Early Development of Ocular Dominance Columns

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The segregation of lateral geniculate nucleus (LGN) axons into ocular dominance columns is believed to involve a prolonged, activity-dependent sorting process. However, visualization of early postnatal ferret LGN axons by direct LGN tracer injections revealed segregated ocular dominance columns <7 days after innervation of layer 4. These early columns were unaffected by experimentally induced imbalances in retinal activity, implying that different mechanisms govern initial column formation and their modification during the subsequent critical period. Instead of activity-dependent plasticity, we propose that ocular dominance column formation relies on the targeting of distinct axonal populations to defined locales in cortical layer 4.

For nearly four decades, the development of ocular dominance—defined as the bias of visual cortical neurons to respond to stimulation of one eye or the other—has been a central model for the development of modular circuitry in mammalian neocortex. Ocular dominance in primates and carnivores results from the segregation of LGN axons into eye-specific columns in layer 4 of the primary visual cortex. The emergence of ocular dominance columns is widely held to involve progression from an initial state in which LGN afferents representing the two eyes overlap extensively to a mature state in which eye-specific afferents occupy largely independent stripe-like territories (*1–3*). In cats and ferrets, this process is believed to take several weeks, and to coincide with the "critical period" during which the organization of these same axons is susceptible to manipulations of visual experience (*1, 4–6*). As elimination of normal retinal activity during this period appears to disrupt segregation (*7*), the emergence of ocular dominance columns has been thought to rely on correlated patterns of neuronal activity that sort geniculocortical axons into eye-specific domains.

This conceptual framework relies heavily on the developmental anatomy of cortical columns as visualized by intraocular injections of transneuronally transported anterograde tracers such as ^{3}H proline (*8*), whose interpretation can be complicated by "spillover" of label in the LGN. As early studies made clear, this was more severe in younger animals and could complicate developmental studies (*1*). Thus, in young animals, the presence of a continuous band of label in layer 4 might represent either the absence of segregation—as subsequent investigations have widely assumed—or result from extensive spillover in the LGN. To circumvent this problem, we visualized the patterns of LGN afferent termination in layer 4 by directly injecting anterogradely transported tracers into eye-specific LGN layers in developing ferrets (*9*). Like cats, ferrets are carnivores with robust ocular dominance columns and a well-defined critical period, but unlike cats, their geniculocortical projection forms postnatally, greatly facilitating experimental manipulations.

Ocular dominance column patches in early postnatal ferrets. By visualizing the dorsal surface of the LGN, tracer injections could be confined to individual LGN layers (Fig. 1A). In animals perfused between postnatal day 9 (P9) and P12 (n = 15) (*10*), which is just as the first geniculocortical axons reach layer 4 (*11*), the geniculocortical innervation of the cortical plate was sparse, with individual axons exhibiting few side branches. In animals perfused between P13 and P15 (n = 15), the density of axons in layer 4 was higher and frequently nonhomogeneous, but the innervation density was too low to determine conclusively whether these fluctuations corresponded to nascent columns (*12*).

However, in animals perfused as early as P16 to P18 (n = 13; equivalent to about embryonic days 58 to 60 in cat), LGN injections produced strikingly segregated patches of geniculocortical axons in layer 4 (Fig. 1B). Similar patches were seen after injections at P19 to P21 (n = 4; Fig. 1, C and D) and P22 to P30 (n = 4) (*12*). The labeled regions of layer 4 extended over 1 to 2 mm and contained three to eight alternating cycles of densely labeled and unlabeled patches of about equal width. Because of its larger size, about three-quarters of the injections were centered in the A layer of the LGN [representing the contralateral eye; P16 to P18 (n = 10), P19 to P21 (n = 3), or P22 to P30 (n = 3)]; however, some of the most striking cases of nascent columns (e.g., Fig. 1D) resulted from injections of layer A1 (which represents the ipsilateral eye). Thus, at the same ages, axons from both layer A and A1 simultaneously assume patchy distributions in layer 4. In a few cases, injections of the LGN were clearly not confined to an individual LGN layer. In such cases, the resulting label consisted of a homogeneous band of label, and patches in layer 4 were absent (n = 3) (*13*). Thus, the segregated, patchy termination patterns observed after restricted LGN injections do not result from a general inhomogeneity of LGN inputs at these early ages. On the basis of their size, shape, and laminar distribution in coronal sections, these patches closely resemble ocular dominance columns in adult ferrets visualized by the same approach (*9, 14*).

