ance (for example, due to enhanced ECM remodeling or local application of cell tension) and thereby couple changes in ECM extension to expansion of cell mass within the local tissue microenvironment. Tissue involution may be promoted in other microenvironments by inducing rapid breakdown of ECM and associated cell retraction. During malignant transformation, progressive loss of shape-dependent regulation also may lead to cell survival in the absence of ECM extension, unrestricted mass expansion, and hence neoplastic disorganization of tissue architecture (*25–27*).

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29. We coated beads with FN (Collaborative Biomedical; 50 μg/ml) using carbonate buffer (*18*). Patterned substrates containing islands coated with FN were fabricated by a microcontact printing method (*13, 14*). Briefly, hexadecanethiol [$HS(CH_2)_{15}CH_3$] was printed onto gold-coated substrates with a flexible stamp containing a relief of the desired pattern. The substrate was immersed immediately in 2 mM tri(ethylene glycol)-terminated alkanethiol [$HS(CH_2)_{11}(OCH_2CH_2)_3OH$ in ethanol], which coated the remaining bare regions of gold. When these substrates were immersed in a solution of FN, vitronectin, or type I collagen (50 μg/ml in phosphate-buffered saline), the protein rapidly adsorbed only to the stamped regions. Antibody-coated substrates were prepared by first immersing surfaces in a solution of goat antibody to mouse immunoglobulin G Fc (50 μg/ml) and washed with 1% bovine serum albumin in Dulbecco's modified Eagle's medium before immobilizing the mouse antibodies to integrin $\alpha_V\beta_3$ (1 μg/ml; LM609; Chemicon), β_1 (1 μg/ml; BD15; Biosource), or a combination of the two (0.5 μg/ml each). Cells cultured on substrates with no mouse antibody or antibodies to intracellular proteins did not adhere under these conditions.

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Lamina-Specific Connectivity in the Brain: Regulation by N-Cadherin, Neurotrophins, and Glycoconjugates

Akihiro Inoue and Joshua R. Sanes*

In the vertebrate brain, neurons grouped in parallel laminae receive distinct sets of synaptic inputs. In the avian optic tectum, arbors and synapses of most retinal axons are confined to 3 of 15 laminae. The adhesion molecule N-cadherin and cell surface glycoconjugates recognized by a plant lectin are selectively associated with these "retinorecipient" laminae. The lectin and a monoclonal antibody to N-cadherin perturbed laminar selectivity in distinct fashions. In contrast, neurotrophins increased the complexity of retinal arbors without affecting their laminar distribution. Thus, cell surface molecules and soluble trophic factors may collaborate to shape lamina-specific arbors in the brain, with the former predominantly affecting their position and the latter their size.

Many parts of the vertebrate brain are organized into parallel laminae that bear distinct neuronal subtypes and receive distinct synaptic inputs (*1–3*). In the optic tectum of the chick, for example, most retinal axons terminate in just 3 of 15 laminae (Fig. 1A). Each individual axon arborizes within a single lamina, even though the dendrites of some postsynaptic cells extend through many laminae (*4–6*). The lamina-selective arborization of incoming axons may be a major determinant of specific synaptic connectivity.

To elucidate the mechanisms underlying lamina-specific retinotectal connectivity, we devised a coculture system in which a transverse tectal section was overlaid with a retinal strip, such that neurites from retinal ganglion cells had equal access to all tectal laminae (Fig. 1B). Outgrowth and arborization on these sections reproduced the lamina-selective patterns observed in vivo, implicating local tectal cues in axonal guidance (*5*). Moreover, lamina-selective growth persisted when sections were chemically fixed before retinal strips were added, which implied that some cues are associated with the cell surface and are independent of activity (*5*). We later assessed the distribution of cell adhesion mol-

Department of Anatomy and Neurobiology, Washington University School of Medicine, 660 South Euclid Avenue, Box 8108, St. Louis, MO 63110, USA.

