

# The Catenin/Cadherin Adhesion System Is Localized in Synaptic Junctions Bordering Transmitter Release Zones

Naoshige Uchida,\* Yasuko Honjo,\* Keith R. Johnson,<sup>§</sup> Margaret J. Wheelock,<sup>§</sup> and Masatoshi Takeichi\*

\*Department of Biophysics, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan; and<sup>§</sup>Department of Biology, University of Toledo, Toledo, Ohio 43606

**Abstract.** Molecular mechanisms linking pre- and postsynaptic membranes at the interneuronal synapses are little known. We tested the cadherin adhesion system for its localization in synapses of mouse and chick brains. We found that two classes of cadherin-associated proteins,  $\alpha$ N- and  $\beta$ -catenin, are broadly distributed in adult brains, colocalizing with a synaptic marker, synaptophysin. At the ultrastructural level, these proteins were localized in synaptic junctions of various types, forming a symmetrical adhesion structure. These structures sharply bordered the transmitter release sites associated with synaptic vesicles, although

their segregation was less clear in certain types of synapses. N-cadherin was also localized at a similar site of synaptic junctions but in restricted brain nuclei. In developing synapses, the catenin-bearing contacts dominated their junctional structures. These findings demonstrate that interneuronal synaptic junctions comprise two subdomains, transmitter release zone and catenin-based adherens junction. The catenins localized in these junctions are likely associated with certain cadherin molecules including N-cadherin, and the cadherin/catenin complex may play a critical role in the formation or maintenance of synaptic junctions.

THE synapse is a site where the axon terminal of a neuron comes into functional contact with a target cell. To generate the synapses, each neuron selectively contacts and communicates with other particular neurons. Despite the importance of understanding how specific neuronal connections are established, little is known about what kinds of adhesion molecules are essential for the formation and maintenance of interneuronal synaptic junctions. This lack of information is contrasted with a great deal of knowledge about other synaptic structures and functions, including synaptic vesicle traffic, channel/receptor function, and signal transduction in synapses (Jessell and Kandel, 1993; Südhof, 1995).

Various electron microscopic studies have revealed the ultrastructural features of interneuronal synaptic junctions. Electron-dense cytoplasmic materials accumulate under both pre- and postsynaptic plasma membranes; and the former is, in general, associated with a cluster of synaptic vesicles. These junctional structures are considered to be the sites where synaptic transmission takes place. The interneuronal synaptic junctions exhibit some variations in morphological characteristics of the cytoplasmic density. Gray (1959) and Colonnier (1968) showed that there are two types of synapses, asymmetric (Gray's type I) and symmetric (Gray's type II). The intercellular space be-

tween the membrane thickenings, called the synaptic cleft, is occupied by filamentous materials.

Molecular analysis of the synaptic junctions has thus far been done mostly for neuromuscular junctions. For these junctions, some intercellular components were identified, which include s-laminin/laminin  $\beta$ 2 and agrin, components of the basal lamina running along between the motor nerve ending and muscle (Hall and Sanes, 1993; Noakes et al., 1995; Gautam et al., 1996). We should stress that the interneuronal and neuromuscular synapses are morphologically distinct from one another. In particular, the interneuronal synapses do not contain a basal lamina. As a consequence, the synaptic cleft is narrower in the interneuronal synapses ( $\sim$ 10 to 20 nm) than in the neuromuscular junctions (50 nm) (Peters et al., 1991). Thus, the interneuronal synapses represent a type of direct cell-cell contact, whereas the neuromuscular junctions are mediated by extracellular matrix structures. These differences strongly suggest that distinct mechanisms operate for the formation and maintenance of these two types of synapses.

The "classic" cadherins are a family of  $\text{Ca}^{2+}$ -dependent cell-cell adhesion molecules and are crucial for intercellular adhesion of most cell types. There are many subtypes of the classic cadherin, and  $>10$  subtypes are known to be expressed in the developing brain (Redies, 1995). Each cadherin shows a homophilic binding activity, and this type of molecular interaction results in the endowment of cells with specific adhesiveness (Takeichi, 1995). Cadherins are highly concentrated in specialized cell-cell junctions, called zonula adherens in epithelia (Boller et al., 1985; Takeichi, 1988), fascia adherens in the intercalated

Address all correspondence to Masatoshi Takeichi, Department of Biophysics, Faculty of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-01, Japan. Tel.: (81) 75 751 2111. Fax: (81) 75 753 4197. e-mail: takeichi@take.biophys.kyoto-u.ac.jp.

disks of cardiac muscles (Volk and Geiger, 1986), and autotypic adherens junction in Schwann cells (Fannon et al., 1995). Recently, M-cadherin was detected in a type of nonsynaptic interneuronal junction designated as the contactus adherens (Rose et al., 1995).