Equivalence of early patches to ocular dominance columns. To ascertain that these patches indeed represent the juvenile form of ocular dominance columns, we reconstructed serial coronal sections (*9*) from three animals to provide a surface view of the labeling patterns after LGN injections (Fig. 2A). These reconstructions yielded stripe-like patterns with areas of dense label roughly 250 μm wide interdigitated with unlabeled zones of equal width. These patterns are very similar to the striped patterns seen in flattened, tangential sections of cortex after transneuronal transport in older animals (*6, 15, 16*). The presence of continuous label across many serial coronal sections and the arrangement of patches into alternating stripes [similar to that seen in the original anatomical descriptions of ocular dominance columns (*17, 18*)] argue against the possibility that these patches arise from sectioning, sampling, or processing artifacts. As no other anatomical feature in developing or mature primary visual cortex has this characteristic, alternating pattern, including the segregation of ON and OFF inputs (*19*), it is difficult to imagine that these stripes could represent a feature other than ocular dominance columns.

To further characterize these patterns, we measured the center-to-center spacing of labeled patches in coronal sections from neonatal animals (P17 to P21 at perfusion; Fig. 2B). Patches had a center-to-center spacing of 530 ± 22 μm (n = 6; mean ± SEM), statistically indistinguishable from the normal adult value of 612 ± 34 μm (n = 5), determined with identical techniques (*9*) [multivariate analysis of variance (MANOVA), Wilks' Lambda, P = 0.13], and comparable to spacing determined with other techniques (*4, 6, 15, 16*). Although not significantly different, younger animals tended to have smaller center-to-center patch spacing, probably reflecting the substantial brain growth that occurs between these neonatal ages and adulthood.

Segregation of early ocular dominance columns. A central feature of ocular domi-

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nance column formation inferred from transneuronal transport studies and descriptions of individual axon arbors (*20, 21*) is an initial state during which the cortical territories occupied by LGN afferents overlap completely, followed by a prolonged period (several weeks) during which afferents segregate into discrete zones. The direct visualization of groups of afferents by LGN injection suggests a different picture. As soon as we can detect ocular dominance columns—about a week after axons first arrive in layer 4—there is little further refinement of the segregated pattern. In many cases, densely innervated columns were adjacent to equivalently sized zones nearly devoid of axon arbors, a pattern similar to, and perhaps even more precise than that observed in adults (*9*).

To approximate the degree of segregation in the early-emerging columns observed with direct LGN injections, we determined the ratio of label density at the center of columns in juvenile (P17 to P21 at perfusion, $n = 6$) and adult animals ($n = 3$). The segregation index (SI) (*22*) varies from a value of 1 for highly segregated label, to 0 for unsegregated labeling. The SIs of juveniles (0.64 ± 0.06; $n = 6$; mean ± SEM) and adults (0.52 ± 0.05; $n = 3$) were indistinguishable (MANOVA, Wilks' Lambda, $P = 0.13$; Fig. 2C), indicating that the initially formed columns were as well segregated as adult columns. Even with the direct LGN injection technique, it is impossible to be certain that the injected tracer was completely confined to a single layer. Thus, the presence of label between patches could be due to either genuine overlap of arbors from the two geniculate layers or tracer diffusion. This problem, which would degrade the apparent specificity of label, should be worse in younger animals, whose LGN layers are smaller and closer together. Therefore, these estimates of segregation in young animals may underestimate the precision of initial segregation. Thus, ocular dominance column formation appears to involve the rapid, selective elaboration of initially well specified connections, rather than prolonged regression of mistargeted axons (*23, 24*).

Effects of unbalanced retinal activity on ocular dominance column establishment. The establishment of ocular dominance columns and their plasticity during the critical period have both been hypothesized to involve competition between groups of afferents on the basis of correlated patterns of neuronal activity (*23, 25, 26*). During the critical period, just 4 days of monocular deprivation significantly alters the morphology and distribution of LGN axon arbors in cortex (*27*). At these ages, monocular enucleation (like pharmacological activity blockade) has even more marked effects on ocular dominance than monocular deprivation by lid suture (*25, 28*). To test the effects of

