Immunoelectron Microscopic Localization of the Neural Cell Adhesion Molecules L1 and N-CAM during Postnatal Development of the Mouse Cerebellum

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Abstract. The cellular and subcellular localization of the neural cell adhesion molecules L1 and N-CAM was studied by pre- and postembedding immunoelectron microscopic labeling procedures in the developing mouse cerebellar cortex. The salient features of the study are: (*a*) L1 displays a previously unrecognized restricted expression by particular neuronal cell types (i.e., it is expressed by granule cells but not by stellate and basket cells) and by particular subcellular compartments (i.e., it is expressed on axons but not on dendrites or cell bodies of Purkinje cells). (*b*) L1 is always expressed on fasciculating axons and on postmitotic, premigratory, and migrating granule cells at sites of neuron–neuron contact, but never at contact sites between neuron and glia, thus strengthening the view that L1 is not involved in granule cell migration as a neuron-glia adhesion molecule. (*c*) While N-CAM antibodies reacting with the three major components of N-CAM (180, 140, and 120 kD) show a rather uniform labeling of all cell types, antibodies to the 180-kD component (N-CAM$_{180}$) stain only the postmigratory granule cell bodies supporting the notion that N-CAM$_{180}$, the N-CAM component with the longest cytoplasmic domain, is not expressed before stable cell contacts are formed. Furthermore, N-CAM$_{180}$ is only transiently expressed on Purkinje cell dendrites. (*d*) N-CAM is present in synapses on both pre- and postsynaptic membranes. L1 is expressed only preterminally and not in the subsynaptic membranes.

These observations indicate an exquisite degree of fine tuning in adhesion molecule expression during neural development and suggest a rich combinatorial repertoire in the specification of cell surface contacts.

THE development of the nervous system proceeds in several distinct, yet coordinated events: neural induction, cell proliferation, migration, aggregation, cytodifferentiation, cell death, and synapse formation and elimination (Cowan, 1982). Several of these events can be studied in the early postnatal cerebellar cortex which is a relatively simply organized and therefore well-known structure (Eccles et al., 1967; Palay and Chan-Palay, 1974, 1982; Ito, 1984). The migration of granule neurons is an outstanding feature in the developing cerebellum (for review see Rakic, 1971, 1982; Mugnaini and Forstrønen, 1967). The following histogenetic steps can be discerned: premigratory granule cell precursors proliferate in the outer part of the external granular layer and then become postmitotic in the inner part of this layer. Postmitotic granule cells associate with the surface of Bergmann glial processes and form axons that interact with already formed granule cell axons in a fasciculated manner to form the parallel fibers in the molecular layer. The granule cell body then extends a leading process along the Bergmann glial surface, which is followed by the granule cell body through the molecular layer into the internal granular layer, where the granule cell takes up its final position.

Cell adhesion molecules have been shown to play an important role during development of the cerebellar cortex.

Among these, the two neural cell adhesion molecules L1 and N-CAM[1] have been implicated in the migration of granule cells from the external to the internal granular layer (Lindner et al., 1983, 1986). Compared with L1, N-CAM appears to play a less dominant, yet significant role in granule cell migration, possibly by mediating contact between granule cells and Bergmann glial processes. The functional role of L1 for granule cell migration is less evident since it does not appear to be a neuron–glia, but neuron–neuron cell adhesion molecule (Keilhauer et al., 1985). On the basis of the observations that L1 mediates aggregation of neuronal cell bodies (Rathjen and Schachner, 1984) and fasciculation of axons (Fischer et al., 1986) of granule cells we have suggested that not only neuron–Bergmann glial contact is necessary for granule cell migration, but sorting out of postmitotic granule cells by aggregation in the internal part of the external granular layer and/or fasciculation of granule cell axons are prerequisites for successful migration.

The present study was undertaken to further investigate the functional roles of L1 and N-CAM during development of the mouse cerebellar cortex. We reasoned that a detailed description of the developmental appearance of the individual