Cadherin function is controlled by proteins associated with the cytoplasmic domain of cadherin, which are collectively called catenins (Kemler, 1993). These include  $\alpha$ -catenin,  $\beta$ -catenin, and plakoglobin. Through these proteins, cadherins are thought to interact with the cytoskeleton, and this interaction seems to be essential for the cadherins to exert their full activity. It is now known that  $\beta$ -catenin directly associates with the cytoplasmic domain of cadherins and that  $\alpha$ -catenin binds to this  $\beta$ -catenin (Aberle et al., 1994; Jou et al., 1995); i.e.,  $\beta$ -catenin is a mediator for the interaction between cadherin and  $\alpha$ -catenin. Two subtypes of  $\alpha$ -catenin were identified: one is  $\alpha$ E-catenin, expressed mainly by nonneural tissues (Nagafuchi and Tsukita, 1994); the other,  $\alpha$ N-catenin, is widely expressed by the nervous system (Hirano et al., 1992; Uchida et al., 1994; Hirano and Takeichi, 1994). Both subtypes can equally support cadherin function (Hirano et al., 1992; Watabe et al., 1994).

Recently, N-cadherin was detected at synaptic clefts of tectal neurons (Yamagata et al., 1995), and also in a postsynaptic density fraction (Beesley et al., 1995). The present study aimed to investigate whether the cadherin/catenin system functions as a general adhesion machinery for interneuronal synaptic junctions. We examined the localization of  $\alpha$ N-catenin and  $\beta$ -catenin in adult and postnatal brains, which are known to associate with multiple subtypes of the classic cadherin. This approach was expected to provide us with a broader view of the localization of this adhesion system in the brain rather than studying particular cadherin subtypes. To confirm colocalization of cadherin and catenins, we also studied N-cadherin expression and their complex formation. Our results demonstrate that the above catenins as well as N-cadherin are localized in a subdomain structure of synaptic junctions that borders the transmitter release zones, but not throughout the junctional spaces, thus in part inconsistent with the previous report (Yamagata et al., 1995). These findings revealed a novel profile of the adhesion structure in interneuronal synaptic junctions.

## Materials and Methods

### Animals

ICR mice and newly hatched chickens were used. The day when the animals were born or hatched was regarded as the postnatal or posthatching day 0 (P0). For adult mice, those older than 1 mo were used. The identification of anatomical sites in the chicken brains was done according to Kuenzel and Masson (1988).

### Antibodies

NCAT2 (Hirano et al., 1992), a rat mAb against  $\alpha$ N-catenin, was used for production of other mAbs to this protein. Immunoprecipitates with NCAT2 were obtained from chick brain lysates as described below. Rats were immunized with these materials (20 brain samples per rat), and their splenocytes were used for production of hybridomas. From the hybridomas thus obtained, three mAbs, NCAT3, 4, and 5, were isolated. All of them reacted with chick, mouse, and rat  $\alpha$ N-catenin and recognized two isoforms of this molecule (Uchida et al., 1995). NCAT4 and 5 reacted spe-

cifically with  $\alpha$ N-catenin but not with  $\alpha$ E-catenin, but NCAT3 crossreacted with  $\alpha$ E-catenin. We mainly used NCAT5 for immunohistochemistry, and NCAT2 for immunoblotting and immunoprecipitation.

To detect  $\beta$ -catenin, we used mouse mAb 5H10 or rabbit polyclonal antibodies (Shibamoto et al., 1995). For detection of N-cadherin, rat mAb NCD2 (Hatta and Takeichi, 1986) was used. Rabbit antibodies to synaptophysin (A010; Dako, Santa Barbara, CA) and mouse mAb to calbindin-D (C-8666; Sigma Chemical Co., St. Louis, MO) were also used. DCAT1 (Oda et al., 1994) was used as a control antibody for immunoprecipitation.

