Activity-Dependent Regulation of N-cadherin in DRG Neurons: Differential Regulation of N-cadherin, NCAM, and L1 by Distinct Patterns of Action Potentials

Kouichi Itoh,* Miwako Ozaki,** Beth Stevens, R. Douglas Fields

Unit on Neurocytology and Physiology, Laboratory of Developmental Neurobiology, UNP, National Institutes of Health, NICHD, Building 49, Room 5A38, 49 Convent Drive, Bethesda, Maryland 20892

Received 28 April 1997; accepted 18 June 1997

ABSTRACT: Cell adhesion molecule (CAM) expression is highly regulated during nervous system development to control cell migration, neurite outgrowth, fasciculation, and synaptogenesis. Using electrical stimulation of mouse dorsal root ganglion (DRG) neurons in cell culture, this work shows that N-cadherin expression is regulated by neuronal firing, and that expression of different CAMs is regulated by distinct patterns of neural impulses. N-cadherin was down-regulated by 0.1 or 1 Hz stimulation, but NCAM mRNA and protein levels were not altered by stimulation. L1 was down-regulated by 0.1 Hz stimulation, but not by 0.3 Hz, 1 Hz, or pulsed stimulation. N-cadherin expression was lowered with faster kinetics

than L1 (1 vs. 5 days), and L1 mRNA returned to higher levels after terminating the stimulus. The RSLE splice variant of L1 was not regulated by action potential stimulation, and activity-dependent influences on L1 expression were blocked by target-derived influences. The results are consistent with changes in firing pattern accompanying DRG development and suggest that functional activity can influence distinct developmental processes by regulating the relative abundance of different CAMs. © 1997 John Wiley & Sons, Inc.* *J Neurobiol* 33: 735–748, 1997

Keywords: cell adhesion molecules; synaptogenesis; fasciculation; neurite outgrowth; activity-dependent plasticity; DRG; N-cadherin; L1; NCAM

INTRODUCTION

In higher vertebrates, the interplay between genetically predetermined and experientially regulated influences on nervous system development is essential for normal development of many brain structures and functions (Fields and Nelson, 1992). The pres-

ent experiments investigate a specific molecular mechanism that could help mediate this process: whether specific patterns of neural impulses can regulate the mixture of different cell adhesion molecules (CAMs) expressed in neurons.

The spatial and temporal expression of specific neural cell adhesion molecules is regulated precisely during development and regeneration of the nervous system. By binding to appropriate CAMs expressed selectively on different neurons, CAMs regulate cell recognition, aggregation, motility, neurite outgrowth, fasciculation, and synaptogenesis, some of the major morphogenic processes in nervous system development (Fields and Itoh, 1996; Goodman, 1996). CAMs produce these effects through a wide range of cell biological mechanisms, including cytoskeletal interactions, intracellular signaling, association with extracellular matrix, and receptor mole-

* Present address: Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113, Japan

** Present address: Laboratory of Cellular Information Processing, Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-shi, Saitama 351-01, Japan

Correspondence to: R. D. Fields

© 1997 John Wiley & Sons, Inc. *This is a US Government work and, as such, is in the public domain in the United States of America. CCC 0022-3034/97/060735-14

cules (Von Bohlen et al., 1992; Schachner, 1992; Gumbiner, 1993). Cell adhesion molecules are broadly divided into two functional classes: calcium-dependent and calcium-independent (Edelman and Crossin, 1991). The present experiments test members of both these families, including immunoglobulin (Ig) superfamily members NCAM and L1, and the calcium-dependent CAM N-cadherin, to determine whether action potentials applied at specific frequencies can regulate the mixture of CAMs expressed in mouse dorsal root ganglion (DRG) neurons in culture.

The class of calcium-dependent CAMs include the cadherins, which bind homophilically (Takeichi, 1990), and integrins, which bind to extracellular matrix proteins (McKerracher et al., 1996). The various cadherins have a highly conserved cytoplasmic region that can interact with ankyrin and other components of the cytoskeleton, and five structurally repeated extracellular domains containing multiple putative calcium-binding motifs. In addition to their adhesive function, cadherins activate intracellular signaling cascades by interacting with catenins and other signaling molecules (Nathke et al., 1995). A specific form of cadherin, N-cadherin, is expressed in neural tissue and muscle, where it participates in selective attachment of neurites to particular cell types and promotes neurite outgrowth (Walsh et al., 1994; Burden-Gully and Lemmon, 1995). Cadherin expression is down-regulated during periods of cell migration (Nakagawa and Takeichi, 1995; Barami et al., 1994; Redies, 1995) and myelination (Cifuentes-Diaz et al., 1994; Shibuya et al., 1995; Scherer, 1996) and increased during peripheral nerve regeneration (Shibuya et al., 1995; Ide, 1996; Hasegawa et al., 1996), and levels change in association with various nervous system diseases (Lagunowich et al., 1994). The normal decrease in N-cadherin in developing myotubes (Hahn and Covault, 1992) can be prevented by blocking activity with *d*-tubocurarine in cell culture (Fredette et al., 1993), but it is not known whether action potential stimulation of controlled frequencies can regulate expression of N-cadherin in neurons.

Chemical depolarization provides support for activity-dependent regulation of members of the Ig superfamily of CAMs, but results vary for different CAMs and different cell types. Members of the Ig superfamily, which include NCAM and L1, are involved in neurite outgrowth (Appel et al., 1995), axonal pathfinding (Sretavan et al., 1994; Honig and Rutishauser, 1996), fasciculation (Magyar-Lehmann et al., 1995; Honig and Rutishauser, 1996), myelination (Seilheimer et al., 1989; Wood

et al., 1990), synaptogenesis (Jorgensen, 1995), and synaptic plasticity in vertebrates (Muller et al., 1996) and invertebrates (Peter et al., 1994; Davis et al., 1996; Schuster et al., 1996a,b). Chemical depolarization increases L1 expression in N2a or cerebellar cells in culture without effects on NCAM, but no change is induced in PC12 cells (Scherer et al., 1992). Conversely, chemical depolarization decreases expression of apCAM (invertebrate homologue of NCAM) in *Aplysia*. This induces defasciculation, which is thought to promote synaptogenesis to sustain facilitation of the gill withdrawal reflex (Mayford et al., 1992). We have shown previously that expression of L1 is down-regulated by specific frequencies of electrical impulses in mouse DRG neurons *in vitro* (Itoh et al., 1995).

The paradox that CAMs can promote such diverse and antagonistic processes as cell adhesion/stabilization versus motility/neurite outgrowth is explained by changes in the mixture of multiple CAMs expressed in cell membranes in particular developmental and functional contexts. For example, postmitotic neurons in the songbird forebrain undergo decreased N-cadherin expression relative to Ng-CAM (the avian L1 homologue) expression prior to cell migration from the subventricular zone (Barami et al., 1994). Therefore, it is important to analyze multiple CAMs simultaneously to address how neural impulse activity of particular patterns may regulate specific developmental processes in association with changes in functional activity of the developing nervous system.