*To whom correspondence should be addressed.

ecules in the tectum and identified a subset concentrated in the retinorecipient laminae (RL), called stratum griseum et fibrosum superficiale (SGFS) laminae B, D, and F (*7*). Here, we tested whether these molecules regulate the formation of retinal arbors. In addition, we assessed the function of neurotrophins, soluble factors believed to regulate axonal growth in several parts of the central nervous system (*8*), including the optic tectum (*9*).

The cell adhesion molecule N-cadherin is involved in numerous aspects of neural development (*10*). In chick tectum, it is broadly distributed at early stages but becomes concentrated in RL by embryonic day 14 (E14) as synapses form (*7*). Electron microscopy showed that N-cadherin is localized to the synaptic cleft, which suggests that it has a function in synapse formation or maintenance (*7, 11*). We cultured retinal strips atop fixed tectal sections (*12*) in the absence or presence of a monoclonal antibody (mAb) to N-cadherin (*13*). After 6 days in vitro, retinal neurites were stained and measured (*14*). In control cultures, neurites extended preferentially along the most superficial tectal lamina, the stratum opticum (SO), through which all retinal axons enter the tectum in vivo. Neurites then turned or branched to enter the RL, wherein they either ended or branched again to form rudimentary arbors (Fig. 1C). Neurites also extended along the SO in the presence of N-cadherin mAb, but their behavior in the RL was affected in two respects. First, the extent to which terminals formed secondary branches (rudimentary arbors) was reduced by N-cadherin mAb. In control cultures, about two-thirds of the neurites that reached the RL branched at least once, and about one-third of these formed multiple branches. In the presence of antibody, these fractions were reduced to 40% and 10% of control values, respectively (Fig. 2B). Second, the laminar distribution of retinal terminals was altered. In controls, only 3% of the neurites that left the SO extended past the RL, whereas ~14% did so in the presence of N-cadherin mAb (Figs. 1D and 2C). Moreover, more neurites terminated in laminae A to C in the presence of N-cadherin mAb than in controls, and fewer terminated in laminae D to F (Fig. 2C). These results suggest that N-cadherin normally promotes or stabilizes contacts of neurites with targets in the RL.

N-cadherin mAb also decreased the mean length of neurite outgrowth on the SO (Fig. 2A). This effect, which is consistent with the ability of N-cadherin to promote neurite outgrowth (*15*), might have led indirectly to aberrant arborization. We therefore tested two other cell adhesion molecules, N-CAM and NgCAM/L1, both

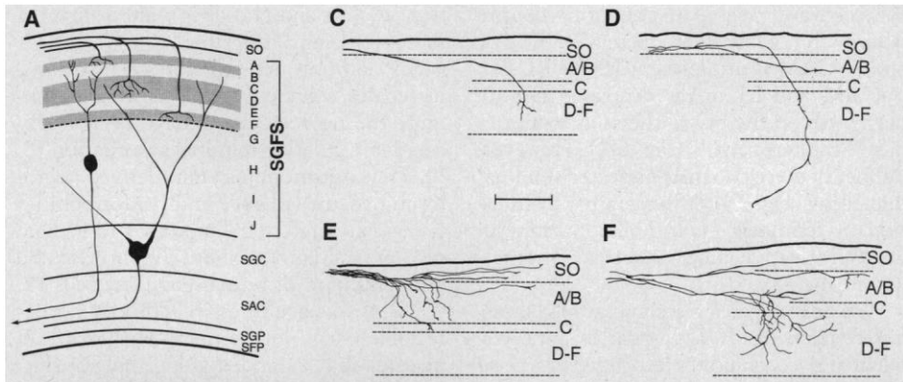
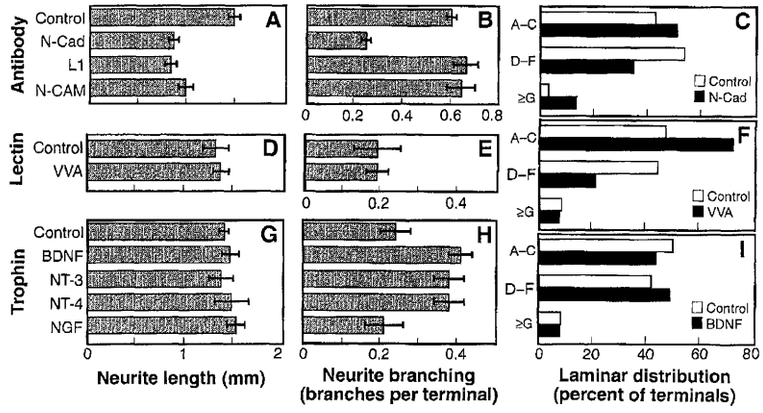


