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Studies on Integrins in the Nervous System

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Abstract

Integrins are of interest to neuroscientists because they and many of their ligands are widely expressed in the nervous system and have been shown to have diverse roles in neural development and function (Clegg *et al.*, 2003; Li and Pleasure, 2005; Pinkstaff *et al.*, 1998, 1999; Reichardt and Tomaselli, 1991; Schmid *et al.*, 2005). Integrins have also been implicated in control of pathogenesis in several neurodegenerative diseases, brain tumor pathogenesis, and the aftermath of brain and peripheral nervous system injury (Condic, 2001; Ekstrom *et al.*, 2003; Kloss *et al.*, 1999; Verdier and Penke, 2004; Wallquist *et al.*, 2004). Using integrin antagonists as therapeutic agents in a variety of neurological diseases is of great interest at present (Blackmore and Letourneau, 2006; Mattern *et al.*, 2005; Polman *et al.*, 2006; Wang *et al.*, 2006). In this chapter, we describe methods used in our laboratory to characterize neuronal responses to extracellular matrix proteins, and procedures for assessing integrin roles in neuronal cell attachment and differentiation.

1. Introduction

In this section, we provide a brief summary of the current state of our knowledge on the roles of integrin receptors in the nervous system, beginning with their roles in neural development. Since the 1980s, the many roles for this fascinating family of adhesion molecules have been documented, which has increased our understanding of neural development, synaptic function, and several neurological diseases.

Much of the initial interest in integrin roles in the nervous system was focused on their roles in the neural crest, a migratory population of cells derived from the dorsal neural tube that populate the sensory, autonomic, and enteric nervous systems as well as contributing to formation of many specialized sense organs, glands, the heart, cranial mesenchyme and bone, and supporting cells in peripheral nerves, including Schwann cells and endoneurial fibroblasts (Le Douarin and Dupin, 2003). Neural crest cells express many integrins and migrate through an extracellular matrix (ECM)-rich environment (Bronner-Fraser, 1994; Kil *et al.*, 1998). Acute inhibition experiments in avian embryos have documented important roles for integrins in migration of the neural crest (Tucker, 2004). In mice, genetic ablation of $\beta 1$ integrins results in severe perturbations of the peripheral nervous system, including failure of normal nerve arborization, delay in Schwann cell migration, and defective neuromuscular junction differentiation (Pietri *et al.*, 2004). In addition to direct effects on migration, it has been shown that absence of specific integrin heterodimers compromises Schwann cell precursor survival, proliferation, and differentiation (Feltri *et al.*, 2002; Haack and Hynes, 2001). Many of these observations are likely to reflect the roles of integrin receptors in regulating activation of MAP kinase, Rac, and other signaling pathways (Campos *et al.*, 2004).

In part because they were characterized long before the identification of the major families of axon guidance molecules, such as the netrins, semaphorins, and ephrins, early studies on axon outgrowth and guidance focused on integrins and the cadherin and immunoglobulin families

of cell adhesion molecules. In these studies, it was demonstrated that virtually all process extension on ECM substrates by neurons requires integrin function and that neuronal growth cones could distinguish between different ECM proteins and respond to orientation or gradients of ECM proteins by directed growth (Dubey *et al.*, 1999; McKenna and Raper, 1988). In addition, integrins have been shown to interact with several of the axon guidance systems. For example, semaphoring 7A-dependent promotion of axon growth requires integrin activity, while semaphoring-mediated activation of plexin signaling reduces integrin-based adhesion (Barberis *et al.*, 2004; Pasterkamp *et al.*, 2003). The A and B ephrins also control integrin activation (Davy and Robbins, 2000; Nakada *et al.*, 2005). Integrins interact genetically with the Slit-Robo pathway in *Drosophila* (Stevens and Jacobs, 2002). Evidence also suggests that two integrins may serve as receptors for netrins in epithelia (Yebra *et al.*, 2003). Despite these intriguing observations, ablation of either $\beta 1$ or αV integrins appear to have only minor effects on axon guidance in the brain although perturbations are most significant in the peripheral nervous system (Blaess *et al.*, 2004; Graus-Porta *et al.*, 2001; McCarty *et al.*, 2005; Proctor *et al.*, 2005). This is likely the result of neurons interacting with many different types of substrates, many of which do not require integrin function.

Despite the absence of major effects on axon guidance, integrin deletion affects many aspects of forebrain and cerebellar development. First, loss of $\beta 1$ integrins results in disruptions of the basal lamina that separates the brain from the overlying mesenchyme (Graus-Porta *et al.*, 2001). As a result, the migration of neurons is perturbed, resulting in abnormal lamination of the cortex and cerebellum. Similar phenotypes are observed in mice lacking another ECM receptor dystroglycan as well as in humans and mice with mutations in basal lamina-encoding genes, such as the laminin $\alpha 5$ subunit (Gleeson and Walsh, 2000). Although some evidence indicates that integrins modulate neuronal interactions with radial glia—which provide the substrate for the tangential migrations that establish the cortical lamination pattern (Sanada *et al.*, 2004; Schmid *et al.*, 2005)—the major phenotype observed in these mutants appears to stem from disruption of signaling pathways controlling neuronal migration that require integrity of the basal lamina (Beggs *et al.*, 2003).

Integrins have a number of additional actions that modulate brain development. Of particular interest, they have been shown to control survival and proliferation of some populations of neural stem cells (Leone *et al.*, 2005). Within the external granule cell layer of the cerebellum, integrin binding to laminin enhances the proliferative responsiveness of granule cells to sonic hedgehog (SHH), probably because association of SHH with laminin facilitates SHH activation of its receptor Smoothened (Blaess *et al.*, 2004).

Integrins have a number of potent, but poorly understood, effects on synaptic function and plasticity. In cell culture, interactions of astroglia with neurons mediated by the glial integrin $\alpha V\beta 3$ results in PKC activation in individual neurons that facilitates excitatory synaptogenesis (Hama *et al.*, 2004). The receptor on neurons mediating PKC activation is not known, but neurons express many proteins, including the ADAMs, L1, and amyloid precursor protein, which are known to interact with integrins and are therefore candidates to mediate this signaling pathway (Mechtersheimer *et al.*, 2001; Wright *et al.*, 2006; Yang *et al.*, 2006). In *Drosophila*, integrins control localization of postsynaptic proteins at the neuromuscular junction through a CamKII-mediated signaling cascade (Burgess *et al.*, 2002). At the vertebrate neuromuscular junction, expression of integrins in muscle, but not nerve, is required for synapse formation (Schwander *et al.*, 2004), possibly through interactions with agrin or promotion of basal lamina assembly/organization (Burgess *et al.*, 2002; Burkin *et al.*, 2000). Integrins have also been localized to the synaptic active zones of motor neuron axon terminals and mediate the enhancement of transmitter release caused by mechanical stretching of muscle fibers (Kashani *et al.*, 2001).

