Relationship between Neuronal Migration and Cell-Substratum Adhesion: Laminin and Merosin Promote Olfactory Neuronal Migration but are Anti-Adhesive

Anne L. Calof,* and Arthur D. Lander‡

*Department of Biology, University of Iowa, Iowa City, Iowa 52242; and ‡Department of Brain and Cognitive Sciences and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Abstract. Regulation by the extracellular matrix (ECM) of migration, motility, and adhesion of olfactory neurons and their precursors was studied in vitro. Neuronal cells of the embryonic olfactory epithelium (OE), which undergo extensive migration in the central nervous system during normal development, were shown to be highly migratory in culture as well. Migration of OE neuronal cells was strongly dependent on substratum-bound ECM molecules, being specifically stimulated and guided by laminin (or the laminin-related molecule merosin) in preference to fibronectin, type I collagen, or type IV collagen. Motility of OE neuronal cells, examined by time-lapse video microscopy, was high on laminin-containing substrata, but negligible on fibronectin substrata. Quantitative assays of adhesion of OE neuronal cells to substrata treated with different ECM molecules demonstrated no correlation, either positive or negative, between the migratory preferences of cells and the strength of cell–substratum adhesion. Moreover, measurements of cell adhesion to substrata containing combinations of ECM proteins revealed that laminin and merosin are anti-adhesive for OE neuronal cells, i.e., cause these cells to adhere poorly to substrata that would otherwise be strongly adhesive. The evidence suggests that the anti-adhesive effect of laminin is not the result of interactions between laminin and other ECM molecules, but rather an effect of laminin on cells, which alters the way in which cells adhere. Consistent with this view, laminin was found to interfere strongly with the formation of focal contacts by OE neuronal cells.

CELL migration is fundamental to the proper development and repair of all organs, and is a particularly dramatic feature of nervous system morphogenesis. Migration of neuronal precursors and immature neurons is responsible for establishing structural features of the nervous system as diverse as the laminae of the cerebral cortex and the ganglia of the sympathetic chain (24, 43).

Much of what is known about the molecules that mediate the migration of neural cells has come from studies of the neural crest, the mesenchymal cell population that gives rise to most of the peripheral nervous system. Experiments performed in vivo and in vitro indicate that the extracellular matrix (ECM)[1] through which these cells move constitutes a rich source of molecular cues that stimulate and guide migration (reviewed in reference 38). Whether the neuronal cells that migrate during morphogenesis of the central nervous system also follow ECM cues is not known, in part because few experimental systems exist for studying the migration of central nervous system cells in vitro, where the effects of defined ECM molecules may be tested readily. Thus far, two studies on the migration of cells in cerebellar microexplant cultures have suggested that the ECM molecules tenascin and thrombospondin play roles in the migration of cerebellar granule neurons (7, 37).

Migration of neurons and their precursors over long distances is a particular dramatic feature of the developing luteinizing hormone-releasing hormone (LHRH) system of the hypothalamus. Several recent reports have shown that the LHRH-containing neurons of the mammalian brain originate in the nasal cavity as components of the olfactory epithelium (OE), and, during fetal life, enter the brain and migrate a considerable distance to the hypothalamus (9, 42, 47, 48). Apparent emigration of neuronal cells from cultured embryonic OE explants has also been seen (3). These findings suggest that OE cultures could provide a useful system with which to study the migratory behaviors of central nervous system neurons and their progenitors.

A study was therefore undertaken to characterize the types of cells migrating from embryonic OE explants, the motile behaviors they display in vitro, and the influences of ECM components on their migration. The results, presented be-

1. *Abbreviations used in this paper*: CMF, calcium and magnesium free; ECM, extracellular matrix; INP, immediate neuronal precursor cells; IRM, interference reflectance microscopy; LCM, low calcium medium; LHRH, luteinizing hormone-releasing hormone; LN, laminin; MN, merosin; OE, olfactory epithelium.

low, indicate that both neurons and neuronal precursors derived from the OE are motile in vitro, and that their migration can be stimulated and guided by either the ECM protein laminin (LN) or the laminin-related molecule merosin (MN) (13). Experiments performed to assess the relationship between the migration of OE neuronal cells and the strength of cell-substratum adhesion uncovered the finding that LN and MN are anti-adhesive for these cells, i.e., cause them to adhere poorly to substrata that would otherwise be highly adhesive. The possibility is discussed that the anti-adhesivity of LN and MN and their effects on neuronal migration may be related processes. Some of these observations have been presented previously in abstract form (Calof, A. L., and A. D. Lander. *J. Cell Biol.* 111:491*a*).

Materials and Methods

Materials

LN was purified from the Engelbreth-Holm-Swarm sarcoma according to published methods (21, 45). Purified FN (from human plasma) was purchased from New York Blood Center (New York, NY). MN (purified from human placenta) was obtained from Telios Pharmaceuticals (San Diego, CA). Type IV collagen (purified from the Engelbreth-Holm-Swarm sarcoma) was obtained from Collaborative Research (Bedford, MA). Type I collagen (purified from rat tail tendon) was the generous gift of Dr. James San Antonio (Department of Pathology, Harvard Medical School, Cambridge, MA). The peptide gly-arg-gly-asp-ser (GRGDS) was purchased from Calbiochem. Tissue culture media and additives were purchased from Gibco-BRL (Grand Island, NY) or Cellgro-Mediatech (Fisher Scientific Co., Pittsburgh, PA). Unless indicated, all other supplies were purchased from Sigma Chemical Co. (St. Louis, MO).

Olfactory Epithelium Explant Culture

OE was dissected from E14 mouse embryos (CD-1 random-bred mice; Charles River) and purified as described in Calof and Chikaraishi (3). For explant culture, finely chopped pieces of OE were cultured in serum-free, low (0.1 mM) calcium medium (LCM) with 5 mg/ml BSA, 10 μg/ml insulin, 10 μg/ml transferrin, 20 nM progesterone, 100 μM putrescine, and 30 nM selenium (= complete LCM) at 37°C in a 5% CO_2/95% air atmosphere, as described (3, 5). EGF (3) was eliminated from these cultures. Explants were cultured on glass coverslips treated with ECM molecules as described below, and fixed for 2 h at room temperature by underlaying the culture medium with a solution of 3.7% formaldehyde/5% sucrose in PBS.

Isolation and Culture of Dissociated Neuronal Precursors and Neurons

In some experiments, neurons and neuronal precursors were dissociated and isolated from the basal cells of the OE as follows: pieces of purified E14 OE were cultured in complete LCM for 13 h in plain petri plastic dishes (to which explants do not attach). During this time, olfactory receptor neurons and their precursors sort out from, but remain loosely attached to, the basal epithelial cells, which form a ball of tightly packed cells. These pieces of tissue were then transferred to 15-ml conical polystyrene centrifuge tubes and spun at 100 *g* for 5 min. The supernatant was discarded, and the tissue resuspended by gentle swirling in 4 ml of calcium- and magnesium-free (CMF) HBSS (CMF-HBSS) containing 0.5 mg/ml trypsin (Sigma type III; Sigma Chemical Co.), and then incubated for 4 min at 37°C. 1 ml of a 1 mg/ml solution of deoxyribonuclease I (Sigma type IV; Sigma Chemical Co.) in DME was added and the mixture was returned to 37°C for 6 min. Trypsin inhibitor (Sigma type I-S; Sigma Chemical Co.) was then added to a final concentration of 1 mg/ml, and the cells were mechanically dissociated by triturating 10 times in a flame-polished pasteur pipette. Large fragments were allowed to settle for 3 min at 1 *g*, and the supernatant was removed and filtered through a piece of sterilized nylon mesh with 20-μm opening size (Small Parts, Inc., Miami, FL). 3 ml of CMF-HBSS containing 1 mg/ml of soybean trypsin inhibitor was added to the fragments, and the trituration, settling, and filtration procedures were repeated. The pooled supernatant was found to contain exclusively neurons and immediate neuronal precursors (see below), while basal cells, which are not dissociated by trypsin alone, were found in the fragments. The pooled supernatant was transferred to a 15-ml conical polystyrene centrifuge tube, underlaid with 2 ml of 4% crystalline BSA in CMF-HBSS, and centrifuged for 10 min at 100 *g*. The supernatant was removed, and the cells were resuspended in complete LCM and used immediately. The dissociation and purification procedures took ~1 h to complete.

To assess the purity of the neuronal cell fraction isolated by this method, the two fractions ("Supernatant" and "Fragments") were analyzed using cell type-specific antibodies. Supernatant cells were collected onto microscope slides coated with Cell-Tak (Collaborative Research, Bedford, MA), and fixed sequentially in acetone (5 min), 95% ethanol/5% glacial acetic acid (10 min), and acetone again (5 min). Cells from the "Fragments" fraction were fully dissociated using trypsin/EDTA (1 mg/ml trypsin in CMF-HBSS containing 1 mM EDTA and 5 mM Hepes, pH 7.4), collected onto Cell-Tak-coated slides, and fixed in the same manner. Slides were then stained with biotinylated AG1 anti-NCAM IgG (8 μg/ml) or Dako rabbit antiserum to keratins (1:400 dilution), as described previously (3). Slides were examined using a 40× objective on an Axiophot microscope (Zeiss, Oberkochen, Germany). A minimum of 20 random fields, containing a minimum of 200 cells, were scored for each analysis. The supernatant fraction was found to consist of 74% neurons (NCAM+) and 26% immediate neuronal precursors (NCAM−); these are the same relative proportions observed in 13-h cultures grown in LCM on adhesive substrata (3; and Table I). The supernatant fraction contained <1% basal cells (keratin+). In contrast, NCAM−, keratin+ cells constituted the vast majority (77%) of cells in the "Fragments" fraction.

Culture Substrata

Glass coverslips (12-mm round, thickness #1; Propper Mfg., Long Island City, NY) were acid cleaned (3), sterilized under ultraviolet light (45 min), covered with 0.1 ml of solution containing one or several ECM proteins (as indicated in the text), and incubated for 3 h at 37°C in a humidified air atmosphere. Coated coverslips were washed three times in CMF-HBSS, then placed into wells of a 24-well tissue culture tray and covered with complete LCM (containing 5 mg/ml BSA) before cells were plated onto them.