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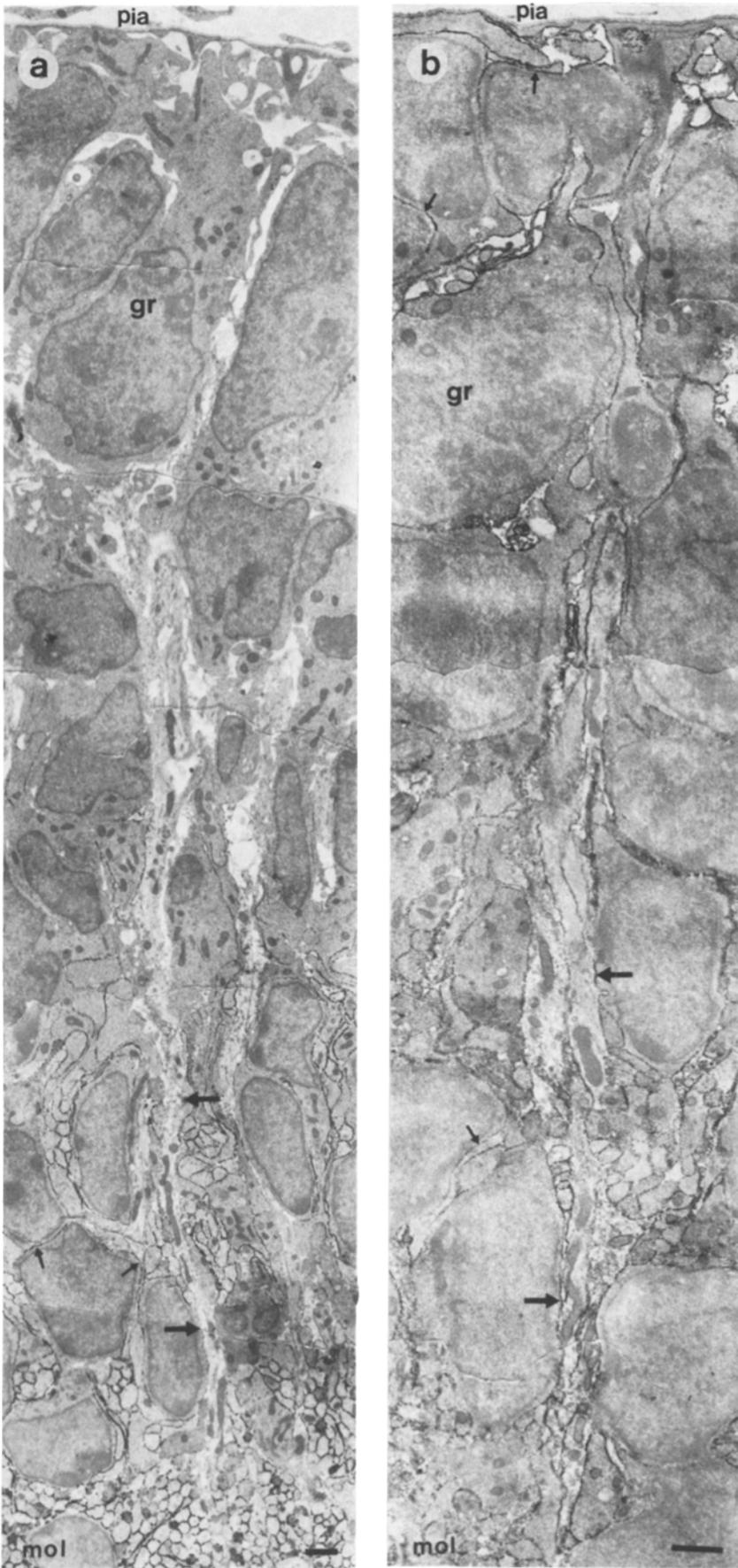1. *Abbreviation used in this paper:* N-CAM, neural cell adhesion molecule.

Figure 1. Immunoelectron microscopic localization of (*a*) L1 and (*b*) N-CAM in the cerebellum of 8-d-old mice. The midsagittal sections show the external granular layer (EGL) from the pial surface (*pia*) to the beginning of the molecular layer (*mol*). (*a*) Pial surface and outer part of EGL are L1 negative, while the inner part of the EGL and the molecular layer are L1 positive. Note the peroxidase reaction product on cell bodies of granule cells in the inner part of the EGL and on parallel fibers (*small arrows*). The Bergmann glial process (*large arrows*) is L1 negative. (*b*) The whole EGL from pial surface to molecular layer is N-CAM positive. Note the peroxidase reaction product on granule cell bodies in the outer and inner part of the EGL (*small arrows*) and on the Bergmann glial processes (*large arrows*). Bars, 1.0 μm.

adhesion molecules on particular cell types and subcellular compartments, such as dendrite, cell body, axon, and synapse, could yield important insights into the cellular mechanisms of their action. Immunoelectron microscopic methods were therefore used for this analysis. Of particular interest in this study was the question about the functional role of the largest, 180-kD component of N-CAM (N-CAM$_{180}$) that is distinguished from the other two components, N-CAM$_{140}$ and N-CAM$_{120}$ by the largest cytoplasmic domain (Cunningham et al., 1983; Gennarini et al., 1984). In contrast to N-CAM$_{140}$ and N-CAM$_{120}$, N-CAM$_{180}$ could be shown to appear relatively late during cerebellar development at or after the appearance of L1 (Pollerberg et al., 1985). Furthermore, N-CAM$_{180}$ is involved in the stabilization of cell contacts by involvement of the cytoskeleton (Pollerberg et al., 1985, 1986). Although the functional interdependence of N-CAM and L1 remains to be determined in molecular terms, it is clear that the two molecules do not act independently of each other in cell aggregation and adhesion with L1 (Faissner et al., 1984; Keilhauer et al., 1985; Lindner et al., 1986; Rathjen and Rutishauser, 1984; Thor et al., 1986). The temporal and spatial relationships between N-CAM, N-CAM$_{180}$, and L1 expression were, therefore, of particular interest for the present study.

Materials and Methods

Animals and Tissue Processing

NMRI mice (8- and 21-d-old) were anaesthetized by intraperitoneal injection of an aqueous solution of chloralhydrate (3%, 0.01 ml/g body wt) and perfused through the left ventricle with ~50-100 ml 4% paraformaldehyde and 0.2% glutaraldehyde in 0.12 M Palay buffer (Palay and Chan-Palay, 1974). Brains were then removed from the skull and immersed in 4% paraformaldehyde in 0.12 M Palay buffer overnight at 4°C. Midsagittal slices of cerebellum, 40-50 µm in thickness, were cut on an Oxford vibratome and rinsed for at least 2 h in PBS, pH 7.3 containing 1% BSA (PBS/BSA) at room temperature.