Although localization studies indicate that integrins are present at many synapses in the brain, genetic and pharmacological studies indicate that integrins are not required for synapse formation, but are required for normal synaptic plasticity. In a particularly elegant series of studies, the presence of integrins in the mushroom body of the *Drosophila* brain was shown to be required for short-term memory (Grotewiel *et al.*, 1998). Conditional expression of an integrin subunit in the adult mushroom body rescued the memory deficits, providing definitive evidence that this was an effect on function, not early development. Studies in the murine hippocampus have demonstrated that $\beta 1$ integrins are required for normal LTP (Chan *et al.*, 2006; Huang *et al.*, 2006). Studies of mice with reduced expression of individual $\beta 1$ integrin heterodimers have suggested that specific integrins have different functions at the synapse (Chan *et al.*, 2003). Acute pharmacological perturbations using inhibitory integrin reagents indicate that integrins are involved in regulation of both NMDA and AMPA receptor function and act through regulation of protein kinases and the actin cytoskeleton (Kramar *et al.*, 2003, 2006; Lin *et al.*, 2003). Clearly, much remains to be understood about interactions between integrins and the signaling pathways known to be fundamental in initiation and maintenance of LTP.

Integrins are also necessary for normal development of non-neuronal cells in the nervous system, including astroglia, oligodendrocytes, and Schwann cells. For example, the presence of $\beta 1$ integrins is required for normal morphological development of radial glia, and abnormalities in the morphology of these glia may underlie the neuronal lamination deficits observed in mice with mutants in genes encoding basal lamina constituents and their receptors, including both integrins and dystroglycan (Forster *et al.*, 2002; Gleeson and Walsh, 2000).

An abundance of studies in culture indicate that integrins have profound effects on the differentiation of the precursors to oligodendrocytes (Colognato *et al.*, 2002, 2004). Despite these effects of integrin absence on oligodendrocyte development and myelination in the brain and spinal cord appear to be modest. In mice lacking $\beta 1$ integrins myelination appears to be completely normal during development and, following injury, in the adult (Benninger *et al.*, 2006). During development there is elevated apoptosis of premyelinating oligodendrocytes, but this does not prevent successful myelination. No obvious reduction in myelination is obvious in mice lacking either the αV or $\beta 8$ integrins, although this has been examined in the same detail as the studies performed using mice lacking the $\beta 1$ integrin subunit (McCarty *et al.*, 2005; Proctor *et al.*, 2005).

$\beta 1$ integrins are clearly essential for many steps in peripheral nerve development, including several aspects of Schwann cell differentiation. $\beta 1$ gene ablation in the neural crest cell lineage delayed the migration of Schwann cell precursors along embryonic peripheral nerves without detectable effects on proliferation or apoptosis (Pietri *et al.*, 2004). At later times, the absence of these integrins interfered with normal sorting of sensory axons with Schwann cells and reduced their myelination (Feltri *et al.*, 2002; Pietri *et al.*, 2004). Integrin deficiency in the neural crest cell lineage also interfered with basal lamina assembly in peripheral nerves and prevented normal differentiation of the neuromuscular junction. No obvious phenotypes in the peripheral nervous system have been reported in mice lacking the αV or $\beta 8$ integrins.

Absence of either αV or $\beta 8$ integrins does, however, have a profound influence on brain development. Expression of $\alpha V\beta 8$ is required for normal vascular development in the brain. In its absence, vascular development is severely perturbed, resulting in massive embryonic hemorrhage. Cell-specific targeting has shown that this integrin must be expressed in the neuroepithelial, not the endothelial lineage (McCarty *et al.*, 2005; Proctor *et al.*, 2005). Intriguingly, $\alpha V\beta 8$ has been shown to promote the activation of TGF β through binding to an RGD sequence in the TGF β latency-associated peptide (Mu *et al.*, 2002). Similarities between the phenotypes of the αV and $\beta 8$ and mutations in the TGF β signaling pathway suggest that

the vascular abnormalities may reflect, at least in part, a reduction in TGF β activation. Surprisingly, in animals that survive hemorrhage, the vasculature recovers so that hemorrhage is no longer visible in young adults. These animals, however, do develop motor deficits and die prematurely, most likely as a result of neurodegeneration in the central nervous system (CNS).

Integrins are also of interest to neuroscientists because they have been implicated in several neurodegenerative disorders. Of particular importance, because integrins regulate the transit of lymphocytes, macrophage, and other cells across the blood–brain barrier in response to inflammatory stimuli, anti-integrin reagents are of great interest as therapeutic agents to control demyelinating diseases, such as multiple sclerosis that involve the immune system and inflammatory responses (Bartt, 2006; Kanwar *et al.*, 2000). The involvement of integrins in inflammatory responses has created interest in the possibility of using anti-integrin reagents to alleviate several neurodegenerative disorders, including Parkinson's and Alzheimer's diseases (Austin *et al.*, 2006). Of special interest, several integrins appear to interact with amyloid precursor protein and these are postulated to mediate deposition or toxic actions of A β and amyloid formation (Bozzo *et al.*, 2004; Koenigsknecht and Landreth, 2004; Sondag and Combs, 2006). Thus future studies on integrin functions in the normal and diseased brain are likely to provide interesting insights, some of which may have practical applications.

2. Neuronal Cell Adhesion and Neurite Outgrowth Assays

2.1. Preparation of substrates

2.1.1. Standard substrate preparation—When substrates such as laminin, fibronectin and the collagens, are available in abundant quantities, substrates are typically prepared in the same way as for other cell types by incubation of substrata with ECM proteins in solution, washing, and blocking, and then neuronal adhesion or neurite outgrowth assays. For example, the laboratory has frequently used the following protocol (Hall *et al.*, 1987) in which sterile Linbro 96-well, flat-bottom tissue culture plates are coated with 100 μ l per well of laminin, collagen IV, other ECM protein or antibody diluted in calcium- and magnesium-free phosphate buffered saline (CMF-PBS; 200 mg/liter KCl, 200 mg/liter K₂SO₄, 8.0 g/liter NaCl, and 2.16 g/liter Na₂HPO₄·7H₂O, pH 7.36 to 7.45) at a concentration typically of 10 to 100 μ g/ml. Fibronectin is applied in 100 μ l per well of sterile 0.1-*M* cyclohexylaminopropane sulfonic acid buffer, pH 9.0. Plates are incubated with proteins overnight in the cold room. After rinsing three to five times with sterile CMF-PBS, the wells are blocked by incubation with 1% BSA for at least 2 h at room temperature. Plates are again rinsed three to five times with sterile CMFPBS and 100 μ l of culture medium is added to each well. At this time antibodies are added to the wells, and plates are stored in the incubator, at 37° and 5% CO₂, until the cells are ready, typically about 1 h, but plates can be stored for several days at 4°.

2.1.2. Modified substrate preparation using limiting reagents—Many of the integrin-binding proteins in the nervous system are difficult to purify or present for other reasons in only limiting quantities. In this case, we recommend first coating the Linbro Titertek 96-well plastic dishes with nitrocellulose (Lagenaur and Lemmon, 1987). In this procedure, 5 cm² of nitrocellulose type-BA 85 (Schleicher and Schuell, Keene, NH) is dissolved in 6 ml of methanol. Aliquots are spread over the surface of microwells and allowed to dry under a laminar flow hood. Test protein samples are applied at 50 μ l per well at concentrations approximately 100-fold lower than the concentrations used for standard substrate preparation. The presence of nitrocellulose on the substrate results in capture of virtually all of the protein in solution. Our laboratory has used this procedure to examine integrin-mediated interactions of neurons with tenascin and purified fragments of tenascin (Varnum-Finney *et al.*, 1995).