In some cases, substrata containing patterns (stripes) of ECM molecules were prepared as follows: pieces of flat silicone (~15-mm square) glued to a glass microscope slide were notched using a razor blade to produce a series of parallel stripes ~0.4 mm in width and 1–2 mm in depth. After sterilization of the silicone with 70% ethanol, acid-cleaned, sterile glass coverslips were pressed against the silicone and allowed to adhere. A given protein solution was applied to one edge of the silicone square and allowed to flow by capillary action into the troughs created by the cuts, and in contact with the glass. After 5 min, the protein solution was removed by suction, and the coverslip separated from the silicone. After air drying briefly, coverslips prepared in this way were washed three times with CMF-HBSS and then further treated over their entire surface with 100 μl of a second protein solution, which was incubated at 37°C and washed as described above. Following culture of OE explants on such coverslips, and subsequent fixation, the positions of the applied stripes were visualized by indirect immunofluorescence, using rabbit antisera to ECM proteins: anti-mouse Collagen IV (Collaborative Research Inc., Lexington, MA), 1:50 dilution; anti-mouse LN (PolySciences Corp., Niles, IL), 1:200 dilution; anti-human FN (gift of Dr. Richard O. Hynes, M.I.T., Cambridge, MA), 1:50 dilution. Binding of primary antisera was visualized with rhodamine-conjugated goat anti-rabbit IgG (Cappell Laboratories, Malvern, PA; 1:100 dilution).

Time-lapse Video Analysis of Cell Motility

Biophysica Technologies (Baltimore, MD) glass coverslips (31-mm diam, 0.07-mm thickness) were cleaned by soaking overnight in concentrated HCl and then washing exhaustively in deionized distilled water. Coverslips were coated with either LN (100 μg/ml) or FN (50 μg/ml), incubated and washed as described above, and then clamped into a Biophysica Microscope Tissue Chamber. OE neuronal cells or explants were resuspended in complete LCM modified by the addition of Hepes (3.75 mM, pH 7.4) and the reduction of $NaHCO_3$ to 4.7 mM, and plated onto the coverslip in the chamber. The chamber was filled completely with medium, a thermistor probe was placed in the chamber, and the chamber was then capped with a second coverslip and sealed with vacuum grease. Temperature was held at 37°C throughout 2–3 h of recording. Cells were visualized using an Axiovert microscope (Zeiss) equipped with a 25× oil immersion objective and Nomarski optics. The image was magnified further through a 4× objective in the

SIT camera (Hamamatsu Phototonics, Hamamatsu City, Japan), processed through a series 151 Image Processor (Imaging Technologies, Woburn, MA), and recorded on 0.5-in video tape with a Panasonic time-lapse VCR.

Interference Reflectance Microscopy

Explant cultures of purified OE were grown on acid-cleaned glass coverslips coated with either LN (100 μg/ml), FN (50 μg/ml), or LN plus FN (50 μg/ml and 25 μg/ml, respectively) by the procedures described above. After 18 h in culture, cells were fixed by underlaying the culture medium with formaldehyde fixative as described above, and the coverslips were then mounted onto glass slides directly out of fixative in 90% glycerol/PBS. Samples were examined under reflected light using a 63× Plan Neofluor Antiflex objective (Zeiss).

Assay of Cell–Substratum Adhesion

Cell-substratum adhesion was measured using a modification of the centrifugal detachment assay of McClay et al. (32). Briefly, a multi-well plate was formed by applying a silicone gasket with round holes (0.1 cm^2) to a sheet of polystyrene (0.030-in thick; Altec Plastics, Boston, MA) that had been previously cleaned with deionized water and 95% ethanol. The silicone was pressed against the polystyrene and adhered well, producing a plate containing multiple flat-bottomed wells into which solutions could be introduced. Wells were coated with ECM proteins by applying 20 μl per well and incubating for 3 h at 37°C in a humidified atmosphere (except in the case of type I Collagen, for which incubations were performed at 4°C to prevent gelation). After washing the wells three times in CMF-HBSS, they were covered in a blocking solution of 10 mg/ml BSA (heat inactivated 2 h at 70°C) in CMF-HBSS, pH 8.2, and held overnight at 4°C. Wells were then washed into complete LCM, and ^{35}S-labeled neuronal cells (see below) were introduced into the wells at a concentration of $\sim 3–6 \times 10^4$/well in a volume of 20 μl/well.

Immediately upon addition of cells, the entire plate was placed into a plate holder in a refrigerated centrifuge (RT6000; Sorvall Instruments, Newton, CT), taken to 600 rpm (56 *g*), and immediately turned off. This process synchronized contact of all cells with the substratum. The plate was then incubated for 30 min at 37°C/5% CO_2 to permit cell attachment. The gasket and plate were then removed from the incubator, flooded with warm PBS, sealed closed with plastic wrap and a polystyrene coverplate, and inverted. The inverted plate was then centrifuged for 8 min at different speeds, as indicated in the text (in the event that a detaching force of only 1 *g* was needed, the inverted plate was not centrifuged, but was simply held at 4°C for 30 min). The entire plate, still inverted, was then immersed in a dish containing 2 liters of ice-cold PBS, and the coverplate and plastic wrap removed while the plate was submerged. The plate (with silicone gasket still attached) was then inverted again, lifted out of the dish of PBS, and immersed in fixative (3.7% formaldehyde/5% sucrose/0.1% Triton X-100/PBS) for 30 min. Fixative was washed away by inverting the plate into a dish of deionized distilled water, and allowing it to remain submerged for 15 min. The plate was then removed from the water, and air dried.

Cell adhesion was expressed as the amount of radioactivity remaining associated with the polystyrene surfaces in each of the wells. This was determined by peeling off the silicone gasket and measuring the radioactivity associated with the plastic plate using either a Betagen Betascope or a Molecular Dynamics Phosphorimager, both of which gave similar results.

Quantification of Protein Binding to Polystyrene

Multi-well plates fashioned from sheet polystyrene and silicone gaskets were coated exactly as described above, except that ECM protein solutions were spiked with either ^{125}I-LN or ^{125}I-FN. Proteins were labeled with ^{125}I by the Iodogen method (Pierce Chemical Co., Rockford, IL) to a specific activity of 11 μCi/μg for FN and 21 μCi/μg for LN. After washing with CMF-HBSS and blocking overnight with BSA, excess BSA was removed by gentle suction, the plate air dried, and the silicone gasket removed. Individual coated areas were cut from the polystyrene sheet and counted in a gamma counter.

Metabolic Labeling of Cells

Cells were metabolically labeled with ^{35}S by growing OE explants in suspension for 13 h (as described above) in complete LCM with no L-cystine and reduced L-cysteine and L-methionine (11.6 mg/l and 1.5 mg/l, respectively) plus 28 μCi/ml Tran^{35}S-label™ (ICN Radiochemicals, Irvine, CA). Isolation of neurons and immediate neuronal precursors was performed as described above. Viability of isolated neuronal cells, assessed by trypan blue exclusion, was always >95% in these studies.

Results

Olfactory Receptor Neurons and Neuronal Precursors Are Motile Cells

Cultures of purified embryonic OE contain two major classes of cells, which can be distinguished from one another by the use of antibodies to keratin filaments. The basal epithelial cells of OE are keratin positive, while the keratin-negative population consists of immature olfactory receptor neurons (which express the marker NCAM) and the immediate neuronal precursor cells (INP), which are NCAM negative (3, 5). As shown previously, most INPs divide during the first 48 h of culture, giving rise to cells that rapidly differentiate into post-mitotic, NCAM-positive neurons (3). In the following sections, the term "neuronal cells" will be used to refer to the combined neuron/INP population.

When OE explants are cultured on substrata treated with LN and FN, basal cells and neuronal cells exhibit distinctive behaviors (Fig. 1). Basal cells remain closely associated with each other, forming contracted sheets or balls of cells. Neuronal cells sort out from the basal cells and, during the first day in culture, become dispersed up to several hundred microns away from the basal cells. These observations suggest that neuronal cells migrate in culture, a fact confirmed by time-lapse microscopy. As shown in Fig. 2, migration of these cells often involves the extension of a wide pseudopodial process into which the cell body is subsequently drawn. Many cells exhibit periods of motility that alternate with periods of relative inactivity, and achieve net cell displacements of >50 μm/h. In some cases, the processes extended by motile cells are narrow and more closely resemble neurites.

To determine whether motile cells are neurons or INPs, cultures were labeled with ^{3}[H]thymidine or BrdU, either during the final 2 h of culture, or throughout the entire culture period (up to 48 h in some experiments). The results (Fig. 3 and data not shown) indicate that the dispersed cell population includes cells that are NCAM-negative, and will incorporate ^{3}[H]thymidine during the final 2 h of culture, as well as some that express NCAM, possess neurites, and cannot be labeled by ^{3}[H]thymidine or BrdU administered throughout the culture period. These results imply that motility is a property of both INPs and postmitotic neurons.

Laminin and Merosin Stimulate and Guide Neuronal Cell Migration

The observations described above on cell migration in OE cultures were made using culture substrata onto which the ECM molecules LN and FN had both been adsorbed. To examine the individual influences of these molecules on cell migration, substrata treated with either LN or FN were tested. As shown in Fig. 4, migration of cells away from the epithelial explant occurred on LN-treated substrata, but not on FN-treated substrata, despite the fact that both substrata permitted attachment of explants and cells.

These results suggested that LN dramatically stimulates the motility of OE neuronal cells. It is possible, however, that LN is required only to permit these cells to free themselves