Antibodies

Production, characterization, and specificity of polyclonal L1 and N-CAM antibodies from rabbits have been described (Rathjen and Schachner, 1984; Faissner et al., 1984). Polyclonal L1 and N-CAM antibodies were mostly used since they stained more strongly than the corresponding monoclonal ones, but with similar results. Polyclonal N-CAM and monoclonal H28.123 antibodies react with the three components of N-CAM with molecular masses of 120, 140, and 180 kD (Gennarini et al., 1984). Immunoaffinity purification of polyclonal L1 antibodies was performed by passing hyperimmune serum over a Sepharose 4B column conjugated to immunoaffinity-purified L1 antigen by cyanogen bromide activation and elution of the bound antibody with glycine buffer at pH 2.5. Preparation of monoclonal antibodies to the 180-kD component of N-CAM (N-CAM$_{180}$) has been described (Pollerberg et al., 1985). Polyclonal rabbit antibodies to human hemoglobin were prepared as described (Martini and Schachner, 1986).

For preembedding staining, polyclonal antibodies were visualized by peroxidase-coupled Protein A (Sigma Chemical GmbH, Munich) at a dilution of 0.75 µg/ml. The monoclonal N-CAM$_{180}$ antibody was visualized by peroxidase-coupled goat antibodies to rat IgG (72 µg/ml; Cappel Laboratories, Inc., Cochranville, PA). For postembedding staining, antibodies adsorbed to colloidal gold (15 nm in diameter; Janssen Pharmaceutica, Beerse, Belgium) were used at dilutions of 1:20. Polyclonal antibodies were visualized by goat antibodies to rabbit IgG and monoclonal antibodies by antibodies to rat IgG.

Pre- and Postembedding Staining

These procedures were carried out essentially as described (Martini and Schachner, 1986). In brief, for preembedding staining vibratome sections

were treated by periodate, NaBH$_4$, and DMSO as described (Schachner et al., 1977). Sections were washed in PBS and incubated overnight at 4°C with primary antibodies. Control sections were immersed in PBS/BSA or in rabbit anti-hemoglobin serum diluted in PBS/BSA to the same concentrations as the experimental antibodies. Sections were washed again and then incubated with peroxidase-coupled Protein A or peroxidase-coupled antibodies to rat IgG. Sections were fixed with 1% glutaraldehyde in phosphate buffer, washed again, and developed with 3,3'diaminobenzidine-4HCl and H$_2$O$_2$ in the dark. Sections were postfixed with 2% OsO$_4$ in phosphate buffer, dehydrated in ethanol and propylene oxide, and embedded in Epon 812 (Agar Aids Stansted, Essex, UK). Some sections were counterstained with uranyl acetate. Thin sections, 50-70-nm thick, were examined in a Zeiss EM10C.

For postembedding staining vibratome sections were dehydrated in an ascending series of ethanol at low temperatures (Roth et al., 1981; Martini and Schachner, 1986). Infiltration with Lowicryl and polymerization by UV light was performed at −30°C and UV irradiation continued at room temperature to improve the sectioning properties. Ultrathin sections were mounted on Formvar-coated copper grids (E. Merck, Darmstadt, FRG) and incubated with first antibodies, washed, and then incubated with second antibodies complexed with colloidal gold. All incubations were carried out at room temperature. Control sections were treated as described under pre-embedding staining. Sections were counterstained with uranyl acetate.

Results

Cerebella of 8- and 21-d-old mice were investigated for immunoelectron microscopic localization of the neural cell adhesion molecules L1 and N-CAM and the largest of the three components of N-CAM, N-CAM$_{180}$. Midsagittal sections were used throughout this study. At each age the characteristic features of granule, stellate, basket, and Purkinje cells and Bergmann glial processes could be discerned in pre- and postembedding staining procedures by the criteria of Palay and Chan-Palay (1974).

L1

L1 is never found on glia (Fig. 1 a). It is not expressed by the neuroblasts in the external part of the external granular layer, which contains the precursors to granule, stellate, and basket cells (Fig. 1 a). In the inner part of the external granular layer postmitotic premigratory granule cells are L1 positive (Fig. 1 a). Parallel fibers are strongly L1 positive (Fig. 1 a) in a somewhat discontinuous, punctate staining pattern (Figs. 2 b and 3, b and e). The migrating granule cell is also L1 positive, but only when in contact with neighboring neuronal structures such as other granule cell bodies or parallel fibers. Interestingly, L1 was never seen at contact sites between Bergmann glial process and granule cell body or leading process (Fig. 2 b, and compare with nonstained control, inset). Granule cell bodies in the internal granular layer are L1 positive in 8-d-old mice, but negative in 21-d-old mice (not shown). L1 is never detectable on stellate (Fig. 2 d) or basket cells (not shown). It is neither observed on Purkinje cell dendrites (Fig. 2 f) or cell bodies, but is detectable on axons (not shown). Contact sites between parallel fibers and Purkinje cell dendrites are always L1 negative (Figs. 2 f and 3 b). L1 is never detectable on subsynaptic membranes. It is always less strongly seen on preterminal parts of axons than on parallel fibers (Fig. 3 e).