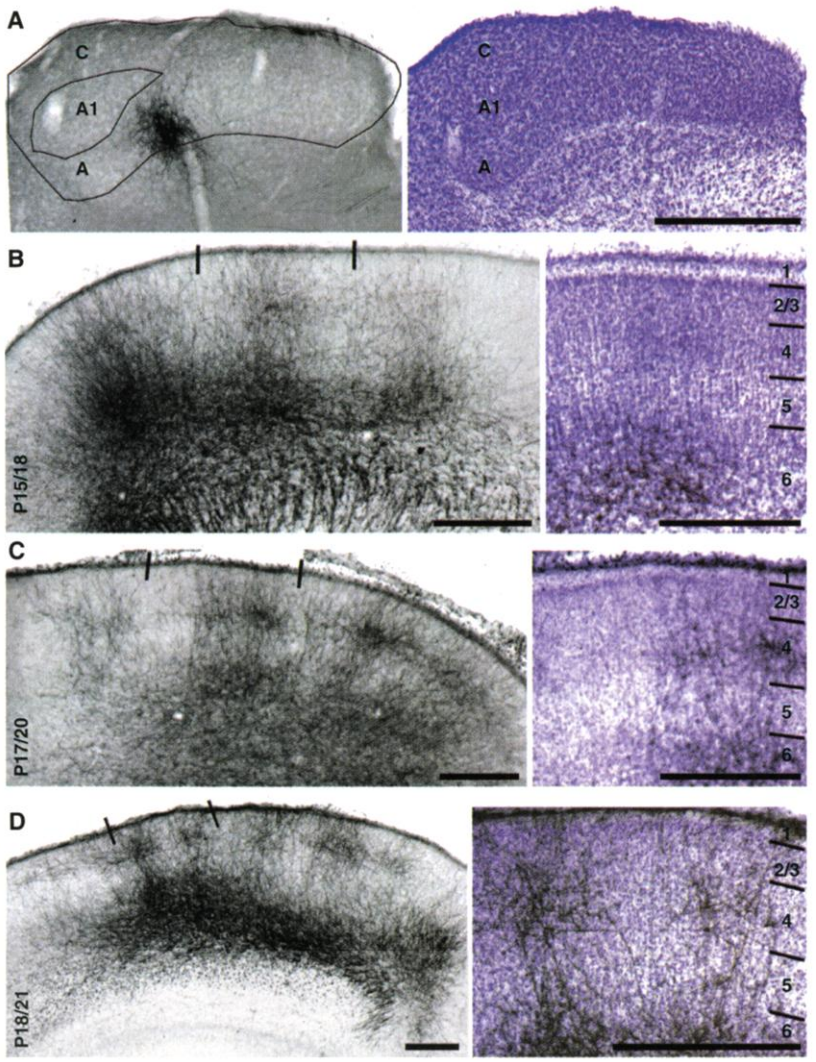

Fig. 1. Ocular dominance columns in neonatal ferret visual cortex. Postnatal ages (P_/_) indicate LGN injection age/perfusion age. (**A**) Anterograde tracer (BDA) injection confined to a single LGN layer (layer A) in a P13/16 ferret. Horizontal section through thalamus on left shows injection site with laminar boundaries, derived from Nissl-stained section on right. (**B** to **D**) At left, coronal sections of ocular dominance patches in layer 4 of visual cortex after LGN injections of BDA. At right, Nissl stains of the same section with laminar boundaries indicated (ticks on left indicate regions enlarged at right). Segregated patches were clearly present in layer 4 and to a lesser extent in layer 6. Scale bars, 500 μm; all sections are 60 μm thick.

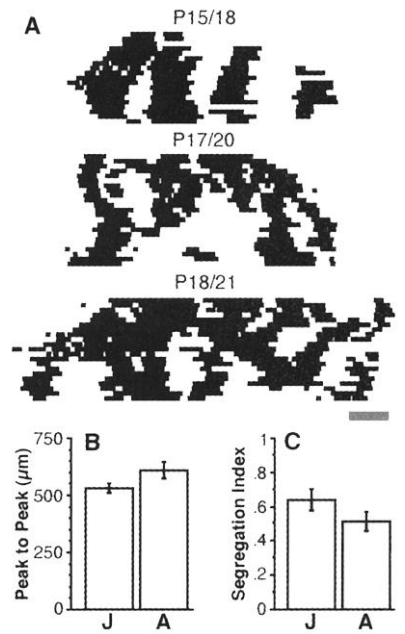

Fig. 2. Stripe-like patterns of juvenile ocular dominance columns and their similarity to adult columns. (**A**) Surface view reconstructions of serial coronal sections from the cortices shown in Fig. 1. Alternating, labeled (black) and unlabeled bands extend for several millimeters along the medial-lateral (left to right) and anterior-posterior axes. Gray scale bar, 500 μm, applies to both axes. (**B**) Mean labeled patch peak-to-peak distances in juvenile (J; P17 to P21; $n = 6$) and adult animals (A; $n = 5$). Values are not significantly different. (**C**) Mean segregation indices for juvenile (P17 to P21; $n = 6$) and adult animals ($n = 3$) are also indistinguishable, indicating that juvenile columns are at least as well segregated as those of adults.