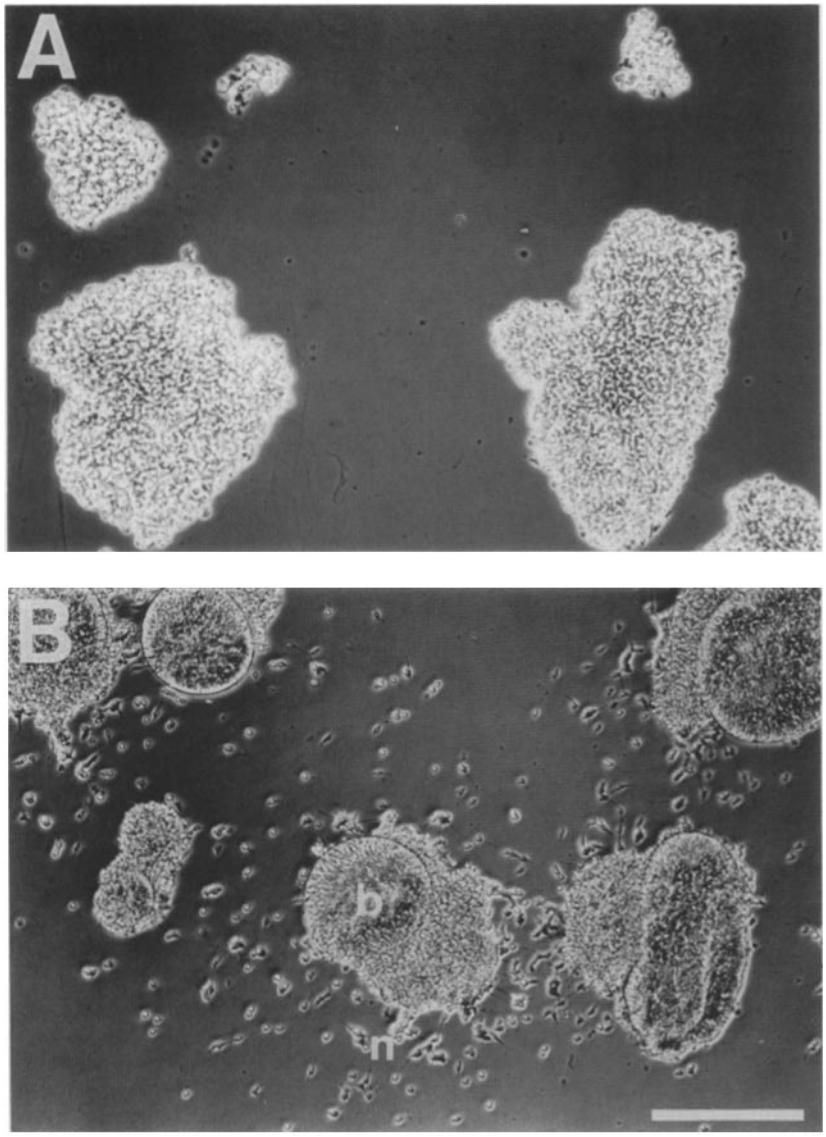

Figure 1. Neuronal cells sort out from basal cells and migrate away from olfactory epithelium explants. Explants of purified OE at 0.5 h (*A*) and 18 h (*B*) following plating onto glass coverslips coated with LN (50 μg/ml) plus FN (25 μg/ml). At 18 h in culture, the basal epithelial cells can be seen in coherent, contracted sheets or balls of cells ("*b*"). OE neuronal precursors and immature neurons ("*n*") have sorted out from the basal cell sheets and can be seen in clumps beside the sheets and widely dispersed on the substratum. Bar, 200 μm.

from the epithelial explant and move onto the substratum; having done so, the cells might then no longer require LN in order to migrate. To test this possibility, OE explants were cultured on substrata patterned with alternating stripes of different ECM molecules. Under these conditions, individual cells migrating onto LN stripes subsequently encounter substrata treated with other molecules, and the ability of neuronal cells to migrate on these other substrata can then be scored.

To produce such patterned substrata, silicone masks were used to form lanes on glass coverslips onto which stripes of a single ECM molecule—either LN, FN, type I collagen, type IV collagen, or MN—were adsorbed. The substrata were then overlayed with a solution containing a second ECM molecule, which could then adsorb between (and potentially also on) the stripes. Explants of embryonic OE were cultured on these substrata for 18 h and fixed. The positions of stripes were identified by immunofluorescence using antibodies directed against the applied molecules, and were compared with the patterns of cell migration.

Typical results are shown in Fig. 5, and summarized in Table I. Explants attached onto all substrata tested, but extensive cell migration was observed only on stripes containing LN or MN. Moreover, cells migrating on LN or MN substrata rarely crossed the border between these and other substrata (e.g., Fig. 5, *a*, *d*, and *e*). When LN was overlaid onto LN stripes, or MN onto MN stripes, cells showed no selective migration, indicating that the procedure for generating stripes does not itself generate artifacts that guide cells. In addition, cells readily migrated from LN stripes onto MN, and vice versa (Fig. 5 *c*). The ability of both LN and MN to promote cell migration, and the inability of these cells to distinguish between stripes of these two molecules, are consistent with the fact that MN is closely related to LN. Both molecules are composed of the same B1 and B2 chains in association with distinct, but homologous, A chains (13).

It was also observed that the outgrowth of neurites from OE neurons was greater on LN and MN than on substrata treated with any of the other ECM molecules tested. In addition, neurites rarely crossed borders between these and other

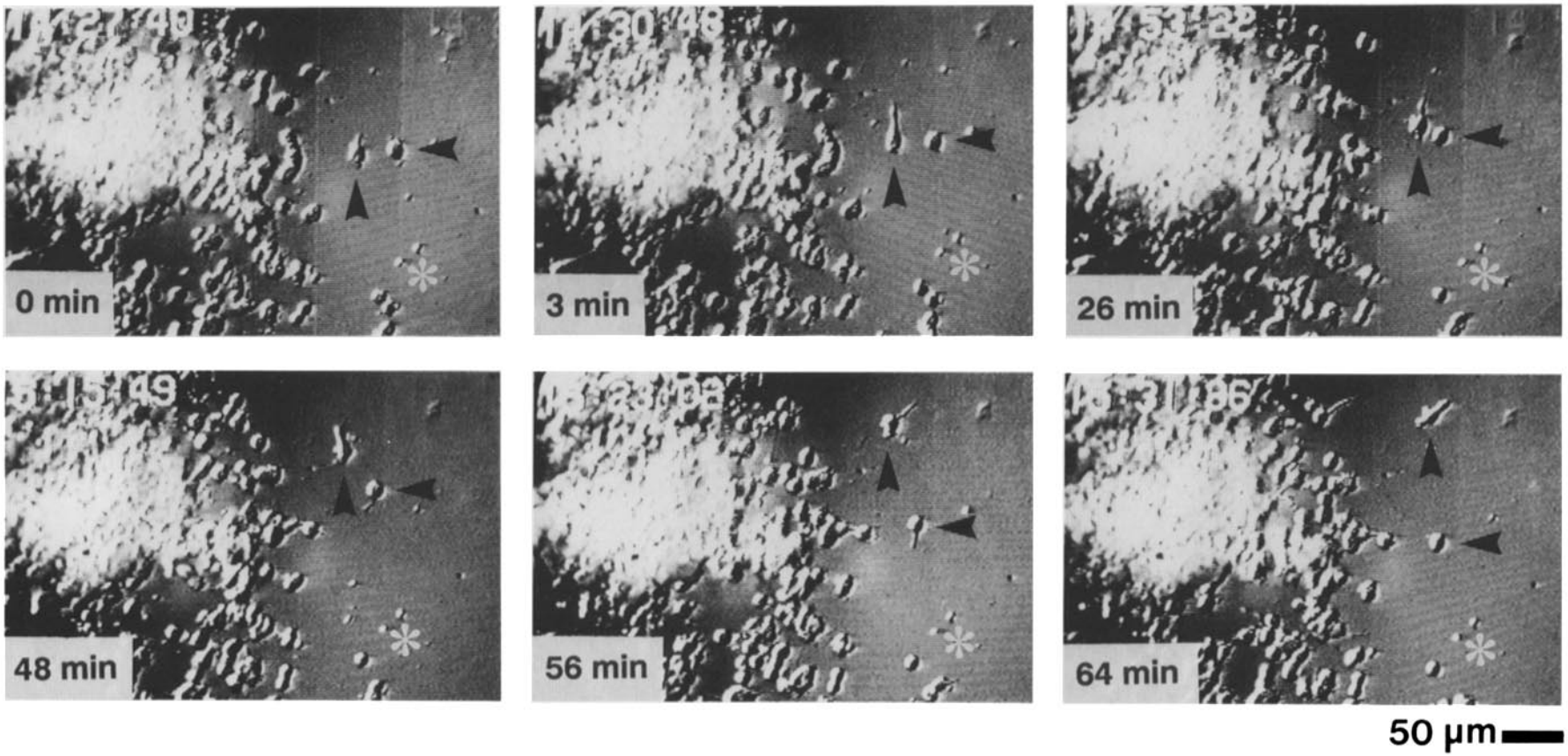

Figure 2. Time-lapse analysis of neuronal cell migration. Pieces of purified OE were plated onto glass coverslips that had been coated with LN (*50 μg/ml*) plus FN (*25 μg/ml*), incubated at 37°C for 12 h, and then mounted on an inverted microscope for time-lapse recording (see Materials and Methods). The locations of two OE neuronal cells over the course of a 1 h recording are indicated by black arrowheads in the six panels. For comparison, an asterisk marks the location of two pieces of debris that remained stationary throughout the period of observation. "0 min" indicates the start of the recording, which was begun at 16 h following the initial plating of the explants. Bar, 50 μm.