N-CAM

Antibodies reacting with all three components of N-CAM stained Bergmann glial processes, mitotic, postmitotic, and premigratory, migratory, and postmigratory granule cells (Figs. 1 b and 2, a and e). In contrast to L1, N-CAM is easily
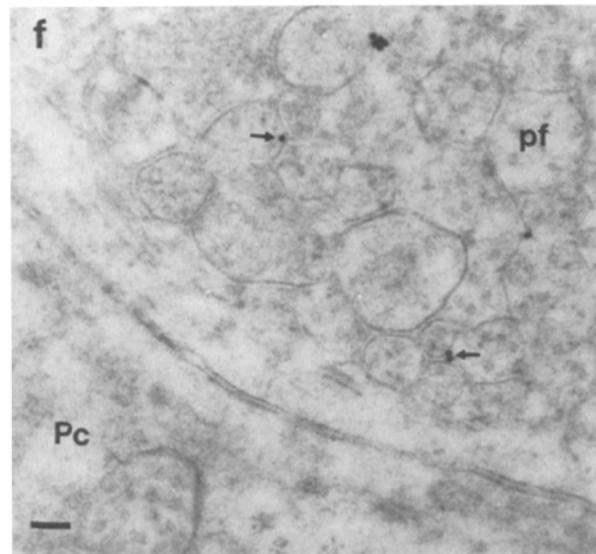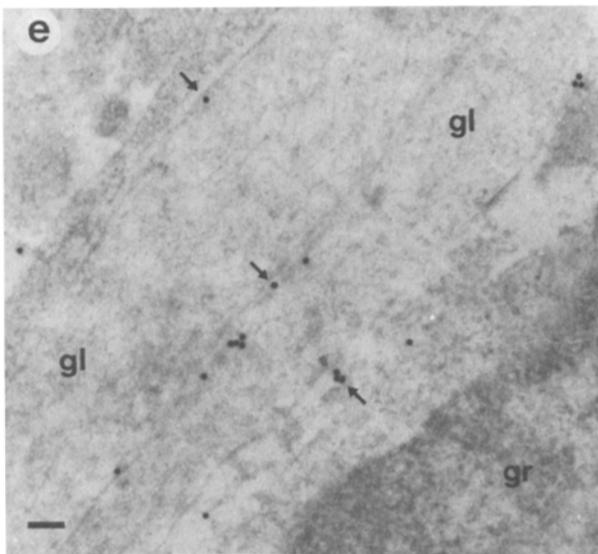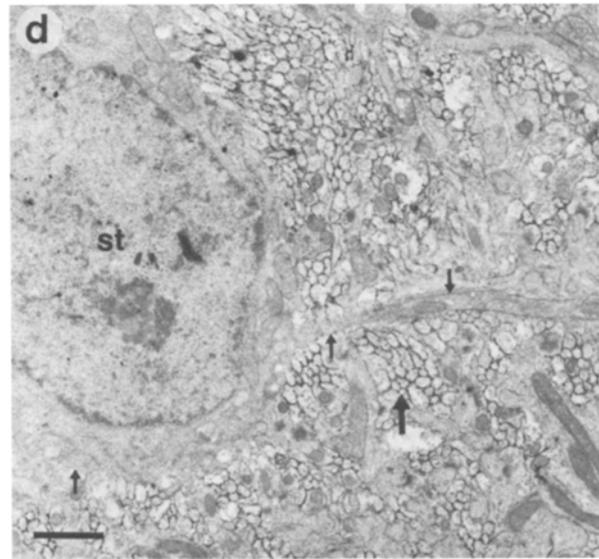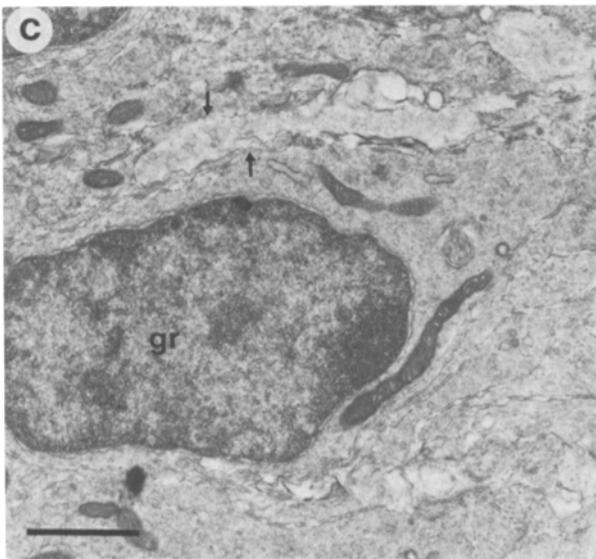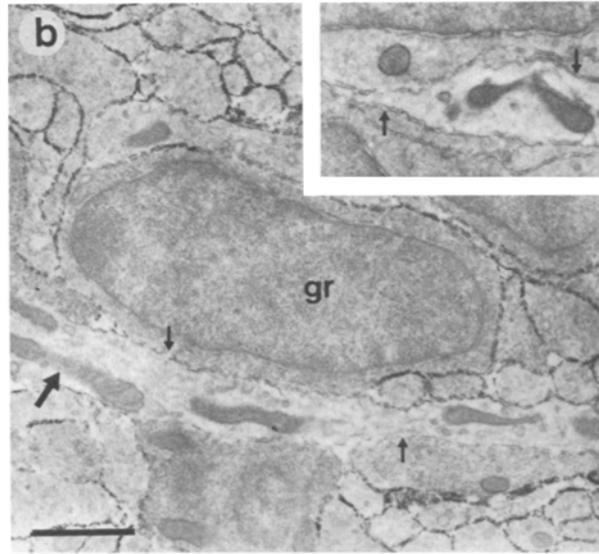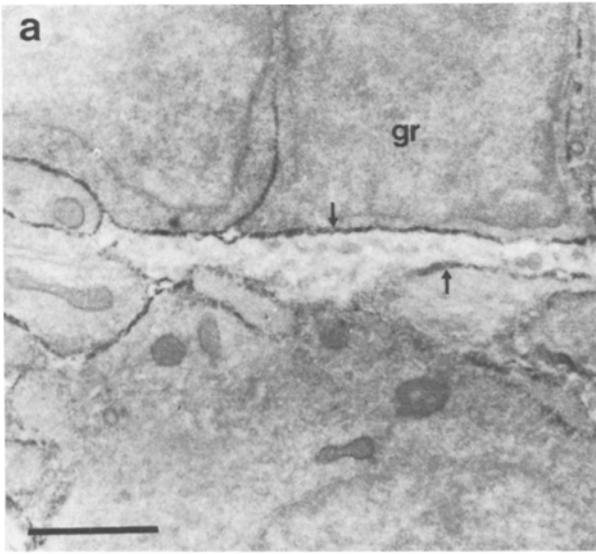
detectable at contact sites between Bergmann glial process and migrating granule cell body and leading process (Figs. 1 *b* and 2, *a* and *e*). Parallel fibers are also N-CAM positive with a more diffuse immunoperoxidase reaction product than Ll (Figs. 2 *a* and 3 *a*). Purkinje cell dendrites are N-CAM positive. They are N-CAM positive at contact sites between parallel fibers (Fig. 3 *a*), on dendritic thorns (spines), and at subsynaptic membranes (Fig. 3 *f*).