MATERIALS AND METHODS

Cell Culture and *In Vitro* Electrical Stimulation

Dorsal root ganglia were dissected from fetal mice at E13.5 days and neurons were dissociated with 0.125% trypsin and maintained in medium containing 5% heat-inactivated horse serum, supplemented with 50 ng/mL NGF according to previously published methods (Fields et al., 1990). Mitosis of nonneuronal cells was inhibited by the addition of 50 μ M FUDR 2 days after plating, and half the volume of culture medium was replaced at 2-day intervals.

Electrical stimulation was provided by field electrodes in multicompartment chambers as described previously (Fields et al., 1992). DRG neurons (50,000 cells/side compartment) were plated in the two side compartments and used 3–4 weeks after plating. The day before stimulation, cultures were washed three times in medium lacking added NGF to eliminate the influence of NGF on L1 expression (Itoh et al., 1995). Neurons were then stimu-

lated for 1–5 days with constant voltage stimulation as described previously (Fields et al., 1992).

Reverse-Transcription Polymerase Chain Reaction

Total cellular RNA was extracted from equal amounts of cultured cells or tissues by the method of Chomcynski and Sacchi (1987). The RNA (0.1–0.5 μ g) was reverse-transcribed by Superscript RNase H-reverse transcriptase (Gibco/BRL) and random primers for 1 h at 37°C and then heated for 10 min at 95°C to stop the reaction. A 10% portion of the synthesized cDNA was amplified in a total volume of 15 μ L with 0.5 U AmpliTaq DNA polymerase (Perkin-Elmer, Branchburg, NJ) in the presence of 0.25 mM dNTP, 1 mM $MgCl_2$, and 25 pmol each of specific primers corresponding to the nucleotides of the extracellular domain 185–216 and 556–577, and intracellular domain 3476–3499 and 3611–3587, respectively of the mouse L1 cDNA sequence of Moos et al. (1988), or corresponding to the nucleotides of mouse NCAM of Satoni et al. (1989), 486–505 and 804–823 of the mouse N-cadherin (NCAD) cDNA (Miyatani et al., 1989), 586–605 and 1018–1037 of the glyceraldehyde 3-phosphate dehydrogenase (GPDH) cDNA (Arcari et al., 1984), or 835–855 and 1368–1348 of the rat neuronal-specific enolase (NSE) cDNA (Sakimura et al., 1985). In pilot experiments, conditions were established in which the amount of PCR products increased linearly with increasing amounts of cDNA and increasing number of cycles (24–34). Reaction mixtures were amplified for 27 cycles, each cycle consisted of 60 s denaturation at 94°C, 60 s annealing at 56°C (for NCAD), 57°C (for L1), 59°C (NCAM), or 60°C (for NSE and GPDH), and 120 s polymerization at 72°C. Ten microliters of PCR products was analyzed on 4% NuSieve 3:1 agarose or 6% polyacrylamide gel and visualized with ultraviolet (UV) illumination after ethidium bromide staining.

Semiquantitative and Competitive PCR

An L1 competitor (225 bases) was constructed from a nonhomologous DNA fragment (*Bam*H1/*Eco*R1 fragment of *v-erbB*) using PCR technique according to the manufacture protocol (Clontech, Palo Alto, CA) for use as an internal standard and for quantitative PCR. For quantitative competitive PCR, 10-fold serial dilutions of the L1 competitor (10^{-4} to 10^{-10} atm) were coamplified with a constant amount of the L1 cDNA reverse transcribed from total RNA using L1-specific primer as described above. PCR products were separated by electrophoresis in 6% polyacrylamide gels, stained with ethidium bromide, and quantified by imaging densitometry (Universal Imaging, West Chester, PA). Expression of L1, NCAM, and N-cadherin was normalized to the expression of neuron-specific enolase (NSE) or GPDH coamplified with the CAM-specific primers. NSE is a molecular marker for differentiated neurons (Zomzely-Neurath, 1983). Stimulation did not influence expression

of NSE or GPDH mRNA. Normalization to NSE expression corrected for variation in density of cell plating and PCR efficiency.

Immunoblot

Cultured DRG neurons were lysed with 0.25 mL of lysate buffer: PBS containing 1% NP-40, 0.5% deoxycholate, 2 mM EDTA, 10 μ g/mL soybean trypsin inhibitor, 10 μ g/mL eggwhite trypsin inhibitor, 0.5 mM AEBSF, 1.0 mM benzamidine, 0.1 mM leupeptin, 10 μ g/mL pepstatin, and 1 μ g/mL aprotinin at pH 8.0 (Calbiochem, La Jolla, CA). The lysate was centrifuged for 20 min at $14,000 \times g$ at 4°C. The pellet was re-extracted and then the supernatant was combined. The protein concentration of each lysate was measured by BCA protein microassay reagent (Pierce, Rockford, IL). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed on 4–12% polyacrylamide gels. Proteins fractionated by SDS-PAGE were electrotransferred to Immobilon-P (Millipore, Bedford, MA) and processed for immunoblot analysis with anti-L1 (A6L1) or an anti-NCAM (Sigma, St. Louis MO) and anti-rabbit IgG-F(ab')₂ antibodies conjugated to horseradish peroxidase (Jackson Immuno-Research Lab., West Grove, PA). Results were quantified by imaging densitometry (Image 1).

Immunocytochemistry

After rinsing cultures with PBS to remove the culture medium, cells were fixed with 1% paraformaldehyde in PBS (pH 7.4) for 10–15 min. Cells were permeabilized for 5 min in ethanol at –20°C and incubated with 1% normal goat serum in PBS for 30 min at room temperature. Cultures were incubated with monoclonal antibody against neurofilament (RT97) for 16 h at 4°C and localized by indirect immunocytochemistry using biotinylated antibodies to mouse IgG-F(ab')₂ as described in the manufacture's protocol using Vectastain ABC method (Vector Lab., Burlingame, CA).