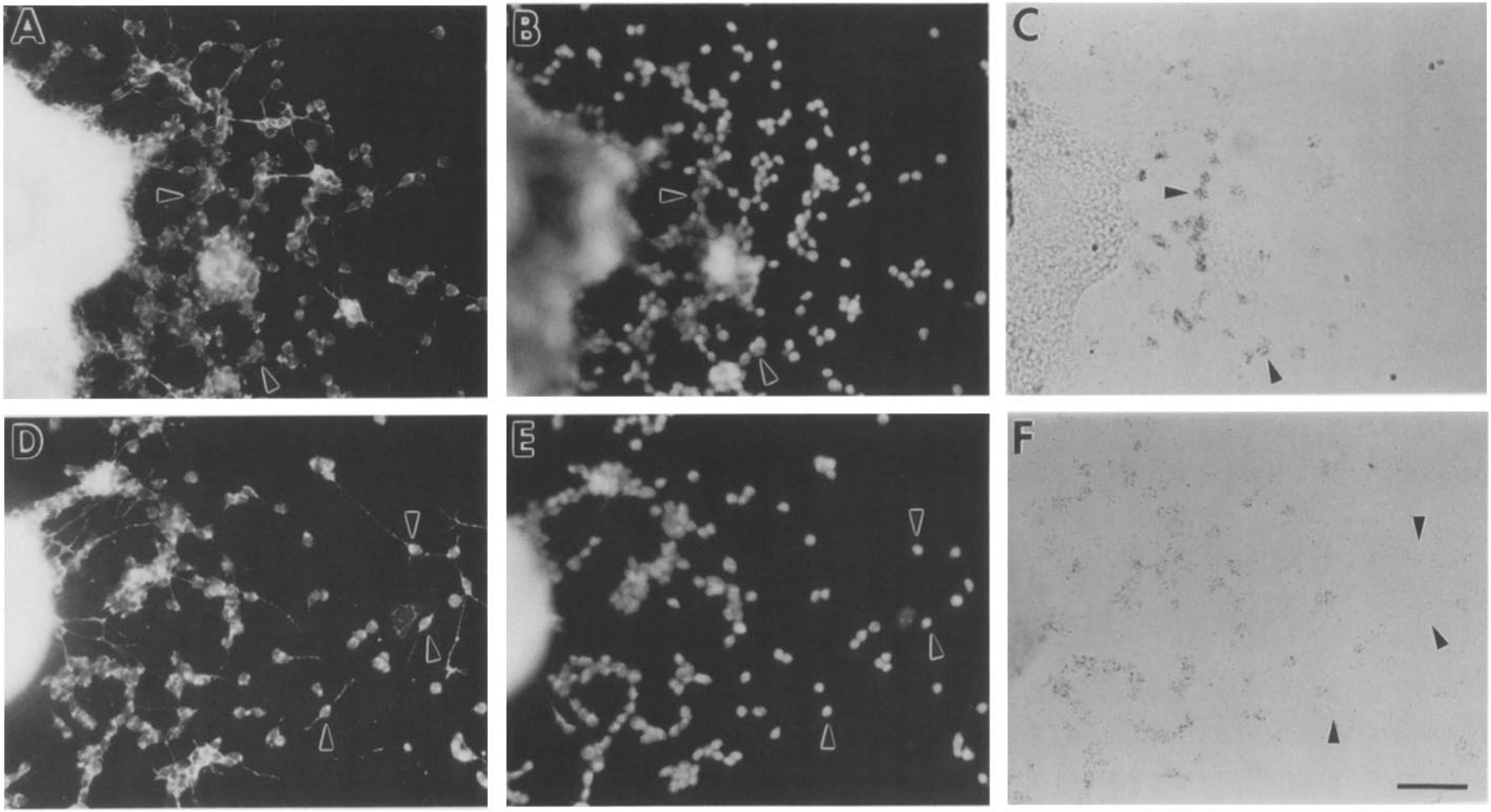

Figure 3. Both neuronal precursors and postmitotic neurons migrate. The immediate neuronal precursors (*INPs*) of the OE can be identified by their ability to incorporate ^{3}[H]thymidine in a pulse-fix assay and by their failure to express the neural cell adhesion molecule, NCAM. In contrast, the postmitotic neurons of the OE express NCAM and do not incorporate ^{3}[H]thymidine (*3*). (*A–C*) INPs migrate. An OE explant was grown for 17 h on a glass coverslip coated with LN (*50 μg/ml*) and FN (*25 μg/ml*). 3 h before fixation, ^{3}[H]thymidine (*80 Ci/mmole*) was added to a final concentration of 5 μCi/ml. After a total of 17 h in culture, cells were fixed and processed for NCAM immunoreactivity (*A*), a nuclear stain (Hoechst 33258; 1 μg/ml; *B*), and autoradiography (*C*) as described previously (3). The presence in the migratory population of ^{3}[H]thymidine–labeled INPs, negative for NCAM staining, is indicated by arrowheads. (*D–F*) Neurons migrate. An OE explant was cultured and processed as described in *A–C*, except that ^{3}[H]thymidine (*0.5 μCi/ml*) was included throughout the culture period. Examples of NCAM+ cells that lack silver grains over their nuclei are indicated by arrowheads. The failure of these neurons to incorporate ^{3}[H]thymidine during the entire 17-h culture period indicates that they must have been postmitotic at the time they migrated from the OE explant. Bar, 50 μm.

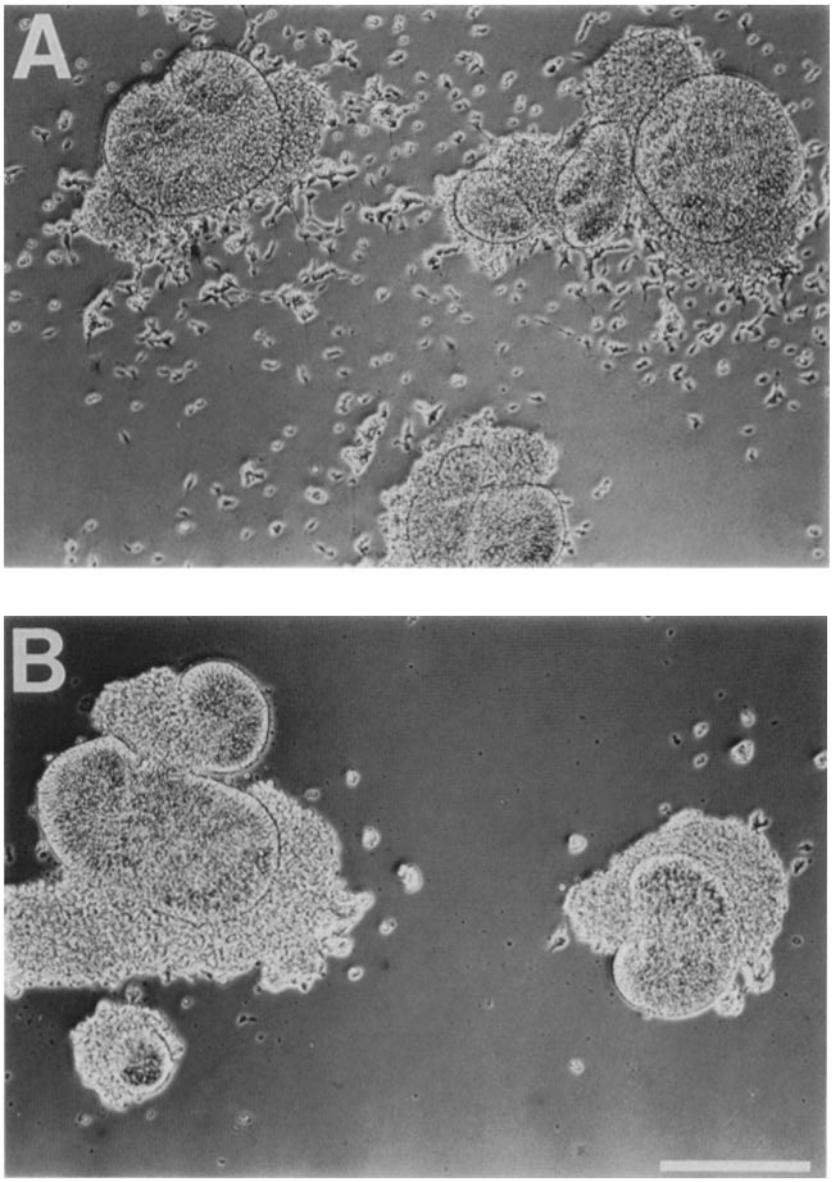

Figure 4. Neuronal cells migrate on laminin substrata but do not migrate on fibronectin substrata. Explants of purified OE were fixed at 18 h following plating onto glass coverslips coated with LN (*A*, *100 μg/ml*) or FN (*B*, *50 μg/ml*). Bar, 200 μm.

ECM molecules (Fig. 5 *e*), indicating that the behaviors of migrating OE cells and their neurites are qualitatively similar.

The Choices Made by Migrating Neuronal Cells Do Not Correlate With Cell Adhesion

Haptotaxis, the ability of migrating cells or neurites to follow pathways of substratum-bound molecules, is frequently attributed to the effects of differential cell–substratum adhesion (e.g., 25, 46). Indeed, in the case of LN, its ability to guide the neurites of many types of neurons has often been taken as evidence that it is especially adhesive for neural cells, although this conclusion has been questioned by some (17, 18, and reviewed in reference 23). To determine whether LN's ability to stimulate and guide migration of OE neuronal cells is related to its adhesivity for these cells, a procedure was devised for isolating dissociated OE neuronal cells (i.e, INPs and neurons) from OE explants cultured in suspensions (see Materials and Methods).

Dissociated OE neuronal cells were plated onto substrata treated with LN or FN, and observed using time-lapse video microscopy. Fig. 6 demonstrates that the cells exhibited very different degrees of motility on the two substrata: on LN substrata ~60% of single cells moved at rates greater than 25 μm/10 min. In contrast, only 1 of 15 cells analyzed on FN substrata exhibited motility in this range, despite the fact that these cells exhibited extensive membrane ruffling and pseudopodial activity. These results indicate that many of the cells in the dissociated neuron/INP population are stimulated to migrate by LN substrata, and do so at rates at least as great as those observed in explant cultures (cf., Fig. 2). The actual fraction of cells capable of migrating on LN substrata is likely to be greater than that observed during 10-min intervals, since cells were often observed to alternate periods of motility with periods of quiescence. It is also possible that damage sustained during enzymatic dissociation might interfere with the motility of some cells.