2.2. Cell adhesion assays

For acute assays, neurons can be isolated and tested in serum-free conditions. In contrast, media conditions are quite important for assays involving long-term survival and differentiation of neurons in culture and conditions for maintenance of several different neuronal populations are described below. For acute assays using freshly isolated neuronal populations, our laboratory performs cell adhesion assays using procedures similar to those used for other cell types with cell substrata precoated with various ECM substrates and single-cell suspensions of neurons (for preparation of individual neuron populations, see below) centrifuged onto these substrates in 96-well plates. For cell attachment assays, after a time of typically 1 h (which can be varied), unattached cells are removed by gentle swirling of the medium and medium removal. Because neurons are typically present in comparatively small numbers, our assays typically measure adhesion by counting of attached neurons although for cell types present in large quantities, such as chick retinal neurons, assays using dyes and optical density measurements may be used instead. Cell counts are performed manually using randomly chosen fields of view, typically at least 15 fields at 200 $\times$ magnification. It is important that fields of view be spaced evenly across the culture substrate surface and that wells be checked to ensure that there is a reasonably uniform distribution of neurons across the entire surface. In these assays, neurons can usually be distinguished from other cells on morphological criteria. Our laboratory also used preplating protocols on which cell suspensions are incubated with tissue culture plastic wells to remove preferentially non-neuronal cells, which adhere preferentially in these conditions.

2.3. Neurite outgrowth assays

Cells on coverslips are fixed with 4% paraformaldehyde and mounted on slides with gelvatol (50% glycerol plus polyvinyl chloride). Cultures are viewed using a Zeiss inverted LSM 5 Pascal confocal microscope using a 10 $\times$, 0.3 n.a. or 20 $\times$, 0.5 n.a. Plan Neufloar objective. All images are captured under the same conditions in each type of experiment. After conversion to TIFF format, the selected images are exported to Image J (National Institutes of Health software) for drawing neuronal images and final quantification.

To determine the percentage of cells with neurites, at least 100 cells are analyzed per culture. The number of cells with a process longer than one cell diameter is determined and compared to the total number of cells counted. In some instances, we have used more stringent criteria to define a neurite, such as requiring an extension longer than two cell diameters.

In some instances, it is important to determine the length and branching properties of neurons grown in conditions where their axons and dendrites are intermingled with those of other neurons. In these instances, we have transfected neurons with plasmids containing a fluorescent protein (e.g., Venus, EGFP, dsRED).

2.4. Axons versus dendrite quantification

For many neuronal populations, it is frequently important to distinguish between axons and dendrites. In this case, we have collected and fixed neurons as described above, but have stained cultures with antibodies to tau to identify axons or antibodies to MAP2 to identify dendrites (Rico *et al.*, 2004).

3. Neuronal Culture Procedures

Procedures used by our laboratory for isolation and culture of several different types of neurons are described below. These assays typically give enriched, not absolutely pure populations of neurons. For studies where highly purified populations of neurons are required, antibodies specific for different cell types have been used in sequence to deplete cell mixtures of unwanted

cell types and to select for specific subpopulations of neurons, such as retinal ganglion cells (Barres and Raff, 1999; Goldberg *et al.*, 2002; Mi and Barres, 1999; Ullian *et al.*, 2004). These procedures have been crucial for advancing our understanding of the roles of cell interactions in differentiation and the functions of ECM proteins in processes such as synaptogenesis (Christopherson *et al.*, 2005). In instances where integrin functions in a defined population of neurons will be examined, readers are referred to the papers cited immediately above for descriptions of useful techniques.

3.1. Rat PC₁₂ pheochromocytoma cell culture

PC12 cells are grown in standard culture flasks in Dulbecco's modified Eagle's medium with 4.5 g/liter of glucose (DME H-21) supplemented with 10% heat-inactivated horse serum, 5.0% newborn calf serum, 2 mM of glutamine, and 100 U/ml penicillin and streptomycin. Cells are passaged by incubating for 5 to 10 min with 0.5 mM EDTA in PBS (8 mM Na₂HPO₄, 2 mM KH₂PO₄, 2 mM KCl, 0.136 M NaCl, pH 7.4). For priming with NGF, PC12 cells are passaged as described above onto fresh plates at a low density (10⁴ cells/cm²) and cultured for 5 to 7 days in DME H-21 supplemented with 1.0% heat-inactivated horse serum, 5.0% newborn calf serum, 2 mM of glutamine, and 100 U/ml of penicillin and streptomycin. In addition, 50 ng/ml NGF is added to prime the cells. PC12 cells attach well to and grow on substrata coated with EHS laminin or collagen I, but exhibit only poor attachment and neurite outgrowth on laminin (Tomaselli *et al.*, 1990; Varnum-Finney and Reichardt, 1994).

3.2. Rodent sympathetic neuron cultures

Superior cervical ganglia (SCGs) are dissected from Sprague-Dawley neonatal (P0) rat pups. SCGs are dissociated by manually removing the outer sheath and then digesting the ganglia for 1 h in 1 mg/ml of collagenase/dispase in CMF-PBS (DeFreitas *et al.*, 1995; Hawrot and Patterson, 1979). Digested SCGs are washed in CMF-PBS and triturated to dissociate the cells. After two washes in CMF-PSS, the cells are resuspended in serum-free L-15 complete medium as described (Hawrot and Patterson, 1979). Alternatively, the N1 serum-free additives (Bottenstein *et al.*, 1980) can be substituted for rat serum. NGF is added to a final concentration of 100 ng/ml, glutamine to a concentration of 2 mM, and BSA to a final concentration of 1 mg/ml. Cells are grown in a 5% CO₂ atmosphere at 37°. Alternatively, to minimize growth of non-neuronal cells, cultures can be grown in L-15 air medium (no bicarbonate) and incubated in a humidified air incubator at 37° (Hawrot and Patterson, 1979).

3.3. Rodent DRG sensory neuron cultures

Newborn rat and mouse dorsal root ganglia (DRG) are dissected into Ca²⁺-Mg²⁺-free Hank's Balanced Salt Solution and incubated with 0.1% trypsin for 30 min at 37°. Ganglia are dissociated with trituration through a flame-polished, siliconized Pasteur pipette in HBSS containing 10% fetal calf serum (FCS). Dissociated cells are depleted of non-neuronal cells by preplating for 1 h at 37° in 60-mm tissue culture dishes in DRG growth medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) (4.5 g/liter of glucose) with 10% FCS, 50 ng/ml 2.5s nerve growth factor, and 100 U/ml of penicillin/streptomycin. Unattached neurons are decanted, washed once in growth medium, and plated in growth medium at about 500,000 cells/35-mm dish precoated with 10 µg/ml EHS laminin (or other chosen substrate). After 3 days, cultures are treated twice for 48 h each with 10 µM cytosine arabinoside in growth medium, followed by 24 h in growth medium alone. Following treatment the cultures should contain more than 95% neurons, as assessed using morphological criteria (Tomaselli *et al.*, 1993).