RESULTS

Influence of Electrical Stimulation and NGF on Survival of Cultured DRG Neurons

Several controls were performed to distinguish activity-dependent effects on gene expression from possible effects on neuronal survival. These cultures were composed primarily of large-type DRG neurons (>70%) as distinguished by computerized morphometry of cell diameters and staining with RT97 antibody against phosphorylated neurofilament, which is diagnostic for large-type DRG neurons (Lawson et al., 1984). Survival of large-type DRG neurons is not dependent on NGF at later stages of development

Histograms of DRG neuron diameters

Figure 1 Electrical stimulation at 0.1 or 1 Hz in the presence or absence of NGF does not induce death of DRG neurons, as indicated by size frequency histograms (A), mean cell body diameter (B), counts of DRG neurons (C), or the total protein content of cultures (D).

or in cell culture (Snider, 1994), but NGF is known to increase expression of L1 (Itoh et al., 1995). Therefore, to study effects of impulse activity on CAM expression independently from effects of neu-

rotrophins, cells were cultured in medium lacking added NGF during the 1–5-day period of electrical stimulation. Morphometric analysis, immunocytochemical staining, and total protein measurements

Figure 2 The influence of different frequencies of impulse activity on mRNA levels of three different cell adhesion molecules. (A) L1 gene expression was reduced by very-low-frequency stimulation (0.1 Hz) for 5 days, but was not affected by higher-frequency stimulation ($n = 34$ experiments). In contrast, NCAM expression (B) was not affected by any frequency of impulse activity ($n = 14$ experiments), and N-cadherin (NCAD) expression (C) was reduced progressively with increasing frequencies of action potentials ($n = 16$ experiments). Linear regression and 95% confidence intervals are shown for (B) and (C).

revealed no changes in cell survival after electrical stimulation at 0.1 or 1 Hz for 5 days in the presence or absence of 50 ng/mL NGF (Fig. 1). These results provide no basis for interpreting changes in CAM expression as a result of stimulus-induced selective death of DRG neurons.

Specific Frequencies of Action Potentials Regulate Expression of Different Cell Adhesion Molecules

Confirming previous reports (Itoh et al., 1995), electrical stimulation at 0.1 Hz down-regulated L1 mRNA significantly, but stimulation at 0.3 or 1 Hz was without measurable effect [Fig. 2(A)]. Quantitative analysis using competitive PCR showed that the magnitude of this down-regulation was nearly 13-fold from unstimulated levels (Itoh et al., 1995). In contrast, expression of NCAM mRNA was not altered significantly after 5 days of 0.1, 0.3, or 1 Hz stimulation [Fig. 2(B)]. N-cadherin mRNA was regulated by neural impulse activity, but with a different pattern of expression compared with L1 and NCAM. All frequencies of stimulation lowered levels of N-cadherin mRNA, in direct proportion to increasing stimulus frequency ($r^2 = 0.93$; $p < 0.01$) [Fig. 2(C)]. Thus, these three CAMs exhibit quite distinct patterns of activity-dependent regulation in response to different frequencies of impulse activity.

Time Course and Bidirectional Control of CAM Gene Expression

Determination of the time course of regulation and response after terminating the stimulus is necessary to interpret the biological significance of this activity-dependent gene regulation. Down-regulation of L1 mRNA by 0.1 Hz stimulation followed slow kinetics, with differences not becoming significant until 5 days of stimulation [Fig. 3(a,b)]. No change in NCAM mRNA was detected after 1, 3, or 5 days stimulation at 0.1 Hz [Fig. 3(c)]. In contrast to L1 and NCAM message, N-cadherin was down-regulated rapidly (<1 day stimulation), and greater down-regulation was produced by higher-frequency stimulation (1 Hz) [Fig. 3(d)], a stimulus frequency that did not affect L1 or NCAM mRNA. No change in neuron-specific enolase mRNA was associated with stimulation for up to 5 days [Fig. 3(e)], indicating no decrease in neuron survival.

The level of L1 mRNA increased to nearly 50% of normal by 3 days after stopping the 5-day stimulus (Fig. 4). This recovery shows conclusively that these activity-dependent effects are due to regulation of L1 mRNA expression in DRG neurons, rather than to stimulus-induced death of a subpopulation of cells having higher levels of L1 message. Moreover, impulse activity does not induce an irreversible change in expression of the L1 gene.

Figure 3 Different cell adhesion molecules are regulated with distinct time courses by 0.1 Hz (triangles) or 1 Hz (squares) impulse activity compared to unstimulated controls (circles). Levels of L1 mRNA were not lowered significantly until 5 days of stimulation at 0.1 Hz measured by semiquantitative RT-PCR (a) or by competitive PCR (b) ($n = 20$ experiments). Levels of NCAM mRNA were not affected by stimulation for 3–5 days at 0.1 or 1 Hz (c) ($n = 25$ experiments). N-cadherin mRNA was lowered in <1 day by 0.1 Hz stimulation, with a significantly greater decrease induced by 1 Hz stimulation (d) ($n = 15$ experiments). Expression of neuron-specific enolase (NSE) mRNA remained constant throughout the experiments, indicating no detectable decrease in neuron survival during the stimulus period (e) ($n = 29$ experiments). Analysis in (a,c–e) is based on densitometry of the PCR product after electrophoresis and is expressed as a ratio of neuron-specific enolase (NSE), to normalize for variation in cell plating density and PCR efficiency. Competitive PCR analysis (b) was performed using 10-fold serial dilutions of an L1 mimic cDNA, which showed a 12.7 ± 1.46 -fold reduction in L1 mRNA levels ($p < 0.001$, 5-day 0.1-Hz stimulation vs. controls; $n = 8$ experiments). *Significant difference compared to controls.

Effects of Activity on Translation of CAM mRNA into Polypeptides

Consistent with earlier reports (Seilheimer and Schachner, 1988), two molecular species of L1

polypeptides (230 and 210 kD) were revealed by immunoblot analysis of cultured DRG neurons [Fig. 5(A)]. These forms are somewhat larger than L1 from brain (200 kD) and Schwann cell (210 kD). Decreased expression of L1 polypeptide levels accompanied the decrease in L1 mRNA after 5 days of 0.1-Hz stimulation [Fig. 5(B)], but recovery was not yet evident at the level of L1 protein by 3 days after terminating the stimulus [Fig. 5(B)].

Three isoforms of NCAM were detected by immunoblot of cultured DRG neurons, with molecular weights of 120, 140, and 180 kDa [Fig. 5(A)]. Consistent with the constant expression of NCAM mRNA after stimulation, no significant change was detected in any NCAM isoform in immunoblots after stimulation (Table 1) or following cessation of stimulation [Fig. 5(B)]. Thus, there is no evidence for transcriptional or translational regulation of NCAM gene expression by neural impulse activity in these experiments.

Availability of specific antibodies recognizing N-cadherin in these neurons will be necessary to test the implication that the sharp decrease in expression at the mRNA level is accompanied by a corresponding decrease at the protein level.

Additional Factors in Activity-Dependent Regulation of L1 mRNA

Experiments with other types of stimulation further illustrate the exquisite sensitivity of gene regulation

Figure 4 L1 mRNA levels recover after terminating stimulation. (A) The level of L1 mRNA was determined by quantitative competitive RT-PCR by linear regression relative to known concentrations of L1 mimic cDNA after 5 days of 0.1 Hz electrical stimulation (open squares) and compared to unstimulated controls (0 Hz) (closed squares), or cultures unstimulated for 3 days after terminating the stimulus (triangles). (B) Pooled data from several experiments ($n = 11$), indicate a 50% recovery to prestimulus levels 3 days after terminating the stimulus ($***p < 0.02$). 1 = unstimulated; 2 = stimulation at 0.1 Hz, 5 days; 3 = 3 days after terminating the 5-day stimulus.