To determine whether the migratory preferences of OE

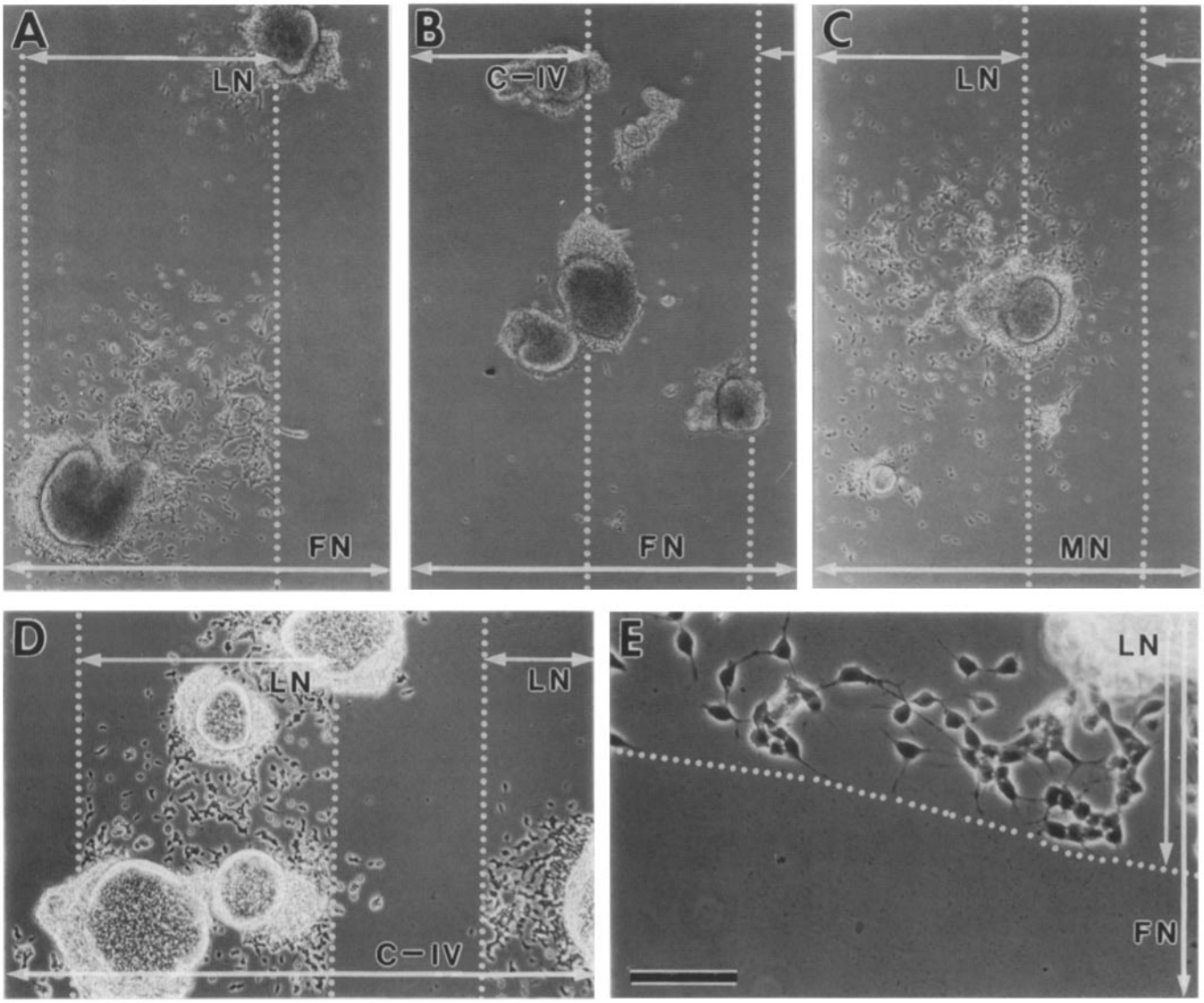

Figure 5. Migration preferences of neuronal cells presented with patterned substrata. Stripes of ECM proteins were prepared on glass coverslips as described in Materials and Methods. OE explants were grown for 18 h and then fixed and processed for immunocytochemistry to allow visualization of applied ECM proteins. Dotted lines mark the boundaries of stripes as determined from fluorescence pictures of the same fields. (*A*) Stripes of LN (*100 μg/ml*) overlaid with FN (*50 μg/ml*). (*B*) Stripes of type IV collagen (*100 μg/ml*) overlaid with FN (*50 μg/ml*). (*C*) Stripes of LN (*100 μg/ml*) overlaid with MN (*100 μg/ml*). (*D*) Stripes of LN (*100 μg/ml*) overlaid with type IV collagen (*100 μg/ml*). (*E*) A higher magnification view of stripes of LN (*100 μg/ml*) overlaid with FN (*50 μg/ml*). Bars: (*A–D*) 200 μm; (*E*) 50 μm.

neurons and INPs correlate with the strength of cell–substratum adhesion, a variation of the centrifugal detachment assay of McClay et al. (32) was used. OE explant cultures were metabolically labeled with ^{35}S-methionine and suspensions of purified, dissociated OE neuronal cells were prepared as described in Materials and Methods. Cells were plated onto a flat polystyrene plate containing multiple substrata treated with ECM molecules. Following incubation for 30 min at 37°C, the plate was inverted and centrifuged at a defined detaching force. The radioactivity remaining associated with the plate bottom was measured to quantify cell adhesion. These measurements correlated well with microscopic counts of cell density.

Fig. 7 *a* shows the results of such an experiment, in which a detaching force of 100 *g* was used. The data are normalized to the value obtained using polylysine (microscopic counts indicated >85% recovery of input cells on this nonspecifically adhesive substratum). Cell adhesion was observed to be strongest to FN, with >60% of the level achieved with polylysine. Adhesion to FN was dose dependent (Fig. 7 *b*). Preliminary experiments indicated that adhesion to FN could be reduced by one-third when the peptide GRGDS (1 mg/ml), derived from one of the cell attachment sites contained within FN, was included with the cells at the time of plating (unpublished observations).

Some adhesion (~25%) was also seen with MN. Strikingly, adhesion to LN, Col I, and Col IV was minimal, and not significantly greater than to untreated plastic. Variations in the experimental protocol such as increasing cell attachment time to 1 h, or decreasing detaching force to as low as

Table I. Migration of Neuronal Cells on Patterned Substrata

Stripe	Overlay	Migration on Stripe	Migration off Stripe
Laminin	Fibronectin	++++	−
Laminin	Collagen IV	+++	−
Laminin	Collagen I	++++	−
Merosin	Fibronectin	++++	−
Merosin	Collagen IV	+++	−
Merosin	Collagen I	++++	−
Collagen IV	Fibronectin	−	−
Laminin	Laminin	++++	++++
Merosin	Merosin	++++	++++
Laminin	Merosin	++++	++++

Stripes of ECM molecules were adsorbed onto glass coverslips as described in Materials and Methods. Solutions were then applied to the coverslips from which additional ECM molecules could adsorb between (and possibly also on top of) the applied stripes. OE explants were cultured for 18 h and then fixed and examined microscopically. The locations of applied stripes were determined by immunofluorescence using specific antibodies (see Materials and Methods). The extent of migration was scored qualitatively.

56 *g* failed to reveal significantly greater adhesion of cells to LN, Col I, and Col IV than to untreated substrata (see Fig. 10).

Laminin and Merosin Are Anti-Adhesive

The data presented in Figs. 5–7 and Table I reveal no discernible relationship between cell adhesion and the migratory preferences of these neuronal cells or the growth of their neurites. In migrating, OE neurons and INPs clearly choose LN and MN over molecules that are more adhesive (e.g., LN vs. FN, Fig. 5 *a*), similarly adhesive (e.g., LN vs. Col. IV, Fig. 5 *d*) or less adhesive (e.g., MN vs. Col. IV or Col. I, Table I), while being unable to choose between LN and MN, which differ in adhesivity (Fig. 5 *c*).

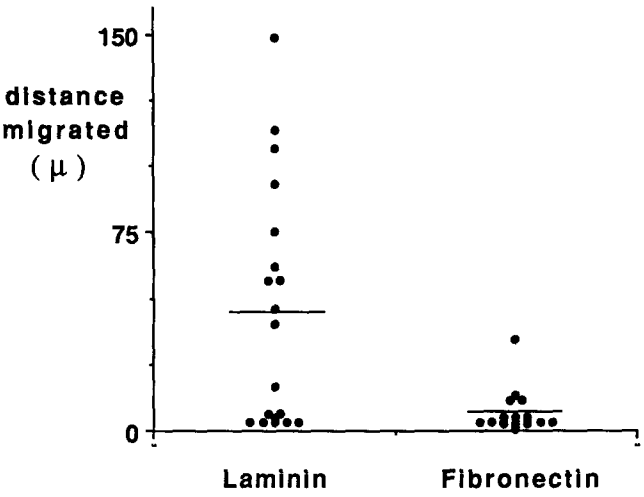

Figure 6. Displacements of neuronal cells on laminin versus fibronectin substrata. Dissociated OE neuronal cells were isolated as described in Materials and Methods, and plated into microscope chambers for time-lapse observation. Cells were allowed to settle for 1 h before video recordings were begun (at 14 h elapsed time following the initiation of suspension cultures; this is the same time point at which adhesion assays were performed; cf., Figs. 7–10). Cell movements were quantified by playing video recordings through a monitor, and manually tracing the movements of randomly chosen cells over 10 min periods during the first 2 h of recording. Only the movements of individual cells that did not contact other cells during a given 10-min period were measured. Data points represent the distances migrated by individual cells. Horizontal lines indicate the mean values, which were 44.7 μm for LN and 6.3 μm for FN.

Although the preference of cells for LN and MN did not correlate with the adhesiveness of these molecules, it did correlate with the striking ability of these two molecules to alter the adhesivity of substrata treated with FN. This ability is demonstrated by two types of experiments.

In the first set of experiments, shown in Fig. 8 *a*, a mixture containing equal concentrations of LN and FN was used to treat a substratum. The adhesion of OE neuronal cells to this substratum was not different from their adhesion to a substratum treated with LN alone. Moreover, even when a mixture containing one-tenth as much LN as FN was used, the level of cell adhesion was nearly as low as to LN alone. Identical mixing experiments performed with MN and FN gave similar results. In contrast, mixing FN with BSA (Fig. 8 *b*) did not alter the level of cell adhesion to FN, suggesting that the decrease in adhesion observed with mixtures containing MN or LN is due to a property specific to these two molecules.

Since the amount of FN that binds to the substratum could potentially be affected by the presence of other molecules during adsorption, a second type of experiment was carried out in which substrata were first treated with FN, and subsequently exposed to LN (Fig. 8 *c*). In this case as well, exposure of a substratum to LN rendered it much less adhesive for OE neuronal cells.

One possible explanation for these data is that LN and MN bind to FN, blocking its ability to interact with cells. If LN acts in this way, then the amount of LN required to decrease cell adhesion to background levels should vary strongly with the amount of FN on the substratum. Moreover, provided that the amount of FN on the substratum is much greater than that necessary to produce maximal adhesion, it should also be the case that the number of LN molecules required to decrease cell adhesion to background levels should be at least as great as the number of FN molecules on the substratum.

To test these predictions, the quantitative dependence of cell adhesion on the amounts of LN and FN bound to the substratum was examined. This was accomplished by first determining the amounts of LN and FN that adsorbed to the plastic substratum under different conditions. Samples of LN and FN were radioiodinated and used as tracers to determine the precise amounts of these molecules that bind to the substratum when they are mixed together in widely varying proportions. These data are shown in Table II. Cell adhesion to substrata treated with LN and FN mixtures, prepared as in Table II but without radioactively labeled proteins, was then measured. The cell adhesion data are presented in Fig. 9, and are plotted in two ways: In Fig. 9 *a*, adhesion is shown as a function of the amount of FN bound, when the FN is applied together with each of five different concentrations of LN. In Fig. 9 *b*, adhesion is shown as a function of the amount of LN bound, when the LN is applied together with each of five different concentrations of FN.