N-CAM₁₈₀

Bergmann glial processes do not express detectable levels of N-CAM$_{180}$ in 8- or 21-d-old mice (Fig. 2 *c*). Only postmigratory granule cell bodies express N-CAM$_{180}$ (not shown). N-CAM$_{180}$ is not detectable on mitotic, postmitotic, and premigratory, or migrating granule cells (Fig. 3 *c*). Purkinje cell dendrites and cell bodies express N-CAM$_{180}$ in an intracellularly localized, diffuse staining pattern at postnatal day 8 (Fig. 3 *c*). At day 21 (Fig. 3 *d*) it is not seen in Purkinje cell dendrites. At both ages parallel fibers are either not N-CAM$_{180}$ positive or if so, only weakly (Fig. 3, *c* and *d*). The immunoperoxidase reaction product tends to appear at restricted sites (Fig. 3, *c* and *d*).

Discussion

The developmental appearance of Ll and N-CAM during early postnatal cerebellar morphogenesis follows a temporal sequence that is best exemplified by the granule neuron. N-CAM$_{140}$ and/or N-CAM$_{120}$ are already expressed on mitotic neurons and remain expressed on these cells at least in the postmitotic, premigratory, and migratory phases. Ll is first detectable on postmitotic, premigratory granule neurons, remains expressed during migration and several days after migration, but ceases to be detectable after ∿2 wk (see also Fushiki and Schachner, 1986; Schachner et al., 1985; Sajovic et al., 1986). It is our impression that the leading process expresses higher levels of Ll antigen than the cell body or trailing process. N-CAM$_{180}$ is the last to appear and is first detectable on postmigratory granule neurons. A prevalent expression of N-CAM$_{180}$ in the internal granular layer has previously been observed by biochemical analysis (Nagata and Schachner, 1986). Since the pattern of immunohistological labeling of N-CAM$_{180}$ resembled that of Ll at the light microscopic level, N-CAM$_{180}$ was thought to be

expressed on granule cells already before migration (Pollerberg et al., 1985). The higher resolution of immunoelectron microscopy of this study revealed, however, that immunoreactivity in the inner part of the external granular layer must be attributed to the presence of N-CAM$_{180}$ on parallel fibers and Purkinje cell dendrites which are N-CAM$_{180}$-positive. The observation that N-CAM$_{180}$ is not detectable before stable cell contacts are formed by the postmigratory granule cells is consistent with our notion that the N-CAM component with the longest cytoplasmic domain (Cunningham et al., 1983; Gennarini et al., 1984) is involved in the stabilization of cell contacts (Pollerberg et al., 1985). A reduced lateral mobility of N-CAM$_{180}$ vs. N-CAM$_{140}$ within the plasma membrane and the specific association of N-CAM$_{180}$ with the membrane-cytoskeleton linker protein brain spectrin (Pollerberg et al., 1986) would be the molecular basis of this stabilization. Stabilization may well result from cooperative activity between N-CAM$_{180}$ and Ll; Ll and N-CAM act in synergism in the adhesion between cerebellar cells (Faissner et al., 1984) and appear to be closely associated with each other in the surface membrane of cells stabilized on a tissue culture substrate (Thor et al., 1986).

Besides the temporal modification of cell adhesion molecule expression, the regulation of expression on particular cell types and even particular subcellular compartments of the surface membrane offers yet other possibilities for specification of cell contacts. Again, Ll and N-CAM show distinct patterns of distribution. Our study shows for the first time that Ll expression appears to be restricted in its ability to detect certain neuronal cell types. In the cerebellar cortex, it is not seen on stellate and basket cells. Also, while N-CAM$_{140}$ and/or N-CAM$_{120}$ are seen expressed by Bergmann glial processes, N-CAM$_{180}$ is not. An even higher level of regulation is required of a cell when expression of a membrane component occurs in one subcellular compartment but not in another. For instance, Ll is expressed by differentiated neurons (e.g., Purkinje and postmigratory granule cells), only on axons, but is not seen on cell body or dendrites. It is likely that the prominent expression of Ll on parallel fibers that are the fasciculating axons of granule cells may constitute the molecular basis for the maintenance of the fasciculative state in adulthood. It is noteworthy that Ll appears on these axons in a more punctate state than N-CAM which is more diffusely localized over the surface membrane of paral-