Figure 5 NCAM and L1 polypeptide levels show different responses to electrical stimulation. L1 and NCAM polypeptide levels were determined in cultured DRG neurons by immunoblot (A) after (1) no stimulation or (2) 0.1 Hz stimulation for 5 days, or for (3) 3 days after stopping the 5-day 0.1-Hz stimulation. Consistent with mRNA measurements, L1 polypeptide levels were lowered after 5 days of 0.1 Hz stimulation (B), but no recovery was detected 3 days after terminating the stimulus ($n = 9$ experiments). None of the NCAM polypeptide levels was affected by electrical stimulation or terminating the electrical stimulus ($n = 12$ experiments). See Table 1.

in response to particular patterns of impulses (Table 2). Chronic membrane depolarization with 60 mM KCl failed to alter L1 mRNA levels. Stimulation with a pulse-train pattern (five impulses at 10 Hz every 2 s) was also without effect (Table 2). Previous results from this laboratory have shown that this phasic pattern of stimulation induces persistent changes in synaptic strength in these multicompartmental cultures when DRG neurons in the side compartments are used to innervate neurons from the ventral spinal cord plated in the central compartment (Nelson et al., 1989). This suggests that regulation of L1 expression by impulse activity is not involved in this activity-dependent synaptic plasticity.

Target-derived influences blocked the activity-

dependent down-regulation of L1 in DRG neurons. This was shown by plating neurons, which were derived from their normal target in the ventral spinal cord, into the central compartment of the three-compartment chamber. DRG neurons were plated into the two lateral compartments and allowed to extend axons into the central compartment, where they form synapses on the ventral spinal cord neurons. The formation of synapses from DRG axons onto spinal cord neurons in this preparation has been confirmed by electrophysiologic recording (Nelson et al., 1989; Fields et al., 1992). Electrical stimulation of DRG neurons in these cocultures failed to alter L1 expression (Table 2). This indicates a functional context prior to synaptogenesis for down-regulation of L1 by neuronal impulse activity.

Table 1 Determination of L1 and NCAM Polypeptides in DRG Neurons after Electrical Stimulation at 0.1 and 1 Hz

Stimulation Frequency	L1 Expression			NCAM Expression			
	230 kD	210 kD	<i>n</i>	180 kD	140 kD	120 kD	<i>n</i>
0 Hz, 5 days	100 ± 11.2	100 ± 18.1	12	100 ± 31.5	100 ± 8.0	100 ± 11.2	5
0.1 Hz, 5 days	58.6 ± 15.3 ^{1,2}	55.0 ± 4.8 ^{2,3}	9	99.9 ± 9.0	91.5 ± 35.9	113 ± 10.3	3
1.0 Hz, 5 days	97.3 ± 2.0	104 ± 4.9	3	101 ± 18.3	84.9 ± 25.9	112 ± 22.5	3

¹ $p < 0.01$ (0 vs. 0.1 Hz).

² $p < 0.05$ (0 vs. 0.1 Hz).

³ $p < 0.005$ (0.1 vs. 1 Hz).

Table 2 Effects of Different Patterns of Stimulation and Innervation of CNS Neurons on Expression of L1 mRNA in DRG Neurons in Multicompartment Cultures

Stimulation Frequency (5 days)	Target Cell in Center Compartment	L1 mRNA Levels (% nonstimulated)	<i>n</i>	<i>p</i>
0 Hz	None	100 ± 13.1	19	
0.1 Hz	None	49.4 ± 7.7	8	<0.001
Phasic ¹	None	102 ± 1.5	2	NS
60 mM KCl	None	99.0 ± 5.9	3	NS
0 Hz	VH	100 ± 9.2	4	
0.1 Hz	VH	114 ± 9.3	4	NS

¹ Five pulses at 10 Hz every 2 s; VH = neurons dissociated from the ventral spinal cord.

Distribution of an Alternative Splice Variant of L1 Message in Cultured Neurons and Schwann Cells

An alternative splice variant of L1 differs by the exclusion of a short RSLE sequence in the intracellular domain (Miura et al., 1991). The short-splice form has been associated with increased cell migration, and the long form with neurite outgrowth (Takeda et al., 1996). It is possible that neural activity could alter the relative expression of these isoforms to regulate these different functions. PCR primers were designed to amplify fragments of L1 corresponding to the cytoplasmic domain of the molecule in the region including the alternatively spliced RSLE sequence (Miura et al., 1991), and these two isoforms were distinguished by the different size of the PCR products (136 and 124 bp). The full-length form was de-

tected in brain and cultured DRG neurons, but L1 lacking the RSLE sequence was not detected in these cells (Fig. 6). This short-splice form was expressed in cultured Schwann cells, however, (Fig. 6). The appearance of the short-splice L1 variant following PCR amplification provided a sensitive method to detect Schwann cell contamination of DRG neuron cultures. No Schwann cell contamination of L1 message was detected in these neuronal cultures. Cultured fibroblasts also showed no L1 mRNA of either splice variant. These results lead to two important conclusions: (a) Any changes in L1 levels observed after stimulation of DRG neurons in culture are due to effects of impulse activity on neuronal expression of L1; and (b) neural impulse activity does not influence the alternative splicing of L1, because the short-splice form was never observed in DRG cultures under any stimulus condition.

Figure 6 The distribution and abundance of an alternatively spliced isoform of L1, which lacks the RSLE insert, varies in different cell types. The short-splice form of L1 (-RSLE) was detected in Schwann cells (lane 3), but not in 8-day postnatal cerebellar neurons (lane 1) or embryonic day 17 DRG (lanes 2). No L1 message was detected in cultured fibroblasts (lane 5). The short-spliced form of L1 was not detected in cultures of DRG neurons (lane 4) in stimulated or unstimulated conditions, indicating that neuronal activity does not regulate the expression of this isoform.

DISCUSSION

The results show that neural impulse activity can regulate expression of CAMs in DRG neurons. This regulation operates at the level of transcription or stability of the mRNA transcripts and affects members of both major families of CAMs: the Ig superfamily and cadherins. The effects are selective for specific types of CAMs, and the regulation is sensitive to different frequencies of electrical stimulation. N-cadherin mRNA levels were decreased rapidly by both 0.1 and 1 Hz stimulation, but L1 expression was lowered after 5 days of 0.1 Hz stimulation, and 1 Hz stimulation had no effect on L1 levels. In contrast, NCAM expression at the mRNA or protein level was not altered by either frequency of stimulation. Activity-dependent plasticity has been associated with changes in an alternative splice variant of NCAM (VASE insert) (Doherty et al., 1994). However, the RSLE splice form of L1 was not regulated by impulse activity in DRG neurons. The dynamics of changes in L1 mRNA levels induced by electrical stimulation *in vitro* differ for L1 and N-cadherin. N-cadherin mRNA levels were significantly lower after 1 day of 1 or 0.1 Hz stimulation, but down-regulation of L1 was relatively slow, with 3–5 days stimulation required to reduce L1 mRNA significantly. Levels of L1 mRNA increase slowly after stopping stimulation.