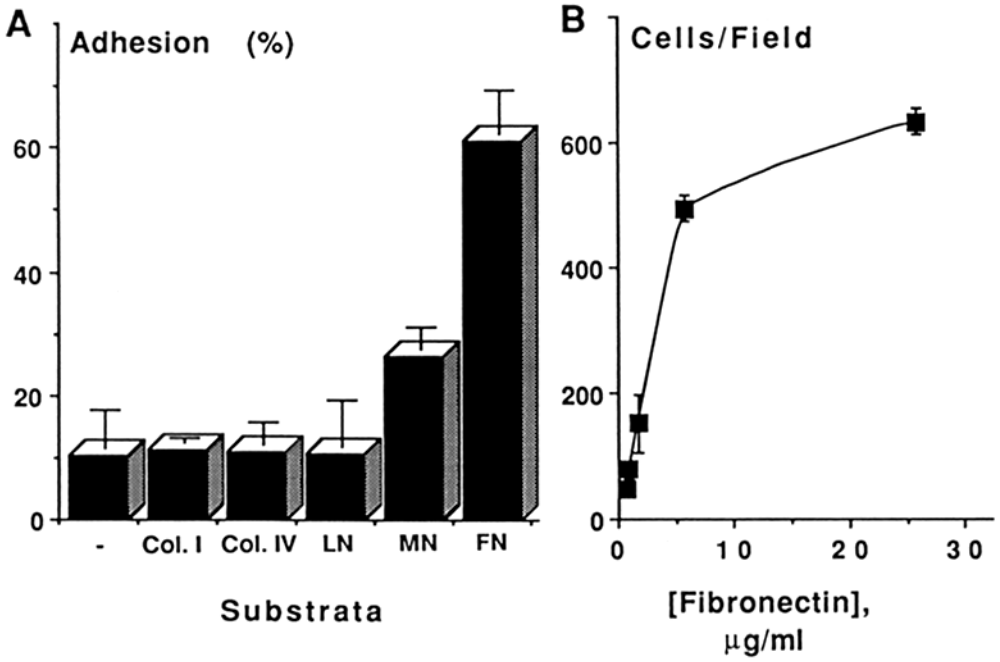

Figure 7. Adhesion of neuronal cells to purified ECM proteins. OE neuronal cells, metabolically labeled with ^{35}[S]-methionine, were isolated and plated onto polystyrene surfaces coated with different ECM proteins. Adhesion was measured after 30 min at 37°C, as described in Materials and Methods. (*A*) Quantification of cell adhesion was performed using a Betascope radioactivity imaging system (Betagen Corp., Waltham, MA.). Detaching force was 100 *g* for this assay. Adhesion is given as percent of polylysine control, and is shown as mean ± standard error. (*B*) Cell adhesion was quantified by counting the total number of cells in 4 fields photographed on Tri-X Pan film using a 10× objective. Detaching force was 625 *g* in this assay. Data are given as mean ± standard error.

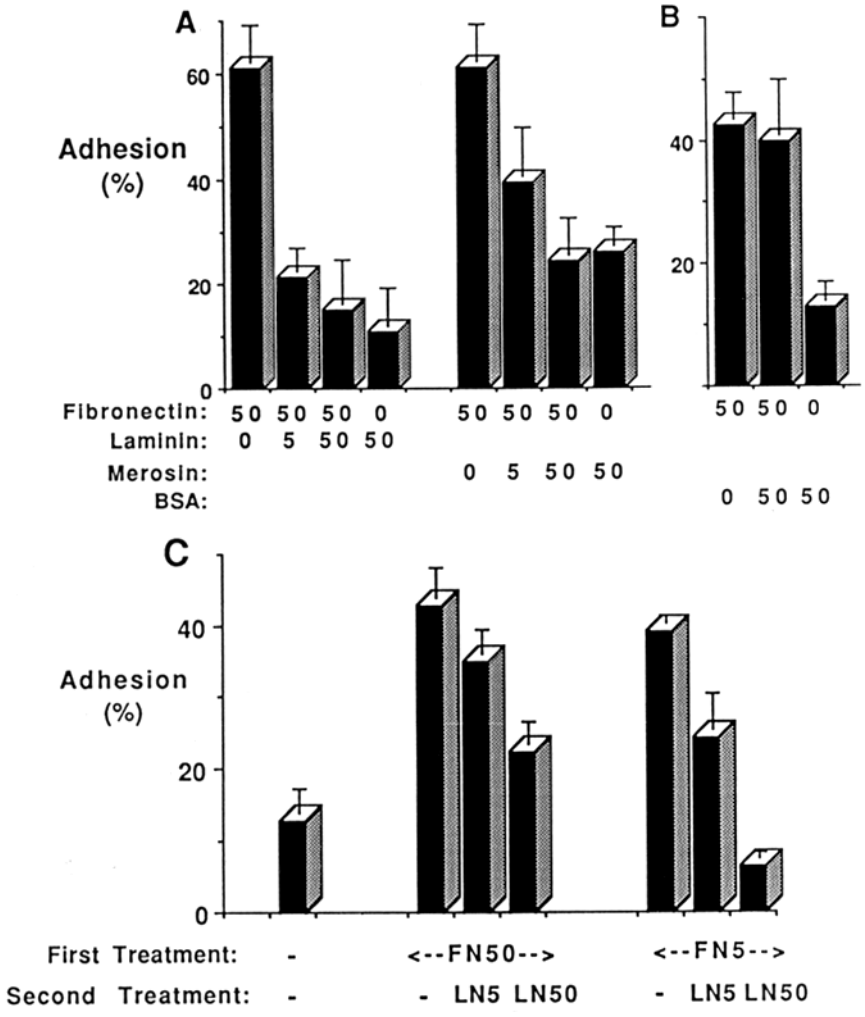

Figure 8. Laminin and merosin are anti-adhesive. (*A–B*) OE neuronal cell adhesion to substrata treated with mixtures of FN with LN, MN, or BSA. (*C*) OE neuronal cell adhesion to substrata treated first with FN (for 3 h at 37°C), then with LN (or buffer alone) for 3 h at 37°C, and finally with blocking BSA overnight. Numbers below the graphs indicate the concentration of applied proteins in μg/ml. Cell adhesion was measured as described in Materials and Methods; quantification was performed using a Phosphorimager (Molecular Dynamics). Detaching force was 100 *g*. Adhesion values are expressed as percent of polylysine control; data are given as mean ± standard error. The data indicate that LN and MN lower the adhesivity of FN-containing substrata, and do so whether they are applied along with FN, or after FN has already adsorbed to the substratum.

Table II. Adsorption of Fibronectin and Laminin to Polystyrene

Concentration applied (μg/ml)		Amount bound (ng/cm²)	
Fibronectin	Laminin	Fibronectin	Laminin
0	0	0	0
0.2	0	4.8	0
1	0	24	0
5	0	122	0
50	0	740	0
0	0.2	0	2.2
0.2	0.2	4.9	2.2
1	0.2	24	2.3
5	0.2	125	2.4
50	0.2	750	1.5
0	1	0	14.2
0.2	1	5.3	14.2
1	1	27.7	13.8
5	1	136	13.0
50	1	847	8.9
0	5	0	79
0.2	5	5.1	77
1	5	23.3	73
5	5	111	63
50	5	832	43
0	50	0	295
0.2	50	3.3	314
1	50	15.7	310
5	50	77.2	320
50	50	648	282

Mixtures of LN and FN spiked with radiolabeled LN or radiolabeled FN were applied to polystyrene microwells, incubated, washed and blocked as described for adhesion assays. Amounts bound were calculated from the specific activities of the labeled molecules, and represent means of quadruplicate data. Standard errors (not shown) were less than 10% for all points.

The data indicate that FN half-maximally promotes cell adhesion when adsorbed at a surface concentration of ~70 ng/cm², while LN half-maximally antagonizes cell adhesion when adsorbed at a surface concentration of ~30 ng/cm². Particularly revealing are the results obtained when FN was applied at 5 and 50 μg/ml (these are represented by the right-most two data points in each of the curves of Fig. 9 *a*, as well as by the top two curves in Fig. 9 *b*). Although the amount of FN that binds the substratum when applied at 50 μg/ml is approximately eightfold higher than when applied at 5 μg/ml (Table II), LN reduces adhesion in both cases with roughly the same dose dependence (Fig. 9 *b*). Moreover, although the amount of FN bound to the substratum when applied at 50 μg/ml is, at 700–900 ng/cm², more than 10 times that required to promote half-maximal adhesion, adhesion can be lowered nearly to background by a much lower amount of LN (~100 ng/cm²). Given that the molecular weight of LN is roughly twice that of FN, the data imply that one molecule of LN is sufficient to overcome the effects of 14–18 molecules of FN.

Laminin Reduces the Strength of Neuronal Cell Adhesion to Fibronectin Substrata

These data argue that binding of LN to FN does not account for the anti-adhesiveness of LN. Instead, it seems likely that LN acts directly on cells and alters the cells' ability to respond to FN. To understand how this might happen, it is useful to know how much of the strength with which cells adhere to FN is lost when LN is present on the substratum. The experiments shown in Figs. 7–9 indicate that, with sufficient LN, strength of cell adhesion to FN can be decreased enough so that the number of cells remaining attached to FN + LN substrata following centrifugation at 100 *g* is reduced to background level (i.e., the level obtained with a BSA-treated

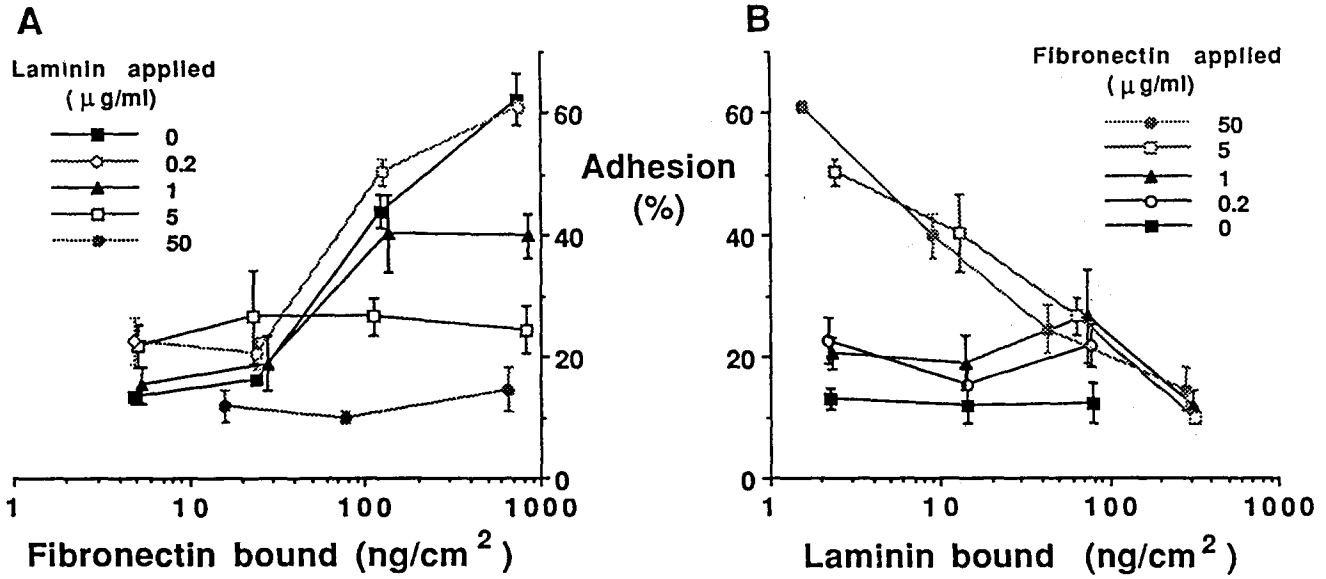

Figure 9. Concentration dependence of anti-adhesive activity of laminin. OE neuronal cell adhesion was measured to substrata treated with mixtures of FN and LN. Amounts of FN and LN bound to the polystyrene substratum were quantified as described in the text, and are given in Table II. Detaching force was 100 *g*. Quantification was performed using a Phosphorimager (Molecular Dynamics, Sunnyvale, CA). Adhesion values are percent of polylysine control; data are given as mean ± standard error. The data are plotted both as a function of the amount of FN bound (*A*) and the amount of LN bound (*B*).