Figure 2. Immunoelectron microscopic localization of Ll (*b, d,* and *f*), N-CAM (*a* and *e*), and N-CAM$_{180}$ (*c*) in the cerebellum of 8-d-old (*a–c, e,* and *f*) and 21-d-old (*d*) mice. Immunolabeling was performed by preembedding (*a–d*) and postembedding (*e* and *f*) staining procedures. Sections were counterstained, except for *a* and *b*. Inset in *b* shows a control section treated with hemoglobin antibody by the preembedding method. (*a–c*) Migrating granule cell (*gr*) in contact with a Bergmann glial process in the inner part of the external granular layer. (*a*) Notice the N-CAM–positive granule cell body and Bergmann glial process (*arrows*). The contact site between migrating granule cell body and Bergmann glial process is N-CAM positive. (*b*) The Bergmann glial process is Ll negative. The surface membrane of the migrating granule cell body is Ll positive except at the contact site with the Bergmann glial fiber. No peroxidase reaction product is seen at contact sites between granule cell body, leading process (*small arrows*), and Bergmann glial process (*large arrow*). Inset on upper right shows a control section in which Ll antibody was replaced by hemoglobin antibody. No difference in staining intensity of membranes at the Bergmann glia–granule cell apposition is seen between Ll and hemoglobin antibody-treated sections. (*c*) The migrating granule cell body and Bergmann glial process are N-CAM$_{180}$ negative. No peroxidase reaction product can be seen at the contact site between granule cell and Bergmann glial process (*arrows*). (*d*) The stellate cell (*st*) in the molecular layer is Ll negative on cell body and axon (*small arrows*). Peroxidase reaction product is only seen on the surface membrane of parallel fibers (*large arrows*). (*e*) Migrating granule cell (*gr*) is N-CAM positive at contact sites with Bergmann glial processes (*gl*). Note the colloidal gold particles (*arrows*). The gold particles are associated mainly with surface membranes. (*f*) Purkinje cell soma (*Pc*) is Ll antigen negative (*arrows*). Parallel fibers cut in cross sections (*pf*) are Ll positive. Ll is detectable exclusively on the surface membrane. Bars: (*a–d*) 1 μm; (*e* and *f*) 0.1 μm.
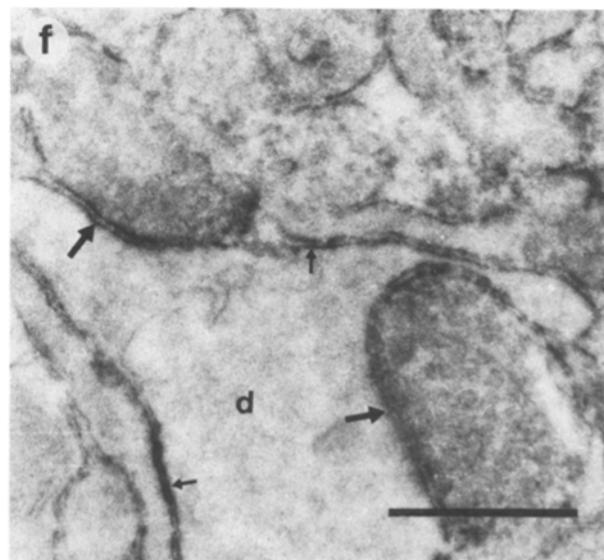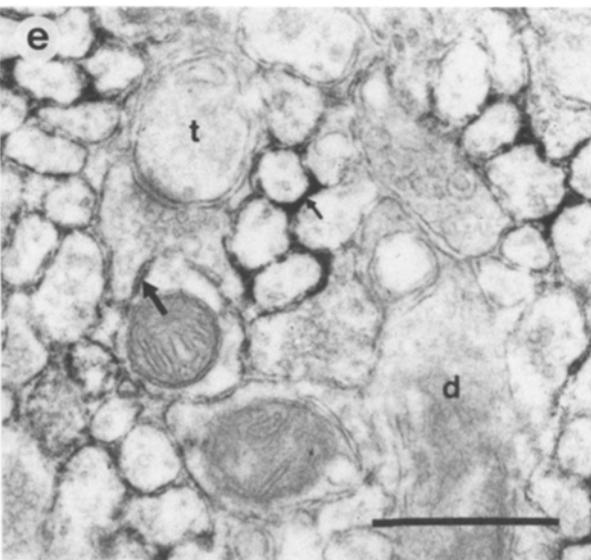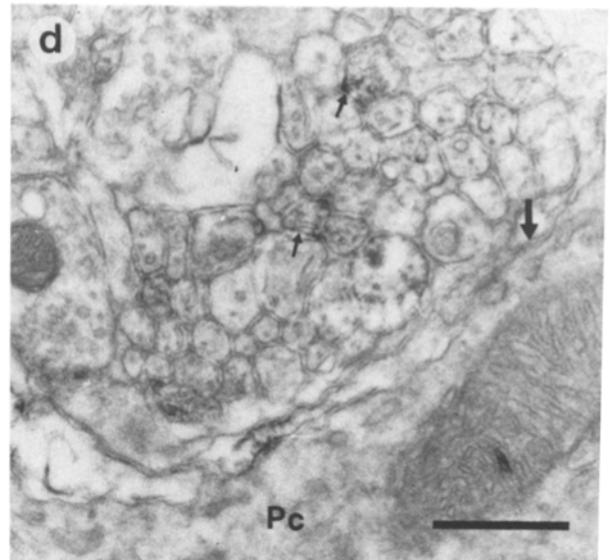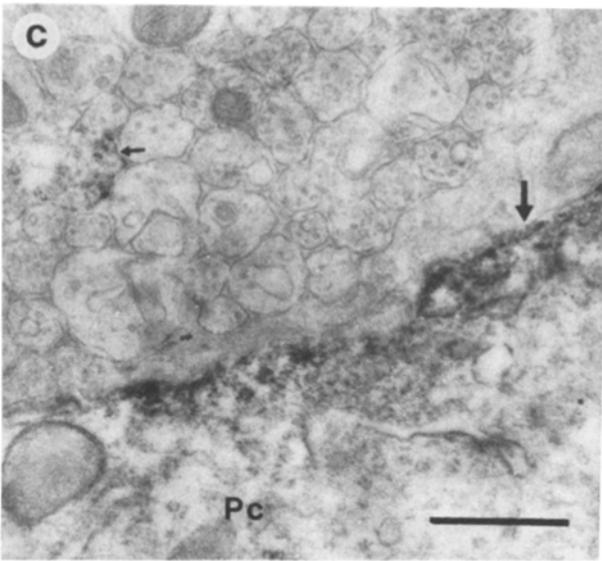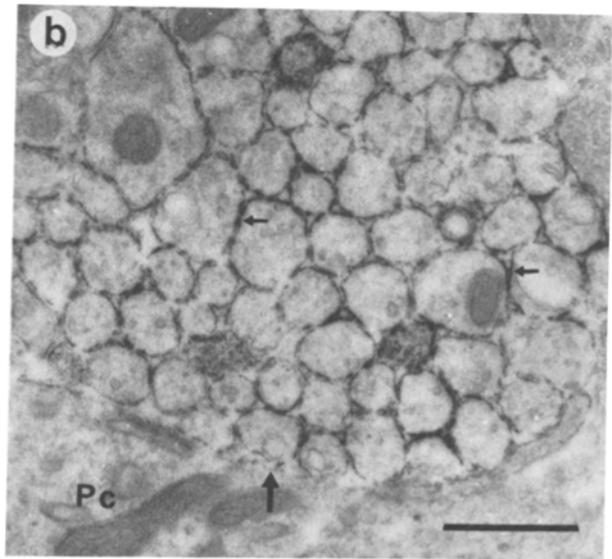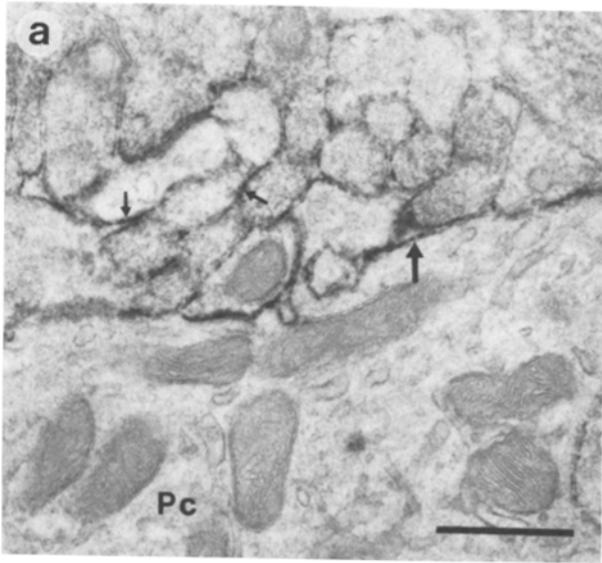
Table I. Presence of Immunoreactivity in Particular Morphological Structures in the Mouse Cerebellar Cortex