Possible Functional Significance of Differences in Activity-Dependent Regulation of N-Cadherin, L1, and NCAM

The 13-fold down-regulation of L1 mRNA produced by 0.1 Hz stimulation would be expected to produce significant functional effects, given that only a twofold change in expression of NCAM or NgCAM (the L1 homologue in chick) produces a 30-fold change in binding rate (Hoffman and Edelman, 1983). We have demonstrated previously that the reduction in L1 expression induced by 0.1 Hz electrical stimulation is sufficient to produce structural and functional effects in cell culture (Itoh et al., 1995). Cell-cell adhesion is reduced, axons defasciculate, and Schwann cell association with axons is inhibited.

Expression of N-cadherin was decreased in direct proportion to the frequency of impulse activity. The specific functional significance of this down-regulation is not yet known, but as impulse activity of any frequency inhibited its expression, the functional significance of high levels of N-cadherin expression

must pertain to early developmental events prior to the onset of spontaneous activity. N-cadherin has been shown to promote initiation of neurites, neurite outgrowth, and growth cone motility in retinal ganglion neurons (Riehl et al., 1996), which are early developmental events.

NCAM mRNA and protein levels were not regulated by impulse activity in these experiments, suggesting that NCAM expression does not have a major role in activity-dependent plasticity in DRG development. NCAM may still have an important activity-dependent influence, however, through post-translational modifications, as has been shown for innervation of neuromuscular junctions (Rutishauser and Landmesser, 1996), or through heterophilic interactions with L1 (Kadmon et al., 1990), as illustrated by cooperative interactions between NCAM and L1 in hippocampal LTP and cell–cell adhesion (Luthi et al., 1994; Muller et al., 1996). There was a weak trend for increased expression of the 120-kD isoform of NCAM and a decrease in the 140-kD isoform with stimulation (Table 1). These differences were small and not statistically significant, but it is worth noting these trends in view of recent research showing that NCAM isoform levels in muscle are regulated by contractile activity (Rafuse and Landmesser, 1996).

Correlation between Impulse Activity and Development of DRG Neurons

Although it is not possible to extrapolate these results to *in vivo* conditions, this is an important consideration for future research. The results in cell culture suggest that L1 and N-cadherin levels would be relatively high prior to development of spontaneous electrical activity in DRG neurons *in vivo*. L1 would also be higher in neurons with higher frequencies of impulse activity (>1 Hz), but N-cadherin levels would be depressed. Very low-frequency impulse activity (0.1 Hz) would reduce L1 expression, except for neurites reaching a source of NGF or contacting neurons in the ventral spinal cord. Current information on the developmental sequence of DRG neurons is consistent with these predictions.

Virtually absent in DRG neurons postnatally, spontaneous electrical activity develops for unknown reasons at about E16 in lumbar DRG neurons of the rat. Initially, firing is very low frequency (<0.5 Hz), but the frequency of firing increases to maximal rates at about E18–19 and then abates completely by birth, to be replaced by high-frequency, stimulus-evoked activity (Fitzgerald, 1987). Several developmental changes in DRG neurons coincide with these changes in activity pattern.

Table 3 Correlations between Action Potential Activity, CAM Expression, and Developmental Events in Mouse DRG Neurons

Action Potential Firing	Relative CAM Expression Levels			Developmental Events
	NCAM	L1	N-cadherin	
None	+	+	+	Cell migration Process initiation Process outgrowth Fasciculation
Low frequency	+	–	–	Defasciculation Target recognition (cessation of outgrowth) Synaptogenesis Gap junction uncoupling
High frequency and phasic	+	+	–	Consolidation of terminals Synapse stabilization Myelination

Thus, action potential activity divides development into three general phases: (a) a preimpulse activity period, (b) a low-frequency spontaneous activity period, and (c) a final phase of high-frequency, phasic, and stimulus-evoked firing. The relative mixture of NCAM, L1, and N-cadherin expressed in DRG neurons stimulated with these three general activity patterns would have functional consequences consistent with the developmental processes accompanying these three phases of activity (Table 3).

Neurite outgrowth occurs largely during the period prior to spontaneous electrical activity, when DRG neurons are extensively coupled electrotonically through gap junctions (Fulton et al., 1990; Fitzgerald and Fulton, 1992). N-cadherin and L1 promote outgrowth of neurites from several types of neurons (Lemmon et al., 1992). Relatively high expression of these two CAMs prior to spontaneous impulse activity would promote neurite outgrowth and fasciculation. Previous work showing that higher frequencies of action potentials (2.5–10 Hz) induce collapse of growth cones in DRG neurons (Fields et al., 1990; Fields, 1994) is also consistent with outgrowth occurring largely prior to spontaneous impulse activity.

Low-frequency spontaneous activity starts at about the time peripheral endings reach the subepidermis (Reynolds et al., 1991; Fitzgerald, 1987). The onset of spontaneous activity coincides with a number of major developmental transitions. The peripheral endings begin to defasciculate to form a diffuse subepidermal plexus (about E17) (Fitzgerald and Fulton, 1992), and DRG neurons become electrotonically uncoupled (cell coupling declines from E15 to E16 until birth) (Fitzgerald and Fulton, 1992). Central DRG terminals, which had reached

the spinal cord by E12, finally begin to extend collaterals into the gray matter (E15) (Ziskind-Conhaim, 1990; Fitzgerald, 1991) at the time spontaneous activity begins.

Defasciculation of peripheral terminals and penetration of central axons into the spinal cord at the time low-frequency spontaneous activity begins may be promoted by down-regulation of L1 and N-cadherin induced by low-frequency spontaneous activity. There is substantial evidence that reduced L1 expression promotes defasciculation and that this can promote synaptogenesis and facilitate changes in direction of neurite outgrowth at critical anatomical loci. Inhibition of L1 function partially defasciculates nerve roots and enhances the growth of axons over myotubes (Landmesser et al., 1988; Dahm and Landmesser, 1988). If expression of the NCAM homologue in *Drosophila* (Fasciclin II) is increased by genetic manipulation, axons of the RP3 motor neuron fail to defasciculate and leave the common motor pathway at a critical choice point to innervate their appropriate muscles (Lin and Goodman, 1995). In summary, the reduction in L1 and N-cadherin mRNA induced by low-frequency action potential firing are compatible with the transition from a neurite outgrowth phase to a synaptogenesis phase, which follows the onset of spontaneous activity in these neurons *in vivo*.