### Immunoprecipitation

Immunoprecipitation was carried out according to Watabe et al. (1994). Briefly, newly hatched chick brains were homogenized and extracted in an ice-cold extraction buffer (1% Triton, 1% NP-40, 1 mM EDTA, 1 mM PMSF in 50 mM TBS, pH 7.6) with gentle rocking for 30 min. The extract was centrifuged, and the supernatant was incubated with NCAT2 or control antibody for 1 h, followed by incubation with Sepharose 4B-linked secondary antibody (Zymed Laboratories, Inc., South San Francisco, CA) for 30 min. The bound antigens were released by incubation with 50 mM triethylamine (pH 11.5).

### Immunofluorescence Staining

Mice or chicks were deeply anesthetized with sodium pentobarbital and perfused through the heart with ice-cold 4% paraformaldehyde in PBS. Their brains were dissected out and postfixed with the same fixative at 4°C for 2 h. The tissues were incubated with 10, 15, 20, and 25% sucrose in PBS at 4°C for >8 h for each step. The samples were next incubated in a 1:1 mixture of 25% sucrose and Tissue Tek compound (Miles Laboratories, Inc., Naperville, IL) at 4°C for 30 min, and then embedded in the Tissue Tek compound and frozen with liquid nitrogen. They were sectioned at 6 to 10  $\mu$ m with a cryostat, collected on glass slides coated with 3-aminopropyltriethoxysilan, and immediately dried under a flow of cold air. The sections were then incubated with PBS for 30 s and fixed again with ice-cold 4% paraformaldehyde in PBS for 5 min, followed by three washes with PBS. These sections were further incubated with TBS for 5 min, and then incubated with TBS containing 5% BSA and 0.02% saponin (pH 7.6) for 15 min. The sections were incubated with a primary antibody in TBS containing 1% BSA and 0.005% saponin for 2 h, followed by three washes with TBS containing 0.005% saponin. They were incubated for 1 h with a fluorescence-labeled second antibody, washed with TBS containing 0.005% saponin, and mounted with a 9:1 mixture of glycerol and TBS containing 1 mg/ml paraphenyldiamine. For double-immunofluorescence staining, we used Cy3-conjugated anti-rat or -mouse antibodies (Chemicon Intl., Inc., Temecula, CA), and FITC-conjugated anti-rabbit antibody (Dako). These samples were observed under a microscope (Axiophot, Carl Zeiss, Inc., Thornwood, NY). Confocal images were collected by a confocal laser scanning microscope (MRC1024; Bio Rad Laboratories, Hercules, CA).

For staining of cultured cells, the cells were fixed with 4% paraformaldehyde at 4°C for 15 min, washed with saline, permeabilized with -20°C methanol for 15 min, and washed with TBS. They were subsequently immunostained as described above.

### Immunoelectron Microscopy

Immunoelectron microscopy was done as described by Burry et al. (1992) and Jongens et al. (1994) with modifications. Brains were perfused and postfixed with 4% paraformaldehyde in PBS at room temperature for 2 h. Cryosections were cut at a thickness of 10 to 15  $\mu$ m and collected on glass slides as described above. The following steps were carried out at room temperature: the sections were incubated with PBS for 30 s, and additionally fixed with 4% paraformaldehyde for 15 min, followed by three washes with PBS. (In preparing samples for N-cadherin staining, the fixation protocol used for immunofluorescence staining [see above] was used to preserve its antigenicity during the above steps.) Then the sections were incubated with TBS for 5 min, and with TBS containing 5% BSA and 0.02% saponin (pH 7.6) for 15 min. The sections were incubated with a primary antibody in TBS containing 1% BSA and 0.005% saponin for 4 h, followed by three washes with TBS containing 0.005% saponin for 20 min in each step. The sections were incubated with a secondary antibody coupled with 1.4-nm gold particles (Nanoprobes, Inc., Stony Brook, NY) for >12 h. After a washing with TBS containing 0.005% saponin, the sections were incubated twice in PBS for 5 min, and then fixed with 2% glutaraldehyde