Antibodies	L1	N-CAM$_{total}$	N-CAM$_{180}$
Bergmann glial processes	−	+	−
Granule cell axons (parallel fibers)	+	+	±
Mitotic granule cells	−	+	−
Postmitotic premigratory granule cells	+	+	−
Migratory granule cells			
Contact site with glia	−	+	−
Contact site with neurons	+	+	−
Postmigratory granule cells	+	+	+
Stellate and basket cells	−	+	ni
Purkinje cells			
Dendrite	−	+	+(P8) −(P21)
Cell body	−	+	+(P8) −(P21)
Axon	+	+	ni
Purkinje cell–parallel fiber synapses	−	+	ni

Indirect immunoelectron microscopy of mouse cerebellar cortex at postnatal days 8 and 21 was performed with L1, N-CAM$_{total}$, and N-CAM$_{180}$ antibodies. Results were the same with pre- and postembedding staining procedures. P, postnatal day; ni, not investigated; N-CAM$_{total}$, antibodies that react with all three components of N-CAM (N-CAM$_{180}$, N-CAM$_{140}$, and N-CAM$_{120}$); −, no immunoreactivity; ±, weak immunoreactivity; +, good immunoreactivity.

lel fibers. That both molecules and their counterpart in chicken, Ng-CAM, indeed mediate neurite fasciculation in vitro is now well documented (Bock et al., 1985; Fischer et al., 1986; Rutishauser et al., 1978; Stallcup and Beasley, 1985). It is worth commenting in this context on the absence of L1 on the nonfasciculating axons of stellate and basket cells which are oriented perpendicularly to the axons of granule cells. It is conceivable that this absence is the molecular basis for the 90° shift in positioning between the inhibitory interneurons (stellate and basket cells) and granule cells, which all happen to derive from a common precursor cell.