Spontaneous activity in DRG neurons declines in later stages of development as high-frequency, sensory-evoked activity develops (Fitzgerald and Fulton, 1992). This period is accompanied by the formation of peripheral sensory end organs (E14–E19), consolidation of the subepidermal plexus of DRG terminals (E17 to birth) (Reynolds et al., 1991), and synaptogenesis of the central terminals

of DRG neurons with dorsal horn and motoneurons (beginning about E17) (Kudo and Yamada, 1987; Ziskind-Conhaim, 1990). Consolidation of peripheral axon terminals and formation of central synapses are coincident with changes in firing pattern that increase levels of L1 in DRG neurons in culture. The increased expression of L1 may help terminate the period of axon terminal defasciculation and thereby promote the consolidation of functional connections. L1 is also necessary for initiation of myelination, which begins in the perinatal period when DRG neurons fire at frequencies higher than those that lower L1 expression.

N-cadherin levels would not increase as the firing pattern became more rapid. This suggests that high levels of N-cadherin expression may be less important after synaptogenesis than for earlier developmental processes in DRG neurons. Inhibition of N-cadherin function in developing retinal ganglion neurons *in vivo* impairs axon and dendrite initiation and outgrowth, and causes growth cone arrest, without inducing errors in axon guidance (Reihl et al., 1996). These events take place early in development prior to synaptogenesis. Other experiments show that cadherin-dependent adhesion in the retina becomes less important at later stages of development as integrins and CAMs of the Ig superfamily assume a larger role in maintaining tissue integrity (Matsunga et al., 1988; Riehl, 1996). In many neurons, cadherin becomes restricted to nodes of Ranvier (Cifuentes-Diaz et al., 1994; Scherer, 1996; Shibuya et al., 1995) and synaptic junctions (Uchida et al., 1996; Fannon and Colman, 1996; Beesley et al., 1995) at later stages of development. It has been proposed that synaptically localized cadherin promotes synaptogenesis with appropriate targets (Fannon and Colman, 1996), and its subcellular distribution bordering the active zone at some CNS synapses suggests possible involvement of cadherin in structural-functional modifications of synaptic junctions (Fannon and Colman, 1996). The restricted distribution after synaptogenesis implies a general reduction in N-cadherin mRNA as expression declines elsewhere throughout the neuronal membrane. The results of controlled patterns of action potential firing show an important influence of impulse activity on the regulation of this gene. The activity-dependent down regulation of N-cadherin expression in DRG neurons is consistent with the changes in distribution reported during development, and with the known functional effects of this CAM in association with neurite initiation, outgrowth, and stabilization of synapses.

Cell adhesion molecules have important and well-established functions in morphogenesis of the

brain. There is wide overlap in functions mediated by various CAMs, but each CAM has distinct properties and functional interactions. Functional specificity is provided by sequential changes in the relative abundance of different CAMs. The present results show that action potential firing pattern can regulate the relative abundance of different CAMs in a sequence that is consistent with the developmental process of these neurons *in vivo*.

The authors thank Dr. Melitta Schachner for generously providing the polyclonal antibody against L1.

REFERENCES

- APPEL, F., HOLM, J., CONSCIENCE, J. F., VON BOHLENUND HALBACH, F., FAISSNER, A., JAMES, P., and SCHACHNER, M. (1995). Identification of the border between fibronectin type III homologous repeats 2 and 3 of the neural cell adhesion molecule L1 as a neurite outgrowth promoting and signal transducing domain. *J. Neurobiol.* **28**:297–312.
- ARCARI, P., MARTINELLI, R., and SALVATORE, F. (1984). The complete sequence of a full length cDNA for human liver glyceraldehyde-3-phosphate dehydrogenase: evidence for multiple mRNA species. *Nucleic Acids Res.* **12**:9179–9189.
- BARAMI, K., KIRSCHENBAUM, B., LEMMON, V., and GOLDMAN, S. A. (1994). N-cadherin and Ng-CAM/8D9 are involved serially in the migration of newly generated neurons into the adult songbird brain. *Neuron* **13**:567–582.
- BEESELEY, P. W., MUMMERY, R., and TIBALDI, J. (1995). N-cadherin is a major glycoprotein component of isolated rat forebrain postsynaptic densities. *J. Neurochem.* **64**:2288–2294.
- BURDEN-GULLEY, S. M. and LEMMON, V. (1995). Ig superfamily adhesion molecules in the vertebrate nervous system: binding partners and signal transduction during axon growth. *Semin. Dev. Biol.* **6**:79–87.
- CHOMCYNski, P. and SACCHI, N. (1987). Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156–159.
- CIFUENTES-DIAZ, C., NICOLET, M., GOUDOU, D., REIGER, F., and MEGE, R. M. (1994). N-cadherin expression in developing, adult and denervated chicken neuromuscular system: accumulations at both the neuromuscular junction and the node of Ranvier. *Development* **120**:1–11.
- DAHM, L. M. and LANDMESSER, L. T. (1988). The regulation of intramuscular nerve branching during normal development and following activity blockade. *Dev. Biol.* **130**:621–644.
- DAVIS, G. O., SCHUSTER, C. M., and GOODMAN, C. S. (1996). Genetic dissection of structural and functional components of synaptic plasticity. III. CREB is neces-