Fig. 1. Lamina-specific arborization of retinal axons in chick optic tectum. (**A**) Schematic drawing of retinal axons entering the tectum through the SO, penetrating deeper layers and arborizing in SGFS laminae B, D, or F (that is, the RL). Two examples of cells postsynaptic to the retinal axons are drawn (*4*). For laminar nomenclature, see (*7*). SGC, stratum griseum centrale; SAC, stratum album centrale; SGP, stratum griseum periventriculare; and SFP, stratum fibrosum periventriculare. (**B**) A retinotectal coculture, in which a strip of retina (at left) was laid perpendicular to a tectal section (*5*). Retinal axons extend along the SO and form rudimentary arbors in the RL. P, pial surface of tectum; V, ventricle. Scale bar, 500 μm. (**C** to **F**) Camera lucida drawings of retinal neurites in cocultures. In (C), rudimentary arbors in the RL in a control culture are shown. In (D), arbors are simplified and some neurites extend beyond the RL in the presence of N-cadherin mAb. In (E), neurites extend only partway into the RL in the presence of VVA. In (F), arbors are increased in complexity in the presence of BDNF. Scale bar in (C), 100 μm.



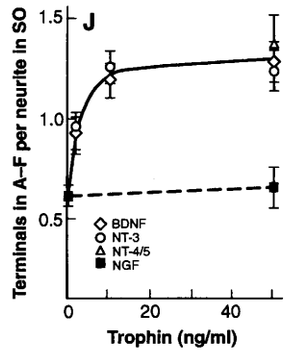


Fig. 2. Effects of cell adhesion molecule mAbs (**A** to **C**), the lectin VVA (**D** to **F**), and neurotrophins (**G** to **J**) on retinal outgrowth and arborization in retinotectal cocultures. Graphs show the extent of neurite outgrowth along the SO (A, D, and G), the number of neurites that extend from the SO into the RL (J), the fraction of neurites in the RL that branch at least once before terminating (B, E, and H), and laminar distribution of retinal terminals on tectal slices (C, F, and I). N-cadherin, N-CAM, and L1 mAbs all partially inhibit neurite outgrowth. N-cadherin mAb also decreases arborization within the RL and promotes extension of neurites beyond the RL. VVA diminishes entry of neurites into SGFS laminae D to F but has little effect on outgrowth or arborization. BDNF, NT-3, and NT-4 stimulate arborization but do not affect the laminar distribution of retinal terminals. Bars represent mean ± SEM of measurements from 13 to 94 cultures (mean = 39). Different culture conditions (*12, 19*) account for the different control values in (A) to (C) versus those in (D) to (I). χ^2 values for differences between control and experimental distributions are 165.9 ($P \ll 0.001$) in (C), 60.4 ($P \ll 0.001$) in (F), and 6.6 ($P > 0.05$) in (I).

of which are capable of promoting neurite outgrowth (*16*); both are present in the SO, and N-CAM is also present in the RL (*7*). N-CAM and L1 mAbs decreased neurite outgrowth on the SO to the same extent as did N-cadherin mAb (Fig. 2A). However, neither of these two mAbs affected terminal branching (Fig. 2B) or the laminar distribution of terminals (*17*). Thus, N-cadherin exerts a selective effect on retinal terminals within the RL.

