, Zetterstrom RH, Young D, Hoffer B, Sara V, Olson L** 1995 IGF-I influences olfactory bulb maturation: evidence from an anti-IGF-I antibody treatment of developing grafts in oculo. Dev Brain Res 84:67–76
25. **Tower DB** 1954 Structure and functional organization of the mammalian cerebral cortex: the correlation of neuron density with brain size. J Comp Neurol 101:19–53
26. **Dehay C, Giroud P, Berland M, Smart I, Kennedy H** 1993 Modulation of the cell cycle contributes to the parcellation of the primate visual cortex. Nature 366:464–466
27. **Kennedy H, Dehay C** 1993 Cortical specification of mice and men. Cereb Cortex 3:171–186
28. **Caviness VS, Talahashi TJ, Nowakowski RS** 1995 Numbers, time, and neocortical neurogenesis: a general developmental and evolutionary model. Trends Neurosci 18:378–382
29. **Rakic P** 1995 Evolution of cortical parcellation: the perspective from experimental neuroembryology. In: Changeux J-P, Chavallon J (eds) Origins of the Human Brain. Claredon Press, Oxford, pp 84–100
30. **Recio-Pinto E, Rechler MM, Ishii DN** 1986 Effects of insulin, insulin-like growth factor-II, and nerve growth factor on neurite formation and survival in cultured sympathetic and sensory neurons. J Neurosci 6:1211–1219
31. **Aizeman Y, De Vellis J** 1987 Brain neurons develop in a serum and glia free environment: effects of transferrin, insulin, insulin-like growth factor-I and thyroid hormones on survival, growth and differentiation. Brain Res 406:32–42
32. **Hicks SP, D'Amato CJ** 1968 Cell migration to the isocortex in the rat. Anat Rec 160:619–634
33. **Heuman D, Leuba G** 1996 Neuronal death in the development and aging of the cerebral cortex of the mouse. Neuropathol Appl Neurobiol 9:297–311
34. **Ferrer I, Tortosa A, Blanco R, Martin F, Serrana T, Planas A, Macaya A** 1994 Naturally occurring cell death in the developing cerebral cortex of the rat. Evidence of apoptosis-associated internucleosomal DNA fragmentation. Neurosci Lett 182:77–79
35. **Waite PM, Li L, Ashwell KW** 1992 Developmental and lesion induced cell death in the rat ventrobasal complex. Neuroreport 3:485–488
36. **Windrem MS, Finlay BL** 1991 Thalamic ablations and neocortical development: alterations of cortical cytoarchitecture and cell number. Cereb Cortex 1:230–240
37. **Killackey HP, Belford GR** 1979 The formation of afferent patterns in the somatosensory cortex of the neonatal rat. J Comp Neurol 183:285–302
38. **Catalano SM, Robertson RT, Killackey HP** 1995 Rapid alteration of thalamocortical axon morphology follows peripheral damage in the neonatal rat. Proc Natl Acad Sci USA 92:2549–2552
39. **Agmon A, Yang LT, O'Dowd DK, Jones EG** 1993 Organized growth of thalamocortical axons from the deep tier of terminations into layer IV of developing mouse barrel cortex. J Neurosci 13:5365–5382
40. **Agmon A, Yang LT, Jones EG, O'Dowd DK** 1995 Topographical precision in the thalamic projection to neonatal mouse barrel cortex. J Neurosci 15:549–561
41. **Van der Loos H, Dorfl J** 1978 Does the skin tell the somatosensory cortex how to construct a map of the periphery? Neurosci Lett 7:23–30
42. **Welker E, Van der Loos H** 1986 Is areal extent in sensory cerebral cortex determined by peripheral innervation density? Exp Brain Res 63:650–653
43. **Killackey HP, Rhoades RW, Bennett-Clarke CA** 1995 The formation of a cortical somatotropic map. Trends Neurosci 18:402–406
44. **Killackey HP, Chiaia NL, Bennett-Clarke CA, Eck M, Rhoades RW** 1994 Peripheral influences on the size and organization of somatotopic representations in the fetal rat cortex. J Neurosci 14:1496–1506
45. **Lee KJ, Woolsey TA** 1975 A proportional relationship between peripheral innervation density and cortical neuron number in the somatosensory system of the mouse. Brain Res 99:349–353
46. **Welker E, Armstrong-James M, Bronchti G, Ourednik W, Gheorghita-Baechler F, Dubois R, Guernsey DL, Van der Loos H, Neumann PE** 1996 Altered sensory processing in the somatosensory cortex of the mouse mutant barreless. Science 271:1864–1867
47. **Cases O, Vitalis T, Seif I, De Maeyer E, Sotelo C, Gaspar P** 1996 Lack of barrels in the somatosensory cortex of monoamine oxidase A-deficient mice: role of a serotonin excess during the critical period. Neuron 16:297–307
48. **Cowan WM, Fawcett JW, O'Leary DDM, Stanfield BB** 1984 Regressive events in neurogenesis. Science 225:1258–1265
49. **Cabelli RJ, Hohm A, Shatz JC** 1995 Inhibition of ocular dominance column formation by infusion of NT-4/5 or BDNF. Science 267:1662–1666
50. **Jensen KF, Killackey HP** 1987 Terminal arbors of axons projecting to the somatosensory cortex of the adult rat. II. The altered morphology of thalamocortical afferents following neocortical infraorbital nerve cut. J Neurosci 7:3544–3553