in PBS for 15 min. The sections were again washed twice with PBS for 5 min and three times with a washing buffer (50 mM Hepes and 200 mM sucrose, pH 5.8) for 20 min. The signals were silver-enhanced by use of HQ silver (Nanoprobes) for 10 to 15 min at 18°C in the dark, followed by three washes with a neutral fixer (20 mM Hepes and 250 mM sodium thiosulfate, pH 5.8) for 5 min each time. Then the sections were rinsed twice in a 100 mM cacodylate buffer (pH 7.3) for 5 min, fixed with 0.1% osmium oxide in this buffer for 30 min on ice, and washed twice with the same buffer. The sections were dehydrated by passage through a graded series of ethanol (50, 70, 90, 95, and 100%) and propylene oxide, and they were embedded in epoxy resin (Polysciences, Inc., Warrington, PA). From these samples, ultrathin sections were cut, stained with uranyl acetate and lead citrate, and then observed with a JEM-1010 (JEOL, Tokyo, Japan). To intensify the signals, we often used the avidin-biotin system using biotinylated secondary antibodies (Amersham Corp., Arlington Heights, IL) and 1.4-nm gold-conjugated streptavidin (Nanoprobes, Inc.).

For immunoperoxidase staining, we used a Vectastain ABC kit (Vector Laboratories, Inc., Burlington, CA). After postfixation with 2% glutaraldehyde in PBS as described above, sections were washed twice with PBS and three times with TBS. The sections were next incubated with 0.5 mg/ml DAB in TBS for 15 min, and then reacted with 0.5 mg/ml DAB and 0.01% hydrogen peroxide in TBS until the reaction products became visible. The sections were dehydrated, embedded, and observed as described above, except that the staining with uranyl acetate and lead citrate was omitted.

### Conventional EM

Mouse brains were fixed by perfusion with 2% paraformaldehyde and 2.5% glutaraldehyde in 100 mM cacodylate buffer. The brains were dissected out and additionally fixed with the same fixative for 1 h at room temperature. The blocks were fixed with 2% osmium oxide for 1 h on ice, and then stained with 0.5% uranyl acetate for 2 h. Next, they were dehydrated and embedded in epoxy resin. The sections were prepared as described above.

### In Situ Hybridization

In situ hybridization was carried out according to Shimamura et al. (1994) with slight modifications. P2 chickens were perfused with ice-cold 4% paraformaldehyde in PBS. Then the brains were dissected out and post-fixed with the same fixative at 4°C for 2 h. They were washed three times with PBS at 4°C for 5 min. The brains were embedded in 3% agarose in PBS and sectioned at 150 to 300  $\mu$ m with a Microslicer (DTK-1000; D.S.K., Kyoto, Japan). The slices were immediately immersed in 100% methanol and subjected to in situ hybridization. The 450-bp PCR fragment of chick N-cadherin subcloned into Bluescript vector (Nakagawa and Takeichi, 1995) was used as a template for antisense or sense probes.