inducing imbalances in retinal activity on early ocular dominance columns, we monocularly enucleated eight ferrets (*9*) between P7 and P14 and injected their LGNs 3 days before perfusion, which occurred between P17 and P21. Thus, these animals sustained 4 to 14 days of unbalanced retinal activity during the initial establishment of ocular dominance columns. In six animals, the LGN injections were successful (Table 1). In all cases, alternating patches of labeled and unlabeled regions were evident and did not obviously differ from columns in normal animals of the same ages (Fig. 3, A and B; compare with Fig. 1, B to D); deprived and nondeprived columns were the same width (deprived columns: 260 ± 33 μm; nondeprived columns: 260 ± 25 μm; means ± SEM; $P = 0.99$, t test; Fig. 3C). Thus, the representation of the removed eye was not reduced, nor was that of the remaining eye expanded. The parceling of cortex by these early-established columns is resistant to the extreme imbalance of retinal activity induced by monocular enucleation, in contrast to the rapid reorganization that similar manipulations produce during the critical period (*5, 28–31*).

Discussion. These observations indicate that the initial appearance of ocular dominance columns occurs nearly 3 weeks before they have been revealed by transneuronal transport, optical imaging, or 2-deoxyglucose labeling in ferrets (P36 to P37) (*4, 6*) or cats of equivalent developmental stages (*32, 33*). Ocular dominance columns not only appear much earlier than previously thought, but they emerge at a markedly different stage of cortical development (Fig. 4). In ferrets, P21 is near the end of the migration period of newly generated layer 2/3 cortical neurons in ferret (*34*), before the onset of cortical visual responses (*35*), before the generation of horizontal collaterals (*36, 37*), well before the critical period (*5*), and during the initial period of synaptogenesis in layer 4 (*11*). It is also a period during which the cortical subplate is still present, which previous studies have linked to the targeting of LGN axons to visual cortex (*38*) and to the formation of ocular dominance columns (*39*).

These findings argue that the establishment and plasticity of ocular dominance columns are temporally and mechanistically distinct phases of visual system development. Several additional lines of evidence support this contention. Even with transneuronal transport, ocular dominance columns with adult-like specificity are clearly evident at birth in monkeys (*40, 41*); thus, in primates, changes in visual experience during the postnatal critical period act on already existing columns. In cats, binocular blockade of retinal activity can eliminate segregation of eye-specific thalamic afferents (*7*). Although originally interpreted as demonstrating that activity was necessary for the segregation of overlapping inputs, subsequent work from the same laboratory showed that this manipulation was performed after ocular dominance columns were already present (*32*). Similar degradation of an already existing, eye-specific pattern has been observed after activity blockade of eye-