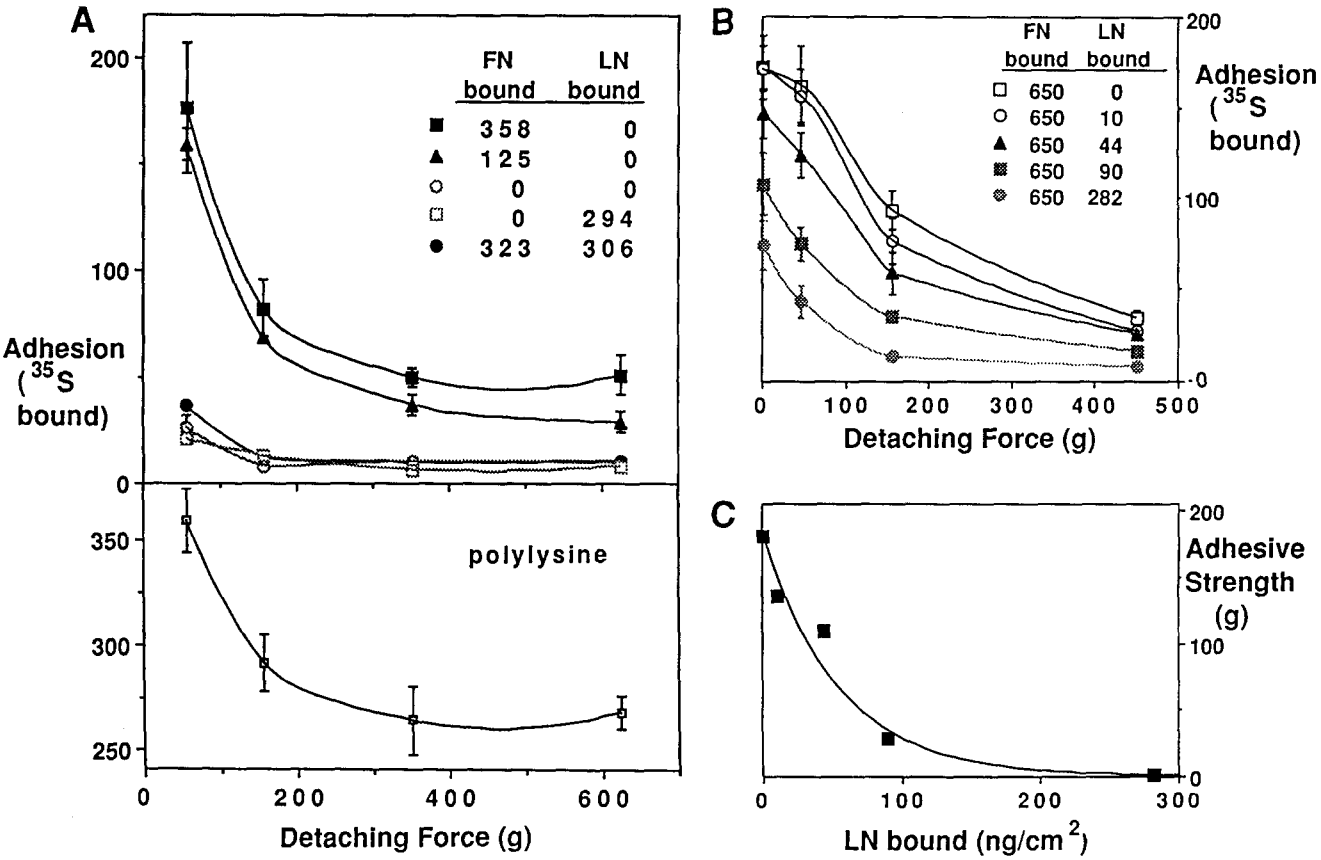

Figure 10. Laminin reduces strength of adhesion of neuronal cells to fibronectin substrata. (*A* and *B*) Adhesion was measured as described in Materials and Methods, except that for each mixture of proteins plated onto the substratum, four different detaching forces were used to assess the strength of cell adhesion. Amounts of protein bound were determined as described in Materials and Methods and are given as ng/cm^2. Adhesion values are given as total radioactivity bound as measured by Betascope (*A*, *counts per minute*) or Phosphorimager (*B*, 10^{-4} × total counts in 2 h); data are presented as mean ± standard error. (*C*) The curves in *B* were used to determine, for each LN concentration, the force required to reduce adhesion to half the level observed with zero LN and a 1 *g* detaching force. This measure of adhesive strength was then plotted versus the concentration of bound LN. A simple exponential curve has been fit to the data points.

substratum). This does not necessarily mean, however, that cell adhesion to FN is completely lost when LN is present. It could be that, with gentler detaching forces, a certain amount of residual, weak adhesion to FN would be detectable. To address this question, direct measurements were made of the strength of cell adhesion to LN, FN, and mixtures of the two. This was accomplished by monitoring cell adhesion over a range of detaching forces from 1 to 625 *g* (Fig. 10). As in Fig. 9, the data have been expressed in terms of the amounts of LN and FN bound to the substratum.

The results shown in Fig. 10 *a* indicate that: (*a*) the strength of cell adhesion to FN is much greater than adhesion to a control substratum (plastic treated with BSA only); (*b*) surface concentrations of FN >125 ng/cm^2 do not substantially increase the strength of cell adhesion; (*c*) adhesion to LN is not detectably stronger than to the control substratum; and (*d*) when a relatively large amount of LN is mixed with FN, adhesive strength is reduced to nearly the same level as the control substratum. These results suggest that LN, in sufficient quantity, virtually eliminates the adhesive response of OE neuronal cells to FN, at least under these assay conditions.

In the experiment shown in Fig. 10 *b*, the amounts of LN and FN that were mixed were varied systemically to produce a series of substrata containing equal amounts of bound FN, but different amounts of bound LN. The results of this experiment indicate that increasing substratum-bound LN decreases the strength of cell adhesion in a progressive manner. The dose dependence of this effect can be illustrated by plotting adhesive strength as a function of laminin concentration. Such a plot is shown in Fig. 10 *c*, where adhesive strength is quantified as the "half-maximal detaching force", i.e., the force required to detach half as many cells as remain adherent to a FN substratum treated with no LN and subjected to minimal detaching force (1 *g*). These data agree with those in Fig. 9 in implying that laminin is half-maximally anti-adhesive at ~35 ng/cm^2, and can have nearly maximal effects at ~100 ng/cm^2. Thus, in this experiment, LN was nearly maximally anti-adhesive at a ratio of one molecule of LN per 13 molecules of FN.

Laminin Inhibits the Formation of Focal Contacts by Neuronal Cells

Lotz et al. (29) have suggested that much of the adhesive strength that nonneuronal cells exhibit toward FN substrata develops over time because of increases in cell area (spreading) and the active formation of focal contacts. It is possible that the same or related processes also control the strength

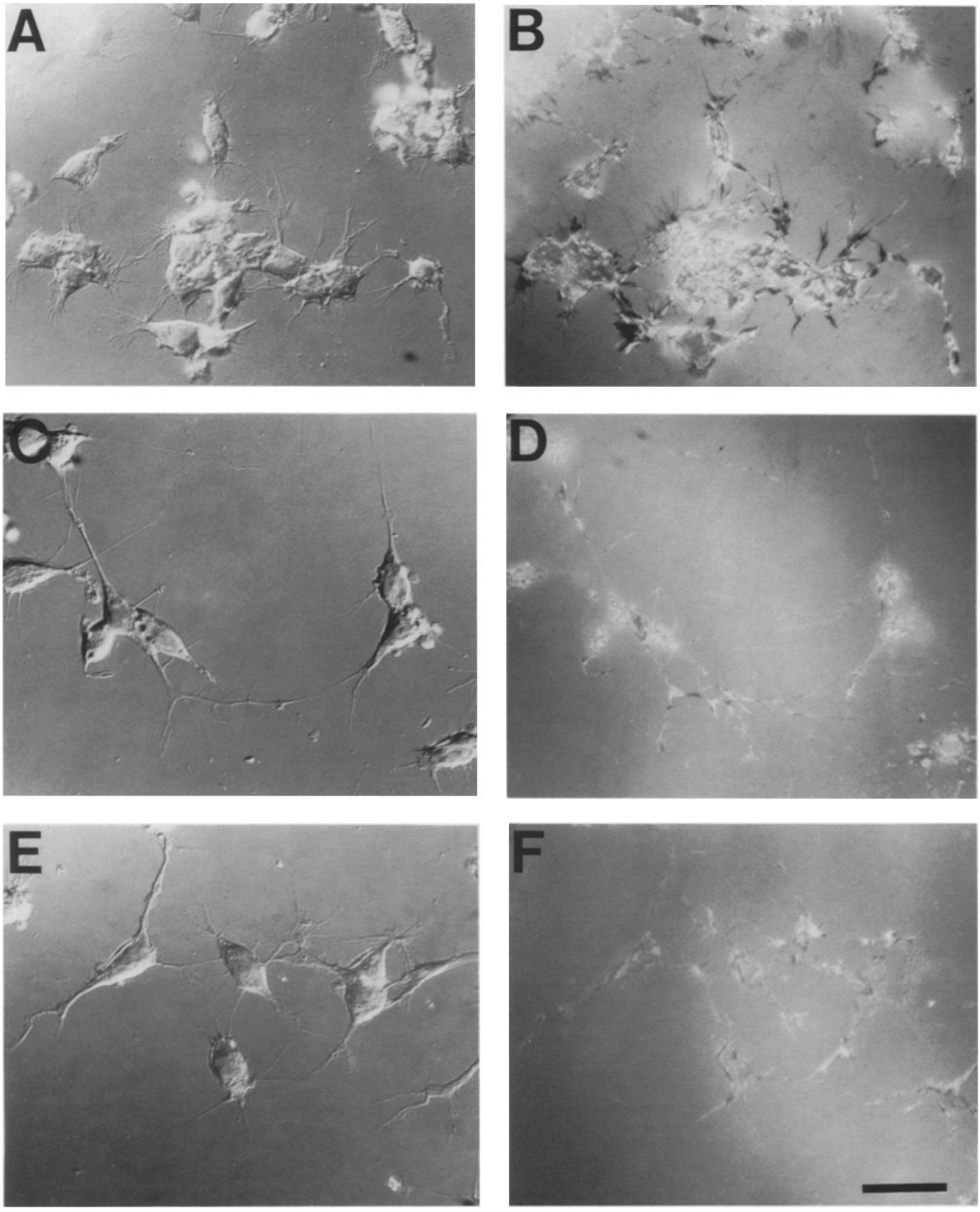

Figure 11. Laminin inhibits the formation of focal contacts. Explants of purified OE were grown for 18 h on acid-cleaned glass coverslips coated with FN at 50 μg/ml (*A* and *B*), LN at 100 μg/ml (*C* and *D*), or a mixture of LN and FN (25 μg/ml and 50 μg/ml, respectively) (*E* and *F*). Cultures were fixed by gentle underlay of culture medium with 3.7% formalin/PBS/5% sucrose warmed to 37°C, and mounted in 90% glycerol/PBS. (*A*, *C*, and *E*) Differential interference contrast optics. (*B*, *D*, and *F*) Interference reflectance microscopy (IRM) of the same fields. Photographs were taken on Kodak TMAX 100 film; IRM exposures were identical in length and were printed identically. Black patches seen in IRM are sites of very close cell-substratum appositions (10–15-nm contacts) that are indicative of focal contacts. Gray patches are sites of less close appositions (15–100-nm contacts). The data indicate that OE neuronal cells form many focal contacts on FN-coated coverslips, but few such contacts on coverslips treated with LN, or with a mixture of LN and FN. Bar, 20 μm.