3.4. Rodent trigeminal sensory neuron cultures

Dissociated trigeminal or DRG sensory neurons are cultured using a published protocol (Buchman and Davies, 1993). Trigeminal or dorsal root ganglia are dissected from E12.5 embryos or P0 newborn mice, and trypsinized for 5 min at 37° with 0.05% trypsin in calcium- and magnesium-free HBSS. After removal of the trypsin solution, the ganglia are washed twice with 10 ml of Hams F12 medium containing 10% HIHS, and are gently triturated with a siliconized Pasteur pipette to give a single-cell suspension. Dissociated neurons are then plated on 16-well chamber slides coated with poly (DL)ornithine (0.5 mg/ml) and laminin (10 µg/ml) in triplicate, at a density of 2000 cells/well in defined F-14 medium, containing thyroxine (400 ng/ml), triiodothyronine (340 ng/ml), progesterone (60 ng/ml), sodium selenite (38 ng/ml), 0.35% bovine serum albumin, and N2 supplement in the presence of 10 ng/ml NGF. For cultures in which it is desired to maintain other populations of neurons, this medium can be supplemented by 10 ng/ml BDNF, 10 ng/ml NT-3, or 50 ng/ml GDNF, optimal concentrations for each of these factors. Cultures are maintained in 5% CO₂ at 37° in a humidified incubator (Huang *et al.*, 1999).

3.5. Rodent hippocampal neuron cultures

Hippocampi are obtained from E-17 to P-2-rat or mouse embryos by dissection using scissors and needles. Dissected hippocampi are incubated for 15 to 20 min in a solution of 2 mg of trypsin per milliliter of Hank's balanced salts solution (HBSS) containing 2.4 g/liter of N-[2-hydroxyethyl]piperazine-N9-[2-ethanesulfonic acid] (HEPES) and 10 mg/liter of gentamicin, pH 7.2, at a concentration of two rat hippocampi per milliliter (three to four mouse hippocampi per milliliter). The hippocampi are then rinsed three times in 10 ml of HBSS, followed by a 5-min incubation in a solution of 1 mg of trypsin inhibitor per milliliter of HBSS, and finally rinsed three times with 10 ml of HBSS. Cells are then dissociated by trituration through the narrowed bore of a fired-polished Pasteur pipette. Neurons are plated on poly-L-lysine-coated cover glasses or tissue culture plastic at the density of 130 cells/mm² in MEM containing 10% horse serum, 1 mM of pyruvate, penicillin-streptomycin and 0.6% glucose ("plating medium"). After 4 h, the plating medium was replaced by neurobasal media, penicillin-streptomycin, and B27 supplements ("maintenance medium") (Elia *et al.*, 2006; Rico *et al.*, 2004; Xie *et al.*, 2000).

3.6. Rodent cortical neuron cultures

These neurons are cultured using the same procedures as used for hippocampal neuron cultures. Typically, cortices are easier to dissociate with trypsin and neurons are more sensitive to trypsin (Xie *et al.*, 2000).

3.7. Chick DRG sensory neuron culture

Embryonic chick dorsal root ganglia at Day 7 or 8 are dissected from the vicinity of the spinal cord manually using fine forceps or glass needles. Ganglia are dissociated into single cells by incubation in 0.05% trypsin in 0.2% versene, 0.10% glucose, 0.02% EDTA, 0.058% NaHCO₂ for 10 min at 37° followed by trituration. Dissociated cells are collected by centrifugation and resuspended in F12 containing 10% fetal bovine serum. To enrich for neuronal cells, cell suspensions are plated onto 60-mm tissue culture dishes (Falcon) for 1 to 3 hr. Neurons are pipetted from the culture dishes, centrifuged, and resuspended in DRG growth medium (F-12 containing 2% BSA (Serva), and 100 ng/ml nerve growth factor) at an appropriate density (1 × 10³ cells per well). 50 µl of this cell suspension is added to the well of a 96-well culture dish coated with 10 µg/ml EHS laminin (or other chosen substrate). Neurons are gently centrifuged onto the dish and incubated at 37° in a 5% CO₂ atmosphere (Varnum-Finney and Reichardt, 1994; Wehrle and Chiquet, 1990).

3.8. Chick ciliary neuron culture

Our laboratory has used procedures developed by others (Nishi and Berg, 1977, 1981; Weaver *et al.*, 1995). E7.5 (stages 31 to 33, Hamburger and Hamilton, 1992) chick ciliary ganglia are dissected manually, after which they are incubated in 0.1% trypsin in Ca^{2+} - Mg^{2+} -free PBS (CMF-PBS) and dispersed by trituration through a fire-polished pipette. Neurons are cultured on plates precoated with laminin (collagen or other ECM substrates can be substituted) in 50% Eagle's MEM with Earl's balanced salts and 50% Ham's F-12 plus 2% chick embryo extract.

3.9. Chick motor neuron culture

Motor neuron cultures are prepared from stage 19 embryos. The dorsal part of the embryo at the brachial level is dissected by removing the dorsal aorta and flanking mesenchyme. This fragment is washed in PBS, and incubated in pancreatin (Gibco, Grand Island NY) for 2 min. Embryos are then placed in cold L-15 medium containing 5 mg/ml bovine serum albumin (BSA) (Sigma, St. Louis MO), and neural tubes are isolated by dissection from remaining sclerotomal tissue. Dorsal and ventral neural tube fragments are separated with a glass needle. Ventral fragments are then placed in 0.25% trypsin for 5 min at 37°, are washed in L-15 containing 10 mg/ml BSA, centrifuged, and resuspended in complete motor-neuron growth medium (F12 medium containing 0.5 mg/ml BSA (Serva, Germany). Brain-derived neurotrophic factor (BDNF) (10 ng/ml) and 10 ng/ml bFGF (Boehringer Mannheim, Indianapolis IN). These ventral neural tube tissue fragments are then gently triturated four times. Dissociated cells are centrifuged, resuspended in growth medium to an appropriate density (1×10^3 cells/well), plated in 100 μl growth medium, and incubated at 37° with 5% CO_2 on appropriate ECM substrates, such as laminin-coated or laminin and poly-D-lysine-coated substrata (Varnum-Finney and Reichardt, 1994; Wehrle and Chiquet, 1990).

3.10. Chick retinal neuron culture

Retinas are dissected from E6 or E12 chick embryos and are incubated for 6 min at 37° in 0.1% trypsin (in Ca^{2+} - Mg^{2+} -free PBS [CMF-PBS]). Digestion is stopped by adding 0.2 volumes of heat-inactivated fetal calf serum. Pellets are washed once in F12 nutrient mixture, and triturated in F12 containing 0.002% DNase I. For cell attachment assays, Linbro/Titer plates (Flow Laboratories, Inc., Maclean, VA) are coated overnight with 20 $\mu\text{g}/\text{ml}$ of LN or collagen IV in CMF-PBS. Coated and uncoated wells are incubated for 2 h at room temperature with 1% BSA in CMF-PBS. Wells are washed twice with CMF-PBS, and about 100,000 retinal cells were added to each well, after preincubation in a sterile tube for 20 min at room temperature in F12 medium with additives (5 mg/ml insulin, 30 nM selenium, 25 mg/ml human transferrin, 100 U/ml penicillin and streptomycin, according to [Bottenstein *et al.*, 1980]). Cells are sedimented to the bottom of wells by centrifugation, and incubated for 1 h at 37° in 5% CO_2 atmosphere in the same medium used during the preincubation. Unattached cells are removed by the brisk addition of warm medium followed by gentle vacuum suction. For neurite outgrowth assays, cells are cultured at 37° in a 5% CO_2 atmosphere, and cultures are examined in the microscope for neurite outgrowth after overnight or longer incubation (de Curtis and Reichardt, 1993; Neugebauer *et al.*, 1991).