We next targeted a second set of cell surface components, *N*-acetylgalactosamine–terminated glycoconjugates recognized by a plant lectin, *Vicia villosa* agglutinin B4 (VVA). Because this lectin selectively stains the RL before retinal axons arrive, its receptors (here called VVARs) may serve as recognition molecules for those axons (*7*). VVARs have also been implicated in the differentiation of neuromuscular synapses (*18*). Addition of VVA to retinotectal cocultures (*19*) had no effect on the number or length of neurites extending on the SO, the number of neurites that entered the RL, or the extent to which retinal neurites formed rudimentary arbors within the RL (Fig. 2, D and E). However, VVA inhibited the extension of retinal neurites into the RL, resulting in an increased number of neurites terminating in SGFS laminae A to C and a decreased number terminating in laminae D to F (Figs. 1E and 2F). Thus, VVA and N-cadherin mAb both inhibited the invasion of RL by retinal neurites, but only the latter decreased secondary branching and permitted growth beyond the RL. These results suggest that VVARs and N-cadherin affect different aspects of laminar targeting.

In addition to cell surface–associated molecules, soluble growth factors, most notably the neurotrophins, have been implicated in regulating terminal arborization (*8*, *9*). The neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are present in embryonic retina and tectum, and both promote the survival and differentiation of retinal ganglion cells (*9*, *20*, *21*). In cocultures, BDNF had no detectable effect on the number or length of neurites that extended along the SO (Fig. 2G), but it increased the number of neurites that entered the RL (Fig. 2J) as well as the fraction of those neurites that formed rudimentary arbors (Figs. 1F and 2H). NT-3 and NT-4 were as effective as BDNF, whereas nerve growth factor (NGF) was ineffective (Fig. 2, H and J). This pattern of efficacy is consistent with reports that retinal ganglion cells express receptors for BDNF, NT-3, and NT-4, but not for NGF (*20*). Thus, neurotrophins promote arborization in the chick, as shown previously in other species (*9*). However, neither the selectivity of initial neurite outgrowth on the SO (Fig. 1F) nor the laminar distribution of terminals within the RL (Fig. 2I) was affected by neurotrophins. These results suggest that neurotrophins and cell surface factors collaborate to shape retinal arbors in the tectum, predominantly affecting their size and position, respectively.

Finally, we exploited the accessibility of the chick embryo to test the roles of N-cadherin, VVARs, and BDNF on laminar specificity in vivo. N-cadherin was blocked by injection of N-cadherin mAb–secreting hybridoma cells into the tectal ventricle, VVARs were blocked by purified VVA, and BDNF was supplied by injection of transfected fibroblasts (*22*). Controls included hybridoma cells producing N-CAM or L1 mAbs and untransfected fibroblasts. Tecta were injected before E11, when retinal axons had extended into the SO but had not yet invaded deeper laminae (*5*). Embryos were then collected at E14, after retinal arbors had formed within the RL. In some embryos, retinal axons were labeled with an antibody to the 200-kD neurofilament subunit, which is selectively associated with retinal axons within the superficial tectum (*7*, *23*). In other embryos, retinal axons were anterogradely labeled by intraocular injection of the lipophilic dye, dil (*24*).

Similar results were obtained with both labels. In control tecta, SGFS laminae B and D to F were densely populated by retinal arbors, but none penetrated into lamina G (Fig. 3, A to C and G). Application of N-cadherin mAb or VVA inhibited invasion of the deeper portion of the RL by retinal axons, restricting retinal arbors to the superficial portion of the zone they normally occupied (Fig. 3, D and E). In addition, N-cadherin mAb, but not VVA, permitted the extension of some retinal axons past their normal boundary and into lamina G (Fig. 3H). In contrast, BDNF increased the density of retinal arbors within the RL without detectably affecting their laminar distribution (Fig. 3, F and I) (*25*). These effects are similar to those seen with fixed tectal sections in cocultures, indicating that they are not indirect consequences of poor tectal health. Likewise, this similarity argues against the possibility that effects observed in vitro are physiologically irrelevant.