with which neuronal cells adhere to FN. If so, one mechanism by which LN could decrease the strength of cell adhesion is by interfering with these processes. To test for this possibility, cells were examined both by differential interference contrast microscopy and by interference reflectance microscopy (IRM). The latter technique reveals areas of close cell–substratum apposition (putative focal contacts) as black patches. The data in Fig. 11 were obtained from cells cultured for 18 h on substrata treated with LN, FN, or a mixture of the two. Numerous black patches are seen with cells on the FN substratum, and appear to occur most often in association with filopodial structures. In contrast, few such patches occur on the LN and mixed substrata, despite the fact that similar filopodial structures can be identified on these cells. Indeed, overall cell body morphology does not appear to be altered significantly on these substrata.

Discussion

These experiments demonstrate that migration of neuronal cells derived from the embryonic OE is strongly dependent on substratum-bound ECM molecules. Postmitotic neurons, as well as their precursors, were found to be highly motile in vitro (Fig. 2–6) and to choose pathways of substratum-adsorbed LN or the LN-related molecule MN over FN, type I collagen, or type IV collagen (Fig. 5; and Table I). The adhesive preferences of these cells for different ECM molecules showed no clear correlation with their migratory preferences, however (Fig. 7). Interestingly, LN and MN were found to be anti-adhesive, i.e., able to reduce the adhesivity of FN-treated substrata (Figs. 8 and 10). The data suggest that LN's anti-adhesiveness is not an effect of LN on FN, but rather a direct effect of LN on neuronal cells, which then alters the cells' response to FN substrata (Figs. 9–11).

Is Anti-Adhesivity Related to the Stimulation of Migration?

The observations that LN and MN promote neuronal cell migration and that both molecules are also anti-adhesive suggests that there may be a connection between these two phenomena. Such a connection could be accounted for in any of several ways:

LN and MN May Stimulate Cell Motility As A Consequence of Decreasing Cell–Substratum Adhesion. The possibility that very strong adhesion is likely to keep cells attached in one place, and thereby prevent cell migration, has been raised by others (cf., 35, 46). However, in the system described in this paper, it is clear that weak adhesion alone is not sufficient to promote cell motility, since substrata treated with type I or type IV collagen, or with BSA, are poorly adhesive, but do not support neuronal migration. It could still be argued that very weak adhesion should also interfere with motility, and that cells are able to migrate only when adhesion is at an intermediate level, neither too high nor too low. This possibility is also argued against by the data reported here: For example, the finding that the strength of neuronal adhesion to LN substrata is not detectably greater than that to BSA-treated plastic (Fig. 10 *a*) argues that level of adhesion alone is not an indicator of a molecule's ability to stimulate cell migration. Indeed, the observation that neuronal cells migrate freely between LN- and MN-containing regions of the substratum (Fig. 5; and Table I), despite the different levels of adhesivity of these two molecules, suggests that neither the motility nor the migratory preferences of these cells is strongly influenced by levels of cell–substratum adhesion.

LN and MN May Decrease Cell–Substratum Adhesion As A Consequence of Stimulating Cell Motility. As McClay and co-workers have pointed out (31), cell-substratum adhesion is a time-dependent process, typically requiring 15 min or more to reach near-final levels. It is possible that LN and MN act primarily to stimulate cell motility, and that the resulting highly motile cells simply do not remain in one place long enough to develop strong adhesion. Although this effect could account for some of the anti-adhesivity of LN and MN, it is unlikely to account for all of it: cell migration was, if anything, more extensive on MN than LN substrata (Table I and unpublished observations), while adhesion to MN was significantly stronger than to LN (Figs. 7–8).

Increased Cell Motility and Decreased Cell–Substratum Adhesion May both be Consequences of An Effect of LN and MN on the Cytoskeleton. It is widely suspected that many ECM components exert effects on cells by influencing the structure or dynamics of the actin-based cytoskeleton. In addition to playing a fundamental role in cellular motility, the actin cytoskeleton can influence cell adhesion by affecting cell shape (and thereby the amount of membrane in contact with the substratum), cell stiffness (and thereby the way in which detaching forces are transmitted) and the formation of localized sites of very strong adhesion ("focal contacts"). Recently, Lotz et al. (29) reported that the time-dependent strengthening of glioma cell adhesion to FN correlates with cell flattening and with the formation of focal contacts (as judged by interference reflectance microscopy). In the present study, interference reflectance images also suggested a correlation between strong adhesion and focal contact formation, although marked changes in cell shape on different substrata were not observed (Fig. 11). Thus, it is possible that LN and MN decrease neuronal cell adhesion to FN by affecting the cytoskeletal machinery involved in the formation of focal contacts.

Like most large ECM proteins, LN is a multidomain molecule, capable of interacting with many types of cell surface receptors, including several integrins, nonintegrin membrane proteins, proteoglycans, galactosyltransferase, and glycolipids (2, 22, 30). In future, it will be important to determine whether LN's effects on cell motility and its anti-adhesivity are mediated through the same domains of LN and the same receptors. If they are, it will suggest that these effects are causally linked. Experiments using fragments of LN and domain-specific inhibitors can be used to address this question. Currently such experiments are underway.

Anti-Adhesivity and other ECM Molecules

The finding that LN and MN are anti-adhesive for the neuronal cells of the OE is interesting in light of studies suggesting that other ECM molecules may be anti-adhesive, notably tenascin (6, 15, 29), the tenascin-related molecules Jl-160/180 (33), and thrombospondin (34). Both tenascin and thrombospondin have also been suggested to inhibit the formation of focal contacts (29, 34). Interestingly, the ability of these ECM molecules to be anti-adhesive appears to depend strongly on the type of cell tested (15, 33). In the case of

tenascin, there is some evidence that adhesive and anti-adhesive domains of the molecule can be separated from one another (44).

Based on these findings, we propose that molecular domains capable of mediating anti-adhesion are common to most, and possibly all, ECM molecules. The response of any cell type to an ECM molecule should then be a function of the number and types of its receptors and whether those receptors recognize adhesion-mediating domains or anti-adhesion–mediating domains. In the event that both types of domains are recognized by a cell, the two effects might antagonize each other. The hypothesis that potentially antagonistic activities coexist in ECM molecules leads to the prediction that fragments of ECM molecules will often exhibit biological activities not observed with intact molecules. Such "cryptic" activities have, in fact, been described for many ECM molecules (e.g., 1, 11, 20, and 41). This hypothesis also leads to the prediction that some reagents, such as antibodies, peptides, and carbohydrates, that are currently used to disrupt cell attachment to ECM molecules may ultimately be found to do so not because they block adhesive function, but because they stimulate anti-adhesive function. Conversely, it might be predicted that similar reagents may be found that stimulate cell adhesion by virtue of inhibiting anti-adhesive function. An example of such a reagent may be the TASC mAb, a recently described integrin-binding antibody that stimulates the adhesion of retinal cells to some, but not all, ECM molecules (36).

Role of ECM in Neuronal Migration

It is interesting to contrast the behaviors of OE neuronal cells with those of other neural cells whose migratory preferences have been studied in vitro. Neural crest cells, the neural cell type whose migratory properties have been studied most extensively, are highly motile in culture, and readily migrate on substrata treated with any of several ECM molecules, including FN, LN, collagen, and tenascin (14, 19, 39). Crest cells exhibit a flattened morphology in culture, and migrate in a manner similar to that of fibroblasts (i.e., through the extension of flattened, lamellipodial leading edges). OE neuronal cells, in contrast, have so far been observed to migrate readily only on substrata treated with LN or MN. These cells do not flatten during migration, and move in a manner more like that described for neutrophils (10) and myoblasts (16), i.e., by extending long pseudopodia (Fig. 2). Cerebeller granule neurons, the only central neurons whose migration has been studied in vitro, adhere to and migrate preferentially along the surfaces of glial cells (12), although some studies suggest that the ECM molecules tenascin (7) and thrombospondin (37) may play roles in cerebellar granule neuron migration.

The pronounced effect of LN and MN on OE neuronal cells raises the possibility that a LN-like molecule guides the migration of these cells in vivo. As mentioned earlier, it has recently been shown that, during normal murine brain development, neuronal cells derived from the OE migrate into the brain and eventually populate parts of the hypothalamus (9, 42, 47, 48). It is not known whether LN is located along the pathway taken by these cells, although LN immunoreactivity has been detected in other parts of the developing brain (8, 26, 28, 40) and, in one case, a correlation between its distribution and sites of neuronal migration has been suggested (27).

It should be pointed out that the OE neuronal cells studied here are not necessarily equivalent to the population of OE cells that has been observed to migrate in vivo. The latter cells, which can be detected histologically by virtue of their expression of the neuropeptide LHRH, appear to represent only a fraction of the total neuronal cells of the OE. Moreover, at least half of the LHRH-positive cells are reported to have already emigrated from the mouse OE by embryonic day 14 (42). These data suggest that LHRH-positive cells represent at most only a small fraction of the OE neuronal cells studied here. Nonetheless, a majority of OE neuronal cells were observed to migrate on LN in vitro (Fig. 6). This observation could be explained in either of two ways: it could be the case that OE neuronal cells other than LHRH-positive cells also migrate in vivo; if so, it will be important to find such cells in vivo and determine where in the brain they end up. Alternatively, it could be the case that OE neuronal cells that do not normally migrate still exhibit the capability to do so in vitro; if so, it will be important to determine what aspect of the tissue environment causes these cells to remain where they do in vivo.

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