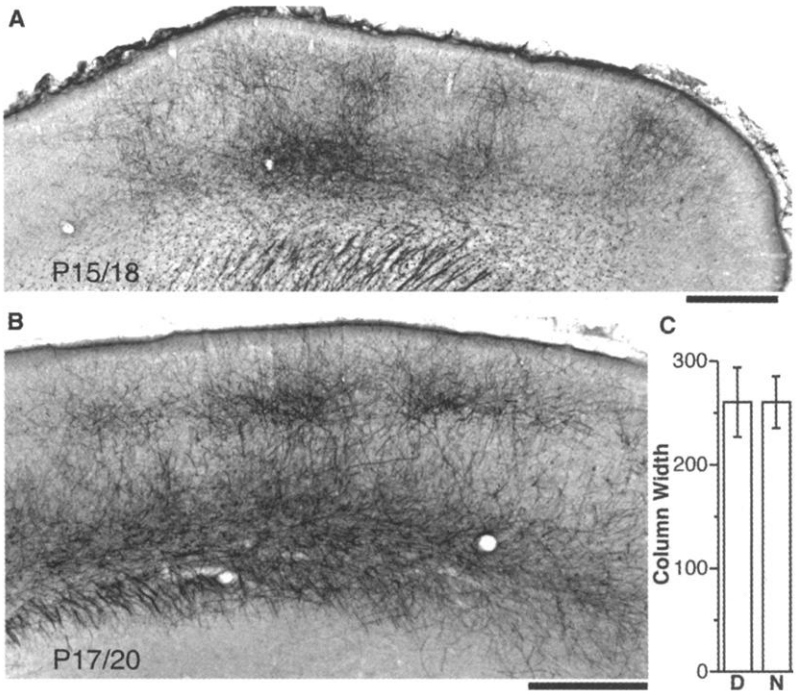

Fig. 3. Persistence of juvenile ocular dominance columns after monocular enucleation. (**A** and **B**) Ocular dominance columns in monocularly enucleated ferrets appear normal (compare with Fig. 1, B to D). Coronal sections of visual cortex showing BDA label after injection of LGN layer A. In both cases, the contralateral eye was removed; hence, the cortical labeling originates from deprived eye afferents. (A) is animal 4 from Table 1 and (B) is animal 5 from Table 1. Scale bar, 500 μm; section thickness, 60 μm. (**C**) The widths of ocular dominance patches representing the enucleated and intact eyes are indistinguishable, implying that early-formed columns do not shrink or expand in response to imbalances in activity.

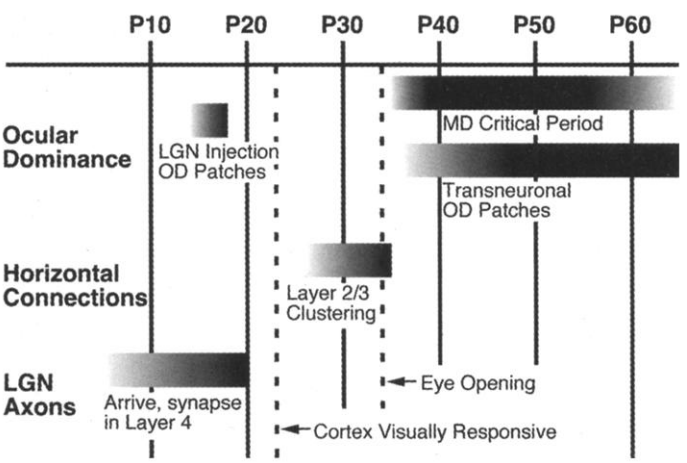

Fig. 4. Developmental timeline for the ferret visual cortex. Derived from (*5*) and numerous earlier references therein (*4–6, 11, 34–37*) and incorporating the current findings. Ocular dominance (OD) column emergence is distinct from the critical period for monocular deprivation (MD) and coincides closely with the initial LGN axon ingrowth into layer 4.

Table 1. Normal ocular dominance columns in monocularly enucleated (ME) ferrets. Eye removed is relative to the LGN injected and the cortex analyzed. LGN layer indicates the LGN layer injected with anterograde tracer. Sections from animals 4 and 5 are shown in Fig. 3.

ME ferret	Eye removed	ME age	Perfusion age	LGN layer
1	Contra	P7	P17	A
2	Ipsi	P7	P21	A
3	Ipsi	P8	P18	A
4	Contra	P14	P18	A
5	Contra	P14	P20	A
6	Ipsi	P14	P21	A

specific retinogeniculate afferents (*42*).

We have previously observed marked correlational differences between spontaneous patterns of activity in the LGN eye-specific layers of P23 to P27 ferrets (*43*), which we now know are present after segregation of geniculate afferents. Thus, endogenous differences in activity patterns at these ages clearly do not produce alterations in anatomical patterning and may in part be the result of ocular dominance segregation. Furthermore, bilateral enucleation, which reduces these differences, has no effect on ocular dominance segregation (*9*). Perhaps most strikingly, the recently established columns shown here are refractory to manipulations in activity patterns that elicit plastic changes later in life.

At P18 in ferret, when we already observe clear ocular dominance columns (Fig. 1B), layer 2/3 cells are still migrating and have not established functional horizontal circuitry (*36, 37, 44, 45*). Because orientation columns, direction selectivity, and horizontal connections all first emerge after P21 in ferrets, it now appears that ocular dominance columns are the first component of modular circuitry to emerge in visual cortex. Therefore, cortical activity patterns present after P16 are likely to represent consequences, not causes, of segregated ocular dominance columns. Moreover, the early presence of ocular dominance columns may constrain the subsequent organization of orientation columns, perhaps explaining why orientation pinwheel centers align with the centers of ocular dominance columns (*46–49*).