4. Biochemical Studies Using Cultured Neurons

Purified neuronal populations are typically present in limited quantities, so sensitive assays are required to detect integrins and other proteins. It is absolutely essential to assess the purity of a culture before making conclusions about cell type-specific expression of integrins or other proteins because non-neuronal cells (both astroglia and endothelial cells that contaminate CNS cultures and Schwann cells and fibroblasts, contaminants of peripheral neuronal cultures) express many integrins. Ideally, neurons should be purified by dye labeling, antibody panning,

or other procedures for biochemical assays (Barres and Raff, 1999; Christopherson *et al.*, 2005; Meyer-Franke *et al.*, 1998).

4.1. Surface labeling of integrins and other neuronal glycoproteins (used for DRG and ciliary ganglion neurons)

4.1.1. ^{125}I labeling—Dissociated neurons are cultured for 24 h on 100 mm² of Falcon tissue culture dishes previously coated with a purified ECM substrate dissolved in PBS. After removal of growth medium and washing with PBS, cultures are surface labeled by adding lactoperoxidase, H₂O₂ and ^{125}I in PBS for 15 min (Tomaselli *et al.*, 1993). Lysates are prepared by adding 1.2 ml of lysis buffer containing 1% Triton-X-100 in PBS and protease inhibitors to the cultures. Lysed material is then scraped from the dish with a cell scraper and centrifuged at 20,000×g for 20 min at 4°. After preclearing twice with 100 µl of protein A-Sepharose, supernatants are incubated for 4 h with specific antibodies coupled to protein A-Sepharose (50 µl beads per culture, Pierce, Rockford IL). Antibodies are coupled to protein A-Sepharose with dimethylpimelimidate using the standard procedure (Harlow and Lane, 1988). Beads are then washed six times by adding 1.0 ml of 1% Triton-X-100 in PBS and pelleting beads at 134×g for 10 sec. Immunoprecipitated proteins are then separated with 6% SDS-PAGE in nonreducing conditions. Gels are dried and exposed to Kodak X Omat R film or are alternatively scanned in a molecular imager (Fujii, Molecular Dynamics).

4.1.2. Biotin labeling—At this juncture, 0.8 mg of sulfo-NHS-biotin (#21217, Pierce, Rockford, IL) is added and rocked with the cells at 4° for 1 h. Cells are then washed three times in TBS and extracted in RIPA (0.01 M Tris-Cl, pH 7.2, 0.15 M NaCl, 1% Na-deoxycholate, 1% Triton X-100, 0.1% SDS, 1% aprotinin, 4 mM PMSF and 10 µg/ml each antipain, leupeptin, pepstatin, and chymostatin) for 1 h at 4°. The extracts are centrifuged at 9170×g for 15 min and DNase is added to the soluble fraction at a final concentration of 100 ng/ml (Weaver *et al.*, 1995).

For immunoprecipitations, all steps are carried out at 4°. Extracts are precleared once with Sepharose CL-4B followed by protein A-Sepharose (each 50 µl/lane for 45 min), and then incubated overnight at 4° with the appropriate antibody coupled directly to protein A-Sepharose (Harlow and Lane, 1988). Beads from all precipitation steps are collected, washed three times each in RIPA, two times in TBS with 0.5% Tween-20, and once in TBS with 0.5% Tween-20 and 0.1% ovalbumin. Precipitates are boiled in nonreducing sample buffer, subjected to SDS-PAGE, and transferred to nitrocellulose (Schleicher and Schuell, Keene, NH). Transfer membranes are blocked for 1 h at room temperature in PBS with 10% BSA, 0.05% Tween-20, then incubated in PBS with 1% BSA, 0.05% Tween-20 (reaction buffer) with streptavidin-HRP (1:4000; Zymed, South San Francisco, CA) for 1 h at room temperature. After a brief rinse in TBS, the transfers are washed twice in 1% Triton X-100, 0.1% SDS, 0.5% Na-deoxycholate in TBS, for 5 min each, and then twice more in TBS. The transfers are then processed for chemiluminescent detection of HRP reaction product according to the manufacturer's specifications.

4.2. Immunocytochemistry

Cultured neurons are extremely fragile on coverslips. Standard procedures and antibodies can be used to visualize integrins and other proteins, but it is critical that the coverslips be handled delicately and not subjected to shear forces during application or removal of reagents. In our laboratory, coverslips are not dipped into reagents, but reagents are added gently to coverslips in horizontal position. Reagents are removed as slowly as possible during washes and coverslips are always left under liquid. Coverslips are carefully transferred to a humidified staining chamber. Cells are fixed with 4% paraformaldehyde for 10 min, washed with PBS, and are then incubated with PBS containing 5% horse or donkey serum and 0.2% Triton-X-100

for 10 min. Coverslips are then incubated overnight at 4° with either a monoclonal antibody or polyclonal antibody in PBS containing 5% horse or donkey serum. Coverslips are then washed with PBS and coverslips previously treated with a monoclonal antibody are incubated with biotinylated horse anti-mouse IgG diluted at 1:200 for 30 min. The coverslips are then washed in PBS and incubated for 30 min with fluorescein-labeled streptavidin-fluorescein diluted at 1:200. Coverslips previously treated with a polyclonal antibody are washed and incubated for 30 min with Texas-red labeled donkey anti-rabbit. Coverslips are mounted on slides with gelvatol and viewed and photographed with a Nikon photomicroscope, using the appropriate filters.