- sary for presynaptic functional plasticity. *Neuron* **17**:669–679.
- DOHERTY, P., FAZELI, M. S., and WALSH, F. S. (1994). The neural cell adhesion molecule and synaptic plasticity. *J. Neurobiol.* **26**:437–446.
- EDELMAN, G. M. and CROSSIN, K. L. (1991). Cell adhesion molecules: implications for molecular histology. *Annu. Rev. Biochem.* **60**:155–190.
- FANNON, A. M. and COLMAN, D. R. (1996). A model for central synaptic junctional complex formation based on the differential adhesive specificities of the cadherins. *Neuron* **4**:423–434.
- FIELDS, R. D. and ITOH, K. (1996). Neural cell adhesion molecules in activity-dependent development and synaptic plasticity. *Trends Neurosci.* **19**:473–470.
- FIELDS, R. D. and NELSON, P. G. (1992). Activity-dependent development of the vertebrate nervous system. *Int. Rev. Neurobiol.* **34**:133–214.
- FIELDS, R. D., NEALE, E. A., and NELSON, P. G. (1990). Effects of patterned electrical activity on neurite outgrowth from mouse sensory neurons. *J. Neurosci.* **10**:2950–2964.
- FIELDS, R. D., YU, C., NEALE, E. A., and NELSON, P. G. (1992). Recording chambers in cell culture. In: *Electrophysiological Methods for In Vitro Studies in Vertebrate Neurobiology*, H. Kettenmann and R. Grantyn, Eds. New York, Liss, pp. 67–76.
- FITZGERALD, M. and FULTON, P. B. (1992). The physiological properties of developing sensory neurons. In: *Sensory Neurons*, S. A. Scott, Ed. Oxford University Press, New York, pp. 287–306.
- FITZGERALD, M. (1991). A physiological study of the perinatal development of cutaneous sensory inputs to dorsal horn cells in the rat. *J. Physiol. Lond.* **432**:473–482.
- FITZGERALD, M. (1987). Spontaneous and evoked activity of fetal primary afferents *in vivo*. *Nature* **326**:603–605.
- FREDETTE, B., RUTISHAUSER, U., and LANDMESSER, L. (1993). Regulation and activity-dependence of N-cadherin, NCAM isoforms, and polysialic acid on chick myotubes during development. *J. Cell Biol.* **123**:1867–1888.
- FULTON, B. P., MILEDI, R., and TAKAHASHI, T. (1980). Electrical synapses between motoneurons in the spinal cord of the newborn rat. *Proc. R. Soc. Lond. Ser. B* **208**:115–120.
- GOODMAN, C. S. (1996). Mechanisms and molecules that control growth cone guidance. *Annu. Rev. Neurosci.* **19**:341–377.
- GUMBINER, B. M. (1993). Proteins associated with the cytoplasmic surface of adhesion molecules. *Neuron* **11**:551–564.
- HAHN, C. G. and COVAULT, J. (1992). Neural regulation of N-cadherin gene expression in developing and adult skeletal muscle. *J. Neurosci.* **12**:4677–4688.
- HASEGAWA, M., SETO, A., UCHIYAMA, N., KIDA, S., YAMASHIMA, T., and YAMASHITA, J. (1996). Localization of E-cadherin in peripheral glia after nerve injury and repair. *J. Neuropathol. Exp. Neurol.* **55**:424–434.
- HOFFMAN, C. G. and EDELMAN, G. M. (1983). Kinetics of homophilic binding by embryonic and adult forms of the neural cell adhesion molecules. *Proc. Natl. Acad. Sci. USA* **80**:5762–5766.
- HONIG, M. G. and RUTISHAUSER, U. S. (1996). Changes in the segmental pattern of sensory neuron projections in the chick hindlimb under conditions of altered cell adhesion molecule function. *Dev. Biol.* **175**:325–337.
- IDE, C. (1996). Peripheral nerve regeneration. *Neurosci. Res.* **25**:101–121.
- ITOH, K., BRACKENBURY, R., and AKESON, R. A. (1995). Induction of L1 mRNA in PC12 cells by NGF is modulated by cell–cell contact and does not require the high affinity NGF receptor. *J. Neurosci.* **15**:2504–2512.
- ITOH, K., STEVENS, B., SCHACHNER, M., and FIELDS, R. D. (1995). Regulation of the neural cell adhesion molecule L1 by specific patterns of neural impulses. *Science* **270**:1369–1372.
- JORGENSEN, O. S. (1995). Neural cell adhesion molecule (NCAM) as a quantitative marker in synaptic remodeling. *Neurochem. Res.* **20**:533–547.
- KADMON, G., KOWITZ, A., ALTEVOGT, P., and SCHACHNER, M. (1990). Functional cooperation between neural cell adhesion molecule L1 and N-CAM is carbohydrate dependent. *J. Cell. Biol.* **110**:209–218.
- KUDO, N. and YAMADA, T. (1987). Morphological and physiological studies of development of the monosynaptic reflex pathway in the rat lumbar cord. *J. Physiol. Lond.* **389**:441–459.
- LAGUNOWICH, L. A., STEIN, A. P., and REUHL, K. R. (1994). N-cadherin in normal and abnormal brain development. *Neurotoxicology* **15**:123–132.
- LANDMESSER, L., DAHM, L., SCHULTZ, K., and RUTISHAUSER, U. (1988). Distinct roles for adhesion molecules during innervation of embryonic chick muscle. *Dev. Biol.* **130**:645–670.
- LAWSON, S. N., HARPER, A. A., HARPER, E. I., GARSON, J. A., and ANDERTON, B. H. (1984). A monoclonal antibody against neurofilament protein specifically labels a subpopulation of rat sensory neurons. *J. Comp. Neurol.* **228**:263–272.
- LEMMON, V., BURDEN, S. M., PAYNE, H. R., ELMSLIE, G. J., and HLAVIN, M. L. (1992). Neurite outgrowth on different substrates: permissive versus instructive influences and the role of adhesive strength. *J. Neurosci.* **12**:818–826.
- LIN, D. M. and GOODMAN, C. S. (1995). Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. *Neuron* **13**:507–523.
- LUTHI, A., LAURENT, J. P., FIGUROV, A., MULLER, D., and SCHACHNER, M. (1994). Hippocampal long-term potentiation and neural cell adhesion molecules L1 and NCAM. *Nature* **372**:777–779.
- MAGYAR-LEHMANN, S., FREI, T., and SCHACHNER, M. (1995). Fasciculation of granule cell neurites is responsible for the perpendicular orientation of small inhibitory interneurons in mouse cerebellar microexplant cultures *in vitro*. *Eur. J. Neurosci.* **7**:1460–1471.
- MATSUNAGA, M., HATTA, K., and TAKEICHI, M. (1988).