The fact that L1 is found only at contact sites between surface membranes that both express L1 (see also Martini and Schachner, 1986) and is always excluded from contact sites between an L1-positive surface membrane and an apposing L1-negative one raises questions as to the underlying molecular mechanisms in terms of polarization of adhesion molecules in the surface membranes of one cell. Particularly pertinent with regard to granule cell migration is, therefore, the

observation that L1 is not seen at contact sites between neurons and glia, thus supporting previous evidence from adhesion experiments that L1 is involved in neuron–neuron, but not neuron–glia adhesion (Keilhauer et al., 1985). A neuron–neuron interaction between granule cells migrating in tandem packages along Bergmann glial processes (Altman, 1976) could be inferred from our present observations. On the basis of immunohistological evidence we have also suggested that L1 may not be involved in the initiation of migration of neurons on radial glial fibers in the telencephalic anlage, but rather in the clustering of postmigratory neurons in the final stages of their migratory pathway and aggregation of neuronal cell bodies at the end of migration (Fushiki and Schachner, 1986). These observations are in contrast to those on Ng-CAM, which is thought to mediate adhesion between neurons and neurons as well as neurons and glia (Grumet et al., 1984a, b; Hoffman et al., 1986). Thus, our present observations would rather support than contradict the contention that the inhibitory effect of L1 antibodies on granule cell migration in vitro is due to the disturbance of tandem alignment of migrating neurons, fasciculation of granule cell axons, or sorting out of postmitotic, premigratory granule cells from mitotic ones (Keilhauer et al., 1985). Furthermore, it is tempting to speculate that L1 on one cell recognizes and interacts with L1 on the partner cell (see also Martini and Schachner, 1986). We have, however, not been able to show a specific and controlled interaction of purified L1 with itself, although L1 has been observed to adhere unusually tightly to itself even in the presence of higher concentrations of detergents and when inserted into liposomes (Sadoul, K., R. Sadoul, and A. Faissner, unpublished observations).

The expression of N-CAM$_{180}$ on Purkinje cell body and dendrites at day 8, but not at day 21 is another example of a highly regulated expression of an adhesive cell surface component. The interpretation of this finding in terms of physiological function remains difficult, however, particularly in view of the notion that N-CAM$_{180}$ is characteristic of stabilized cell surface contacts. Thus, climbing fiber synapses on Purkinje cells are transient on the cell body at early postnatal stages and move to dendrites at later developmental stages to form stable synapses (Mariani and Changeux, 1981a, b).

The present study indicates that the temporal and spatial expression of only two cell adhesion molecules can give rise to an already complex level of functional combinations. This includes the independent regulation of expression of one of

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Figure 3. Immunoelectron microscopic localization of L1 (*b* and *e*), N-CAM (*a* and *f*), and N-CAM$_{180}$ (*c* and *d*) in the cerebellum of 8- (*a–f*) and 21-d-old (*d* and *e*) mice. Immunolabeling was performed by preembedding staining procedures. Sections were not counterstained. (*a–d*) Purkinje cell (*Pc*) dendrite and parallel fibers. (*a*) The surface membrane of the Purkinje cell dendrite is N-CAM positive at contact sites (*large arrow*). Note the peroxidase reaction product on the surface membrane of parallel fibers with a somewhat diffuse and less distinct appearance than the L1 antibody–dependent reaction product (*small arrow*, and compare with *b*). (*b*) The contact sites between Purkinje cell dendrite and parallel fibers are L1 negative (*large arrow*). Note the somewhat punctate peroxidase reaction product on the surface of parallel fibers (*small arrows*). (*c*) Purkinje cell dendrite of 8-d-old mouse is N-CAM$_{180}$ positive (*large arrow*). The mostly intracellularly localized peroxidase reaction product is diffusely distributed. Parallel fibers are either N-CAM$_{180}$ negative or weakly stained (*small arrow*). (*d*) Purkinje cell dendrite of 21-d-old mouse is N-CAM$_{180}$ negative (*large arrow*). Parallel fibers are N-CAM$_{180}$ negative or weakly stained (*small arrows*). (*e*) Dendritic spine or thorn (*t*) of Purkinje cell in the molecular layer is L1 negative. Synaptic membranes are also L1 negative. The preterminal part of the axon is always less strongly L1 positive (*large arrow*) than the parallel fibers (*small arrow*). Purkinje cell dendrite (*d*) is L1 negative. (*f*) The Purkinje cell dendrite (*d*) in the molecular layer is N-CAM positive (*small arrows*). Parallel fibers and synaptic membranes (*large arrows*) show the diffuse peroxidase reaction product characteristic of N-CAM (see also *a*). Bars, 0.5 μm.

the components of N-CAM, N-CAM$_{180}$, from the other(s) and from L1. Taken together with the indication that L1 and N-CAM influence each others' adhesive potencies, a very exquisite network of interactions appears to be possible merely with these two molecules. In addition, the two cell adhesion molecules express the functionally important L2 carbohydrate epitope that is developmentally regulated independently of the protein backbones (Keilhauer et al., 1985; Kruse et al., 1984; Wernecke et al., 1985). Furthermore, other adhesion molecules are involved in the migration of granule cells, such as the novel neuron–glia adhesion molecule, AMOG (Antonicek et al., 1986). Unraveling this already enormous complexity in possibilities of cell surface interactions has grown into an overwhelming task for the future.

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