REFERENCES

- Austin SA, Floden AM, Murphy EJ, Combs CK. Alpha-synuclein expression modulates microglial activation phenotype. *J. Neurosci* 2006;26:10558–10563. [PubMed: 17035541]
- Barberis D, Artigiani S, Casazza A, Corso S, Giordano S, Love CA, Jones EY, Comoglio PM, Tamagnone L. Plexin signaling hampers integrin-based adhesion, leading to Rho-kinase independent cell rounding, and inhibiting lamellipodia extension and cell motility. *FASEB J* 2004;18:592–594. [PubMed: 14734633]
- Barres BA, Raff MC. Axonal control of oligodendrocyte development. *J. Cell Biol* 1999;147:1123–1128. [PubMed: 10601327]
- Bartt RE. Multiple sclerosis, natalizumab therapy, and progressive multifocal leukoencephalopathy. *Curr. Opin. Neurol* 2006;19:341–349. [PubMed: 16914971]
- Beggs HE, Schahin-Reed D, Zang K, Goebbels S, Nave KA, Gorski J, Jones KR, Sretavan D, Reichardt LF. FAK deficiency in cells contributing to the basal lamina results in cortical abnormalities resembling congenital muscular dystrophies. *Neuron* 2003;40:501–514. [PubMed: 14642275]
- Benninger Y, Colognato H, Thurnherr T, Franklin RJ, Leone DP, Atanasoski S, Nave KA, Ffrench-Constant C, Suter U, Relvas JB. Beta1-integrin signaling mediates premyelinating oligodendrocyte survival but is not required for CNS myelination and remyelination. *J. Neurosci* 2006;26:7665–7673. [PubMed: 16855094]
- Blackmore M, Letourneau PC. L1, beta1 integrin, and cadherins mediate axonal regeneration in the embryonic spinal cord. *J. Neurobiol* 2006;66:1564–1583. [PubMed: 17058193]
- Blaess S, Graus-Porta D, Belvindrah R, Radakovits R, Pons S, Littlewood-Evans A, Senften M, Guo H, Li Y, Miner JH, Reichardt LF, Muller U. Beta1-integrins are critical for cerebellar granule cell precursor proliferation. *J. Neurosci* 2004;24:3402–3412. [PubMed: 15056720]
- Bottenstein JE, Skaper SD, Varon SS, Sato GH. Selective survival of neurons from chick embryo sensory ganglionic dissociates utilizing serum-free supplemented medium. *Exp. Cell Res* 1980;125:183–190. [PubMed: 7351213]
- Bozzo C, Lombardi G, Santoro C, Canonico PL. Involvement of beta(1) integrin in betaAP-induced apoptosis in human neuroblastoma cells. *Mol. Cell Neurosci* 2004;25:1–8. [PubMed: 14962735]
- Bronner-Fraser M. Neural crest cell formation and migration in the developing embryo. *FASEB J* 1994;8:699–706. [PubMed: 8050668]
- Buchman VL, Davies AM. Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. *Development* 1993;118:989–1001. [PubMed: 8076530]
- Burgess RW, Dickman DK, Nunez L, Glass DJ, Sanes JR. Mapping sites responsible for interactions of agrin with neurons. *J. Neurochem* 2002;83:271–284. [PubMed: 12423238]
- Burkin DJ, Kim JE, Gu M, Kaufman SJ. Laminin and alpha7beta1 integrin regulate agrin-induced clustering of acetylcholine receptors. *J. Cell Sci* 2000;113:2877–2886. [PubMed: 10910772]
- Campos LS, Leone DP, Relvas JB, Brakebusch C, Fassler R, Suter U, Ffrench-Constant C. Beta1 integrins activate a MAPK signalling pathway in neural stem cells that contributes to their maintenance. *Development* 2004;131:3433–3444. [PubMed: 15226259]
- Chan CS, Weeber EJ, Kurup S, Sweatt JD, Davis RL. Integrin requirement for hippocampal synaptic plasticity and spatial memory. *J. Neurosci* 2003;23:7107–7116. [PubMed: 12904471]

- Chan CS, Weeber EJ, Zong L, Fuchs E, Sweatt JD, Davis RL. Beta 1-integrins are required for hippocampal AMPA receptor-dependent synaptic transmission, synaptic plasticity, and working memory. *J. Neurosci* 2006;26:223–232. [PubMed: 16399691]
- Christopherson KS, Ullian EM, Stokes CC, Mullen CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* 2005;120:421–433. [PubMed: 15707899]
- Clegg DO, Wingerd KL, Hikita ST, Tolhurst EC. Integrins in the development, function and dysfunction of the nervous system. *Front. Biosci* 2003;8:d723–d750. [PubMed: 12700040]
- Colognato H, Baron W, Avellana-Adalid V, Relvas JB, Baron-Van Evercooren A, Georges-Labouesse E, Ffrench-Constant C. CNS integrins switch growth factor signalling to promote target-dependent survival. *Nat. Cell Biol* 2002;4:833–841. [PubMed: 12379866]
- Colognato H, Ramachandrapa S, Olsen IM, Ffrench-Constant C. Integrins direct Src family kinases to regulate distinct phases of oligodendrocyte development. *J. Cell Biol* 2004;167:365–375. [PubMed: 15504915]
- Condic ML. Adult neuronal regeneration induced by transgenic integrin expression. *J. Neurosci* 2001;21:4782–4478. [PubMed: 11425905]
- Davy A, Robbins SM. Ephrin-A5 modulates cell adhesion and morphology in an integrin-dependent manner. *EMBO J* 2000;19:5396–5405. [PubMed: 11032807]
- de Curtis I, Reichardt LF. Function and spatial distribution in developing chick retina of the laminin receptor alpha 6 beta 1 and its isoforms. *Development* 1993;118:377–388. [PubMed: 8223267]
- DeFreitas MF, Yoshida CK, Frazier WA, Mendrick DL, Kypta RM, Reichardt LF. Identification of integrin alpha 3 beta 1 as a neuronal thrombospondin receptor mediating neurite outgrowth. *Neuron* 1995;15:333–343. [PubMed: 7544141]
- Dubey N, Letourneau PC, Tranquillo RT. Guided neurite elongation and schwann cell invasion into magnetically aligned collagen in simulated peripheral nerve regeneration. *Exp. Neurol* 1999;158:338–350. [PubMed: 10415141]
- Ekstrom PA, Mayer U, Panjwani A, Pountney D, Pizzey J, Tonge DA. Involvement of alpha7beta1 integrin in the conditioning-lesion effect on sensory axon regeneration. *Mol. Cell Neurosci* 2003;22:383–395. [PubMed: 12691739]
- Elia LP, Yamamoto M, Zang K, Reichardt LF. p120 catenin regulates dendritic spine and synapse development through Rho-family GTPases and cadherins. *Neuron* 2006;51:43–56. [PubMed: 16815331]
- Feltri ML, Graus Porta D, Previtali SC, Nodari A, Miglia vacca B, Casseti A, Littlewood-Evans A, Reichardt LF, Messing A, Quattrini A, Mueller U, Wrabetz L. Conditional disruption of beta 1 integrin in Schwann cells impedes interactions with axons. *J. Cell Biol* 2002;156:199–209. [PubMed: 11777940]
- Forster E, Tielsch A, Saum B, Weiss KH, Johanssen C, Graus-Porta D, Muller U, Frotscher M. Reelin, disabled 1, and beta 1 integrins are required for the formation of the radial glial scaffold in the hippocampus. *Proc. Natl. Acad. Sci. USA* 2002;99:13178–13183. [PubMed: 12244214]
- Gleeson JG, Walsh CA. Neuronal migration disorders: From genetic diseases to developmental mechanisms. *Trends Neurosci* 2000;23:352–359. [PubMed: 10906798]
- Goldberg JL, Espinosa JS, Xu Y, Davidson N, Kovacs GT, Barres BA. Retinal ganglion cells do not extend axons by default: Promotion by neurotrophic signaling and electrical activity. *Neuron* 2002;33:689–702. [PubMed: 11879647]
- Graus-Porta D, Blaess S, Senften M, Littlewood-Evans A, Damsky C, Huang Z, Orban P, Klein R, Schittny JC, Muller U. Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. *Neuron* 2001;31:367–379. [PubMed: 11516395]
- Grotewiel MS, Beck CD, Wu KH, Zhu XR, Davis RL. Integrin-mediated short-term memory in *Drosophila*. *Nature* 1998;391:455–460. [PubMed: 9461212]
- Haack H, Hynes RO. Integrin receptors are required for cell survival and proliferation during development of the peripheral glial lineage. *Dev. Biol* 2001;233:38–55. [PubMed: 11319856]
- Hall DE, Neugebauer KM, Reichardt LF. Embryonic neural retinal cell response to extracellular matrix proteins: Developmental changes and effects of the cell substratum attachment antibody (CSAT). *J. Cell Biol* 1987;104:623–634. [PubMed: 3493247]