Our results suggest that retinal arbors are shaped by a series of discrete cues. VVARs are present on numerous cell surfaces within the RL, become localized to the RL before retinal axons invade, and remain lamina-specific even in enucleated tecta (*7*). These glycoconjugates may serve as laminar markers that promote arborization in the RL; when they are blocked, retinal axons fail to enter or arborize in the RL. N-cadherin becomes concentrated in synaptic clefts once arbors form, and it remains diffusely distributed in enucleated tecta (*7*), which suggests that its effects may predominate at a later stage of arborization. Interference with N-cadherin may inhibit stabilization of newly formed contacts, leading to withdrawal of some arbors and extension of others beyond their normal laminar boundaries. Finally, activity-dependent release of neurotrophins (*26*) from tectal tar-

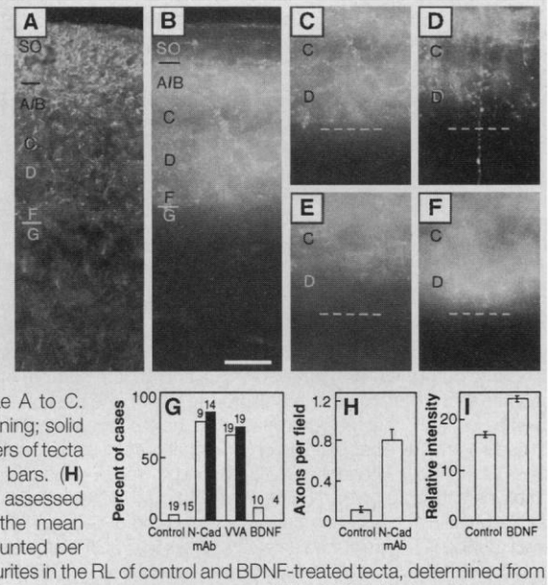


Fig. 3. Determinants of laminar selectivity in optic tectum in vivo. (**A** to **F**) Sections from tecta that had been injected with control cells (A to C), hybridomas producing N-cadherin mAb (D), fibroblasts producing recombinant BDNF (E), or VVA (F). Retinal axons were then stained with antibodies to neurofilaments (A) or by anterograde transport after intraocular injection of dil (B to F). Both methods reveal the restriction of retinal axons to the RL. Scale bars, 50 μm [(A) and (B)], 45 μm (C to F). (**G**) Fraction of tecta subjected to each treatment in which the density of retinal arbors in SGFS laminae D to F was significantly less than that in laminae A to C. Open bars, tecta analyzed by immunostaining; solid bars, tecta analyzed by dil staining. Numbers of tecta in each group are indicated within the bars. (**H**) Growth of retinal neurites beyond the RL, assessed in dil-labeled preparations. Bars show the mean number ± SEM of "escaped" axons counted per 330-μm-wide field. (**I**) Density of retinal neurites in the RL of control and BDNF-treated tecta, determined from neurofilament antibody–stained sections (*25*). Values represent means ± SEM of 87 fields from five control embryos and 121 fields from 10 BDNF-treated embryos. N-cadherin mAb restricts most retinal axons to the upper portion of the RL (D) and permits extension of some axons past the RL (H). VVA decreases penetration of axons into the RL but does not provoke overgrowth (E). BDNF has no effect on laminar selectivity (F) but enhances arborization within the RL (I). Differences between means in (H) and (I) (600% and 43%, respectively) are significant at $P < 0.001$ by Student's t test.

gets may provide a means of regulating the size of arbors without affecting their laminar specificity, thereby providing independent control over quantitative and qualitative aspects of connectivity. Because cadherins (*10*, *11*), VVARs (*27*), and neurotrophins (*8*, *26*) are all expressed in numerous laminated portions of the vertebrate brain, molecular mechanisms elucidated in tectum may regulate connectivity elsewhere as well.