- Role of N-cadherin cell adhesion molecules in histogenesis of neural retina. *Neuron* **1**:289–295.
- MAYFORD, M., BARZILAI, A., KELLER, F., SCHACHER, S., and KANDEL, E. R. (1992). Modulation of an NCAM-related adhesion molecule with long-term synaptic plasticity in *Aplysia*. *Science* **256**:638–644.
- McKERRACHER, L., CHAMOUX, M., and ARREGUL, C. O. (1996). Role of laminin and integrin interactions in growth cone guidance. *Mol. Neurobiol.* **12**:95–116.
- MIURA, M., KOBAYASHI, M., ASOU, H., and UYEMURA, K. (1991). Molecular cloning of cDNA encoding the rate neural cell adhesion molecule L1: two L1 isoforms in the cytoplasmic region are produced by differential splicing. *FEBS Lett.* **289**:91–95.
- MIYATANI, S., SHIMAMURAK, K., HATTA, M., NUGAFUCHI, A., NOSE, A., MATSUNAGA, M., HATTA, K., and TAKEICHI, M. (1989). Neural cadherin: role in selective cell–cell adhesion. *Science* **245**:631–635.
- MOOS, M., TACKE, R., SCHERER, H., TELOW, D., FRUH, K., and SCHACHNER, M. (1988). Neural cell adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. *Nature* **334**:701–703.
- MULLER, D., WANG, C., SKIBO, G., TONI, N., CREMER, H., CALAORA, V., ROUGON, G., and KISS, J. Z. (1996). PSA-NCAM is required for activity-induced synaptic plasticity. *Neuron* **17**:413–422.
- NAKAGAWA, S. and TAKEICHI, M. (1995). Neural crest cell–cell adhesion controlled by sequential and subpopulation-specific expression of novel cadherins. *Development* **121**:1321–1332.
- NATHKE, S. I., HINCK, L., and NELSON, W. J. (1995). The cadherin/catenin complex: connections to multiple cellular processes involved in cell adhesion, proliferation and morphogenesis. *Semin. Dev. Biol.* **6**:89–95.
- NELSON, P. G., YU, C., FIELDS, R. D., and NEALE, E. A. (1989). Competition between convergent inputs in vitro: electrical activity modulates efficacy and number of synapses. *Science* **244**:585–587.
- PETER, N., ARONOFF, B., WU, F., and SCHACHER, S. (1994). Decrease in growth cone-neurite fasciculation by sensory and motor cells in vitro accompanies down-regulation of *Aplysia* cell adhesion molecules by neurotransmitters. *J. Neurosci.* **14**:1413–1421.
- RAFUSE, V. F. and LANDMESSER, L. (1996). Contractile activity regulates isoform expression and polysialylation of NCAM in cultured myotubes: involvement of calcium and protein kinase C. *J. Cell Biol.* **132**:969–983.
- RANSCHT, B. (1994). Cadherins and catenins: interactions and functions in embryonic development. *Curr. Opin. Cell Biol.* **6**:740–746.
- REDIES, C. (1995). Cadherin expression in the developing vertebrate CNS: from neuromeres to brain nuclei and neural circuits. *Exp. Cell Res.* **220**:243–256.
- REYNOLDS, M. L., FITZGERALD, M., and BENOWITZ, L. I. (1991). GAP-43 expression in the developing rat hindlimb. *Neuroscience* **41**:201–211.
- RIEHL, R., JOHNSON, K., BRADLEY, R., GRUNWALD, G. B., CORNEL, E., LILIENBAUM, A., and HOLT, C. E. (1996). Cadherin function is required for axon outgrowth in retinal ganglion cells in vivo. *Neuron* **17**:837–848.
- RUTISHAUSER, U. and LANDMESSER, L. (1996). Polysialic acid in vertebrate nervous system: a promoter of plasticity in cell–cell interactions. *Trends Neurosci.* **19**:422–427.
- SAKIMURA, K., KUSHIYA, E., OBINATA, M., ODANI, S., and TAKAHASHI, Y. (1985). Molecular cloning and the nucleotide sequence of cDNA for neuron-specific enolase messenger RNA of rat brain. *Proc. Natl. Acad. Sci. USA* **82**:7453–7457.
- SANTONI, M. J., BARTHELS, D., VOPPER, G., BONED, A., GORIDIS, C., and WILLE, W. (1989). Differential exon usage involving an unusual splicing mechanism generates at least eight subtypes of NCAM cDNA in mouse brain. *EMBO J.* **8**:385–392.
- SCHACHNER, M. (1992). Neural recognition molecules and their influence on cellular functions. In: *The Nerve Growth Cone*, P. C. Letourneau, S. B. Kater, and E. R. Macagno, Eds. Raven Press, New York, pp. 237–254.
- SCHERER, M., HELLER, M., and SCHACHNER, M. (1992). Expression of the neural recognition molecule L1 by cultured neural cells is influenced by K⁺ and the glutamate receptor agonist NMDA. *Eur. J. Neurosci.* **4**:554–562.
- SCHERER, S. S. (1996). Molecular specializations at nodes and paranodes in peripheral nerve. *Microsc. Res. Technol.* **34**:452–461.
- SCHUSTER, C. M., DAVIS, G. W., FETTER, R. D., and GOODMAN, C. S. (1996a). Genetic dissection of structural and functional components of synaptic plasticity. I. Fasciclin II controls presynaptic structural plasticity. *Neuron* **17**:567–570.
- SCHUSTER, C. M., DAVIS, G. W., FETTER, R. D., and GOODMAN, C. S. (1996b). Genetic dissection of structural and functional components of synaptic plasticity. II. Fasciclin II controls synapse stabilization and growth. *Neuron* **17**:655–667.
- SEILHEIMER, B., PERSOHN, E., and SCHACHNER, M. (1989). Antibodies to the L1 adhesion molecule inhibit Schwann cell ensheathment of nerves in vitro. *J. Cell Biol.* **109**:3095–3103.
- SEILHEIMER, B. and SCHACHNER, M. (1988). Studies of adhesion molecules mediating interactions between cells of peripheral nervous system indicate a major role for L1 in mediating sensory neuron growth on Schwann cells in culture. *J. Cell Biol.* **107**:341–351.
- SHIBUYA, Y., MIZOGUCHI, A., TAKEICHI, M., SHIMADA, K., and IDE, C. (1995). Localization of N-cadherin in the normal and regenerating nerve fibers of the chicken peripheral nervous system. *Neuroscience* **67**:253–261.
- SNIDER, W. D. (1994). Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* **77**:627–638.
- SRETAVAN, D. W., FENG, L., PURE, E., and REICHARDT, L. F. (1994). Embryonic neurons of the developing optic chiasm express L1 and CD44, cell surface mole-

- cules with opposing effects on retinal axon growth. *Neuron* **12**:975–975.
- TAKEDA, Y., ASOU, H., MURAKAMI, Y., MIURA, M., KOBAYASHI, M., and UYEMURA, K. (1996). A nonneuronal isoform of cell adhesion molecule L1: tissue-specific expression and functional analysis. *J. Neurochem.* **66**:2338–2349.
- TAKEICHI, M. (1990). Cadherins: a molecular family important in selective cell–cell adhesion. *Annu. Rev. Biochem.* **59**:237–252.
- UCHIDA, N., HONJO, Y., JOHNSON, K. R., WHEELLOCK, M. J., and TAKEICHI, M. (1996). The catenin/cadherin adhesion system is localized in synaptic junctions bordering transmitter release zones. *J. Cell Biol.* **135**:767–779.
- VON BOHLENUND HALBACH, F., TAYLOR, J., and SCHACHNER, M. (1992). Cell type-specific effects of the neural adhesion molecules L1 and N-CAM on diverse second messenger systems. *Eur. J. Neurosci.* **4**:896–909.
- WALSH, F. S., SKAKPER, S. D., and DOHERTY, P. (1994). Cell adhesion molecule (NCAM and N-cadherin)-dependent neurite outgrowth is modulated by gangliosides. *Prog. Brain Res.* **101**:113–118.
- WOOD, P. M., SCHACHNER, M., and BUNGE, R. P. (1990). Inhibition of Schwann cell myelination in vitro by antibody to the L1 adhesion molecule. *J. Neurosci.* **10**:3635–3645.
- ZISKIND-CONHAIM, L. (1990). NMDA receptors mediate poly- and monosynaptic potentials in motoneurons of rat embryos. *J. Neurosci.* **10**:125–135.
- ZOMZELY-NEURATH, C. E. (1983). In *Handbook of Neurobiology*, 2nd ed., Vol. 4. A. Lajtha, Ed. Plenum Press, New York, pp. 403–433.