- Hama H, Hara C, Yamaguchi K, Miyawaki A. PKC signaling mediates global enhancement of excitatory synaptogenesis in neurons triggered by local contact with astrocytes. *Neuron* 2004;41:405–415. [PubMed: 14766179]
- Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. *Dev. Dyn* 1992;195:231–272. [PubMed: 1304821]
- Harlow, E.; Lane, D. *Antibodies: A Laboratory Manual*. Laboratory Press; Cold Spring Harbor: 1988.
- Hawrot E, Patterson PH. Long-term culture of dissociated sympathetic neurons. *Methods Enzymol* 1979;58:574–584. [PubMed: 423793]
- Huang EJ, Zang K, Schmidt A, Saulys A, Xiang M, Reichardt LF. POU domain factor Brn-3a controls the differentiation and survival of trigeminal neurons by regulating Trk receptor expression. *Development* 1999;126:2869–2882. [PubMed: 10357931]
- Huang Z, Shimazu K, Woo NH, Zang K, Muller U, Lu B, Reichardt LF. Distinct roles of the beta1-class integrins at the developing and the mature hippocampal excitatory synapse. *J. Neurosci* 2006;26:11208–11219. [PubMed: 17065460]
- Kanwar JR, Harrison JE, Wang D, Leung E, Mueller W, Wagner N, Krissansen GW. Beta7 integrins contribute to demyelinating disease of the central nervous system. *J. Neuroimmunol* 2000;103:146–152. [PubMed: 10696909]
- Kashani AH, Chen BM, Grinnell AD. Hypertonic enhancement of transmitter release from frog motor nerve terminals: Ca²⁺ independence and role of integrins. *J. Physiol* 2001;530:243–252. [PubMed: 11208972]
- Kil SH, Krull CE, Cann G, Clegg D, Bronner-Fraser M. The alpha4 subunit of integrin is important for neural crest cell migration. *Dev. Biol* 1998;202:29–42. [PubMed: 9758701]
- Kloss CU, Werner A, Klein MA, Shen J, Menuz K, Probst JC, Kreutzberg GW, Raivich G. Integrin family of cell adhesion molecules in the injured brain: Regulation and cellular localization in the normal and regenerating mouse facial motor nucleus. *J. Comp. Neurol* 1999;411:162–178. [PubMed: 10404114]
- Koenigsnecht J, Landreth G. Microglial phagocytosis of fibrillar beta-amyloid through a beta1 integrin-dependent mechanism. *J. Neurosci* 2004;24:9838–9846. [PubMed: 15525768]
- Kramar EA, Bernard JA, Gall CM, Lynch G. Integrins modulate fast excitatory transmission at hippocampal synapses. *J. Biol. Chem* 2003;278:10722–10730. [PubMed: 12524441]
- Kramar EA, Lin B, Rex CS, Gall CM, Lynch G. Integrin-driven actin polymerization consolidates long-term potentiation. *Proc. Natl. Acad. Sci. USA* 2006;103:5579–5584. [PubMed: 16567651]
- Lagenaur C, Lemmon V. An L1-like molecule, the 8D9 antigen, is a potent substrate for neurite extension. *Proc. Natl. Acad. Sci. USA* 1987;84:7753–7757. [PubMed: 3478724]
- Le Douarin NM, Dupin E. Multipotentiality of the neural crest. *Curr. Opin. Genet. Dev* 2003;13:529–536. [PubMed: 14550420]
- Leone DP, Relvas JB, Campos LS, Hemmi S, Brakebusch C, Fassler R, Ffrench-Constant C, Suter U. Regulation of neural progenitor proliferation and survival by beta1 integrins. *J. Cell Sci* 2005;118:2589–2599. [PubMed: 15928047]
- Li G, Pleasure SJ. Morphogenesis of the dentate gyrus: What we are learning from mouse mutants. *Dev. Neurosci* 2005;27:93–99. [PubMed: 16046842]
- Lin B, Arai AC, Lynch G, Gall CM. Integrins regulate NMDA receptor-mediated synaptic currents. *J. Neurophysiol* 2003;89:2874–2878. [PubMed: 12740418]
- Mattern RH, Read SB, Pierschbacher MD, Sze CI, Eliceiri BP, Kruse CA. Glioma cell integrin expression and their interactions with integrin antagonists. *Cancer Ther* 2005;3A:325–340. [PubMed: 16467916]
- McCarty JH, Lacy-Hulbert A, Charest A, Bronson RT, Crowley D, Housman D, Savill J, Roes J, Hynes RO. Selective ablation of alphaV integrins in the central nervous system leads to cerebral hemorrhage, seizures, axonal degeneration and premature death. *Development* 2005;132:165–176. [PubMed: 15576410]
- McKenna MP, Raper JA. Growth cone behavior on gradients of substratum bound laminin. *Dev. Biol* 1988;130:232–236. [PubMed: 3181628]
- Mechtersheimer S, Gutwein P, Agmon-Levin N, Stoeck A, Oleszewski M, Riedle S, Postina R, Fahrenholz F, Fogel M, Lemmon V, Altevogt P. Ectodomain shedding of L1 adhesion molecule