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19. To initiate neurite outgrowth, we incubated cocultures for 18 hours in serum-containing medium (*12*) and then transferred the culture-bearing filters to serum-free Neurobasal medium with B27 supplement (Gibco-BRL) with or without VVA-B4 (Sigma), mouse NGF, human BDNF, NT-3, or NT-4/5 (PeproTech, Rocky Hill, NJ), all at 5 to 20 μg/ml.
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22. QT6 quail fibroblasts were transfected with a BDNF expression vector. Expression of BDNF in 30 to 40% of the transfected cells was demonstrated by staining of brefeldin-treated cells with anti-BDNF (Promega). QT6 or hybridoma cells were resuspended at 10^4 cells per microliter. Left tecta were injected with 5 to 7 μl of cell suspension or VVA-B4 (1 mg/ml) at E6–E10 (hybridomas) or at E10–E11 (QT6 cells or VVA-B4). Manipulated embryos were killed at E14. Only embryos that had reached stage 40 [V. Hamburger and H. L. Hamilton, *J. Morphol.* **88**, 49 (1951)] were analyzed.
23. Cryostat sections were incubated with antibody to the 200-kD neurofilament subunit (Sigma), L1 mAb, or N-CAM mAb (*16*), followed by second antibodies and Hoechst 33342. Application of second antibody alone confirmed that N-cadherin mAb had penetrated the full thickness of the tectum. Likewise, injection of fluorescein–VVA-B4 (Sigma) demonstrated penetration of the lectin.
24. The right eye was exposed, and a hole was made with a tungsten needle at a temporal-dorsal position. DiI (Molecular Probes) was then injected through the hole with a microelectrode. One day later, embryos were killed, staged, and fixed in 4% paraformaldehyde. Vibratome sections (90 μm) were counterstained with Hoechst 33342.
25. Digital images were analyzed with IP Lab Spectrum, version 2.5.7 (Signal Analysis, Vienna, VA). Laminar boundaries were determined by staining with Hoechst 33342. The increased density in BDNF-treated tecta could reflect either a greater number of retinal axons or a greater degree of arborization. We could not distinguish these alternatives in vivo, but we favor increased arborization on the basis of the in vitro results.
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Stochastic Dynamics and Deterministic Skeletons: Population Behavior of Dungeness Crab

Kevin Higgins,* Alan Hastings, Jacob N. Sarvela, Louis W. Botsford

Ecologists have fiercely debated for many decades whether populations are self-regulated by density-dependent biological mechanisms or are controlled by exogenous environmental forces. Here, a stochastic mechanistic model is used to show that the interaction of these two forces can explain observed large fluctuations in Dungeness crab (*Cancer magister*) numbers. Relatively small environmental perturbations interact with realistic nonlinear (density dependent) biological mechanisms, to produce dynamics that are similar to observations. This finding has implications throughout population biology, suggesting both that the study of deterministic density-dependent models is highly problematic and that stochastic models must include biologically relevant nonlinear mechanisms.

Dungeness crab life-history features are well known (*1–4*) and have been the basis for many mechanistic models of its population behavior (*5–8*). Females extrude up to 2 million eggs in the fall. After hatching in winter and pelagic dispersal of larvae in spring, juvenile crabs settle near shore in late spring and early summer. Reproduction is delayed until crabs reach about 100-mm carapace width (about 2 to 3 years of age). Adults reproduce once per year and may do so repeatedly. Fecundity declines with age and is related to molting, with molting probability dropping precipitously at older ages. Male and female survivorship declines with age. Males become legally vulnerable to harvest at 159-mm carapace width (about 4 years of age). Dungeness crab juveniles are cannibalized by adults and other juveniles.

Data for this study consist of yearly catch records of males (in kilograms), spanning 42 years (1951 to 1992), at eight locations from California, Oregon, and Washington

K. Higgins and A. Hastings, Division of Environmental Studies and Institute of Theoretical Dynamics, University of California, Davis, Davis, CA 95616, USA.
J. N. Sarvela, Department of Computer Sciences, University of Texas, Austin, Austin, TX 78712, USA.
L. W. Botsford, Department of Wildlife, Fisheries and Conservation Biology, University of California, Davis, Davis, CA 95616, USA.

*To whom correspondence should be addressed at Department of Ecology and Systematics, Division of Population Biology, University of Helsinki, Post Office Box 17 (Arkadiankatu 7), FIN-00014, Helsinki, Finland. E-mail: higgins@itd.ucdavis.edu