What signals lead to the initial establishment of columns? The experiments reported here cannot exclude contributions by neuronal activity before P16. Thus, there is a window of about 1 week during which activity in the thalamocortical circuit could provide a template for ocular dominance column patterning, perhaps by involving circuits outside of layer 4 (*38, 39*). However, we have argued previously that the persistence of segregated columns after bilateral enucleation at birth suggests that molecular cues may guide column formation (*9*), an assertion reinforced by the early specificity of geniculate axons and their resistance to monocular enucleation as reported here. Although commonly referred to as "left" and "right" eye-specific, ocular dominance columns are in fact the cortical representation of retinal ganglion cells from the nasal portion of one retina and the temporal portion of the other retina. The nasal/temporal distinctions in the retina and at the optic chiasm are almost certainly mediated by molecular cues (*50, 51*). A similarly high degree of developmental specificity has been observed in the development of the magnocellular and parvocellular pathways in the primate retinogeniculate projection (*52*). We propose that between P10 and P15, axon guidance cues on LGN axons, cortical neurons, or both are sufficient to initially establish columns, and that neuronal activity is subsequently required for their maintenance and plasticity during the critical period.

References and Notes

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10. All experiments used normally pigmented, sable ferrets (P7 to adult), *Mustela putorius furo* (Marshall Farms, North Rose, NY), of both sexes. Surgical procedures were performed aseptically, in accordance with a Duke University Institutional Animal Care and Use Committee Protocol (*9*). LGN layers were injected with BDA [biotinylated dextran amine, molecular weight (MW) = 10,000; Molecular Probes, Eugene, OR] and/or TMRD (tetramethylrhodamine dextran, MW = 3000; Molecular Probes, Eugene, OR). TMRD was pressure-injected with a Picospritzer (General Valve, Fairfield, NJ) through glass micropipettes (5- to 30-μm-diameter tip). BDA was iontophoresed (+ 1 to 5 μA, pulsed for 5 to 15 min) into the LGN through glass micropipettes (5- to 30-μm diameter tip). After 3 to 7 days of transport (3 days was typical for young animals; 7 days was typical for adults), ferrets were perfused and tissue was sectioned (*9*). BDA-labeled tissue was processed for bright-field microscopy (*9*) or for epifluorescence microscopy. BDA label was reacted for epifluorescence microscopy with streptavidin, Alexa Fluor 488 conjugate (Molecular Probes), after pretreatment with 2% bovine serum albumin and 0.25% Triton X-100. Images of histological specimens were acquired with a cooled charge-coupled device (CCD) camera (Princeton Instruments, Trenton, NJ) and analyzed with Macintosh G3 computers and IPLab (Scanalytics, Fairfax, VA).
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14. Dorsal visual cortex in ferret contains both V1 and V2, whereas the tentorial surface contains V1 exclusively (*5, 6, 15, 16*). The anterior region of dorsal geniculorecipient visual cortex has large ocular dominance patches whose relation to typical mammalian ocular dominance columns is not well characterized. We observed well-segregated termination patterns in both anterior and posterior dorsal geniculorecipient visual cortex as well as tentorial visual cortex.
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22. Twelve-bit images of coronal sections across the anterogradely labeled, patchy region in each animal were used to estimate mean label intensity for particular areas of the image. Measurements were pixel value means for ~100-μm^2 areas (comprising 625 pixels; 4.04 μm per pixel), inverted so that larger values indicated denser labeling. Measurements were taken for all patches of label (p) and their neighboring interpatch area (i) on a given section as well as an unlabeled region of tissue (tissue background, t). From these measurements, three values were calculated for each patch-interpatch pair:

$$\text{Patch intensity } (P) = (p - t)$$

$$\text{Interpatch intensity } (I) = (i - t)$$

$$\text{Segregation index (SI)} = 1 - (I/P)$$

P and I indicate label intensity at their respective regions, and SI indicates the difference between P and I for a patch-interpatch pair (segregation increases as SI approaches a value of 1). If $I < 10$, it was set to a fixed value of 10. All measurements for an individual animal contributed to an animal mean SI. Mean animal SI values were then compared by age group. Adult animals used were a subset of adults used for the analysis of center to center spacing and a previous study (*9*).
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