- promotes cell migration by autocrine binding to integrins. *J. Cell Biol* 2001;155:661–673. [PubMed: 11706054]
- Meyer-Franke A, Wilkinson GA, Kruttgen A, Hu M, Munro E, Hanson MG Jr, Reichardt LF, Barres BA. Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. *Neuron* 1998;21:681–693. [PubMed: 9808456]
- Mi H, Barres BA. Purification and characterization of astrocyte precursor cells in the developing rat optic nerve. *J. Neurosci* 1999;19:1049–1661. [PubMed: 9920668]
- Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H, Sheppard D, Broaddus VC, Nishimura SL. The integrin alpha(v) beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1. *J. Cell Biol* 2002;157:493–507. [PubMed: 11970960]
- Nakada M, Niska JA, Tran NL, McDonough WS, Berens ME. EphB2/R-Ras signaling regulates glioma cell adhesion, growth, and invasion. *Am. J. Pathol* 2005;167:565–576. [PubMed: 16049340]
- Neugebauer KM, Emmett CJ, Venstrom KA, Reichardt LF. Vitronectin and thrombospondin promote retinal neurite outgrowth: Developmental regulation and role of integrins. *Neuron* 1991;6:345–358. [PubMed: 1705807]
- Nishi R, Berg DK. Dissociated ciliary ganglion neurons *in vitro*: Survival and synapse formation. *Proc. Natl. Acad. Sci. USA* 1977;74:5171–5175. [PubMed: 270756]
- Nishi R, Berg DK. Two components from eye tissue that differentially stimulate the growth and development of ciliary ganglion neurons in cell culture. *J. Neurosci* 1981;1:505–513. [PubMed: 7346565]
- Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL. Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature* 2003;424:398–405. [PubMed: 12879062]
- Pietri T, Eder O, Breau MA, Topilko P, Blanche M, Brakebusch C, Fassler R, Thiery JP, Dufour S. Conditional beta1-integrin gene deletion in neural crest cells causes severe developmental alterations of the peripheral nervous system. *Development* 2004;131:3871–3883. [PubMed: 15253938]
- Pinkstaff JK, Detterich J, Lynch G, Gall C. Integrin subunit gene expression is regionally differentiated in adult brain. *J. Neurosci* 1999;19:1541–1556. [PubMed: 10024342]
- Pinkstaff JK, Lynch G, Gall CM. Localization and seizure-regulation of integrin beta 1 mRNA in adult rat brain. *Brain Res. Mol. Brain Res* 1998;55:265–276. [PubMed: 9582434]
- Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A, Toal M, Lynn F, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N. Engl. J. Med* 2006;354:899–910. [PubMed: 16510744]
- Proctor JM, Zang K, Wang D, Wang R, Reichardt LF. Vascular development of the brain requires beta8 integrin expression in the neuroepithelium. *J. Neurosci* 2005;25:9940–9948. [PubMed: 16251442]
- Reichardt LF, Tomaselli KJ. Extracellular matrix molecules and their receptors: Functions in neural development. *Annu. Rev. Neurosci* 1991;14:531–570. [PubMed: 1851608]
- Rico B, Beggs HE, Schahin-Reed D, Kimes N, Schmidt A, Reichardt LF. Control of axonal branching and synapse formation by focal adhesion kinase. *Nat. Neurosci* 2004;7:1059–1069. [PubMed: 15378065]
- Sanada K, Gupta A, Tsai LH. Disabled-1-regulated adhesion of migrating neurons to radial glial fiber contributes to neuronal positioning during early corticogenesis. *Neuron* 2004;42:197–211. [PubMed: 15091337]
- Schmid RS, Jo R, Shelton S, Kreidberg JA, Anton ES. Reelin, integrin and DAB1 interactions during embryonic cerebral cortical development. *Cereb. Cortex* 2005;15:1632–1636. [PubMed: 15703255]
- Schwander M, Shirasaki R, Pfaff SL, Muller U. Beta1 integrins in muscle, but not in motor neurons, are required for skeletal muscle innervation. *J. Neurosci* 2004;24:8181–8191. [PubMed: 15371519]
- Sondag CM, Combs CK. Amyloid precursor protein cross-linking stimulates beta amyloid production and pro-inflammatory cytokine release in monocytic lineage cells. *J. Neurochem* 2006;97:449–461. [PubMed: 16539666]
- Stevens A, Jacobs JR. Integrins regulate responsiveness to slit repellent signals. *J. Neurosci* 2002;22:4448–4455. [PubMed: 12040052]
- Tomaselli KJ, Doherty P, Emmett CJ, Damsky CH, Walsh FS, Reichardt LF. Expression of beta 1 integrins in sensory neurons of the dorsal root ganglion and their functions in neurite outgrowth on two laminin isoforms. *J. Neurosci* 1993;13:4880–4888. [PubMed: 7693896]

- Tomaselli KJ, Hall DE, Flier LA, Gehlsen KR, Turner DC, Carbonetto S, Reichardt LF. A neuronal cell line (PC12) expresses two beta 1-class integrins—alpha 1 beta 1 and alpha 3 beta 1—that recognize different neurite outgrowth-promoting domains in laminin. *Neuron* 1990;5:651–662. [PubMed: 2223092]
- Tucker RP. Antisense knockdown of the beta1 integrin subunit in the chicken embryo results in abnormal neural crest cell development. *Int. J. Biochem. Cell Biol* 2004;36:1135–1139. [PubMed: 15094128]
- Ullian EM, Christopherson KS, Barres BA. Role for glia in synaptogenesis. *Glia* 2004;47:209–216. [PubMed: 15252809]
- Varnum-Finney B, Reichardt LF. Vinculin-deficient PC12 cell lines extend unstable lamellipodia and filopodia and have a reduced rate of neurite outgrowth. *J. Cell Biol* 1994;127:1071–1084. [PubMed: 7962069]
- Varnum-Finney B, Venstrom K, Muller U, Kypta R, Backus C, Chiquet M, Reichardt LF. The integrin receptor alpha 8 beta 1 mediates interactions of embryonic chick motor and sensory neurons with tenascin-C. *Neuron* 1995;14:1213–1222. [PubMed: 7541634]
- Verdier Y, Penke B. Binding sites of amyloid beta-peptide in cell plasma membrane and implications for Alzheimer's disease. *Curr. Protein Pept. Sci* 2004;5:19–31. [PubMed: 14965318]
- Wallquist W, Zelano J, Plantman S, Kaufman SJ, Cullheim S, Hammarberg H. Dorsal root ganglion neurons up-regulate the expression of laminin-associated integrins after peripheral but not central axotomy. *J. Comp. Neurol* 2004;480:162–169. [PubMed: 15514929]
- Wang AG, Yen MY, Hsu WM, Fann MJ. Induction of vitronectin and integrin alphav in the retina after optic nerve injury. *Mol. Vis* 2006;12:76–84. [PubMed: 16479252]
- Weaver CD, Yoshida CK, de Curtis I, Reichardt LF. Expression and *in vitro* function of beta 1–integrin laminin receptors in the developing avian ciliary ganglion. *J. Neurosci* 1995;15:5275–5285. [PubMed: 7542700]
- Wehrle B, Chiquet M. Tenascin is accumulated along developing peripheral nerves and allows neurite outgrowth *in vitro*. *Development* 1990;110:401–415. [PubMed: 1723942]
- Wright S, Malinin NL, Powell KA, Yednock T, Rydel RE, Griswold-Prenner I. alpha2beta1 and alphaVbeta1 integrin signaling pathways mediate amyloid-beta-induced neurotoxicity. *Neurobiol. Aging*. January 18;2006
- Xie C, Markesbery WR, Lovell MA. Survival of hippocampal and cortical neurons in a mixture of MEM + and B27-supplemented neurobasal medium. *Free Radic. Biol. Med* 2000;28:665–672. [PubMed: 10754261]
- Yang P, Baker KA, Hagg T. The ADAMs family: Coordinators of nervous system development, plasticity and repair. *Prog. Neurobiol* 2006;79:73–94. [PubMed: 16824663]
- Yebra M, Montgomery AM, Diaferia GR, Kaido T, Silletti S, Perez B, Just ML, Hildbrand S, Hurford R, Florkiewicz E, Tessier-Lavigne M, Cirulli V. Recognition of the neural chemoattractant netrin-1 by integrins alpha6beta4 and alpha3beta1 regulates epithelial cell adhesion and migration. *Dev. Cell* 2003;5:695–707. [PubMed: 14602071]