## Results

### Codistribution of Catenins and Synaptophysin

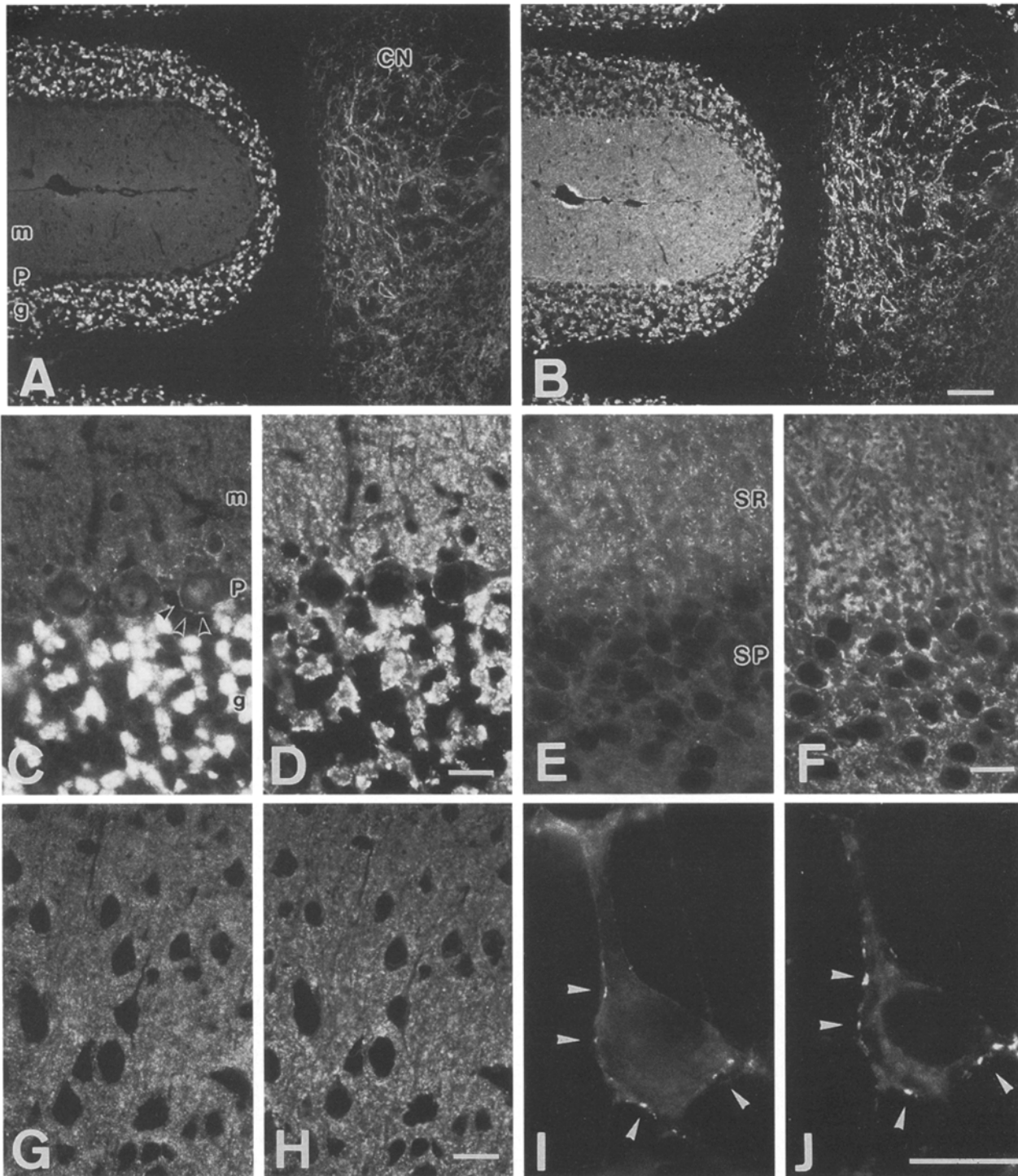
We immunostained the cerebellum, hippocampus, and cerebral cortex of adult mouse brains for  $\alpha$ N-catenin and found that this cadherin-associated protein is widely distributed in these brain regions. Its staining pattern was then compared with that of synaptophysin, a synaptic vesicle protein, which is known to be concentrated in virtually all nerve terminals (Navone et al., 1986). We found that their distributions closely overlapped each other, even though they were not identical. In the cerebellum, the granular layer and cerebellar nuclei strongly stained for both  $\alpha$ N-catenin and synaptophysin (Fig. 1, A and B). In the granular layer, their intense signals were localized in the cerebellar glomeruli. The surfaces of Purkinje cell perikarya also showed sharp, discontinuous signals of these two proteins, which were strictly colocalized with each other (Fig. 1, C and D). In the hippocampus (Fig. 1, E and F) and cerebral cortex (Fig. 1, G and H),  $\alpha$ N-catenin and synaptophysin signals were broadly codistributed. Es-

entially all the areas of the brain showed coexpression of these molecules. There were, however, local variations in the level of  $\alpha$ N-catenin expression; for example, the molecular layer of the cerebellum exhibited only faint  $\alpha$ N-catenin expression, represented by scattered dotted signals, although it had strong synaptophysin signals (Fig. 1, C and D). Axon fascicles stained little for this catenin in the adult brain. We also localized  $\beta$ -catenin and found that its expression pattern was similar to that of  $\alpha$ N-catenin, except that  $\beta$ -catenin was more widely distributed; for example,  $\beta$ -catenin was detected in the choroid plexus and endothelial cells of blood vessels, but  $\alpha$ N-catenin was not.

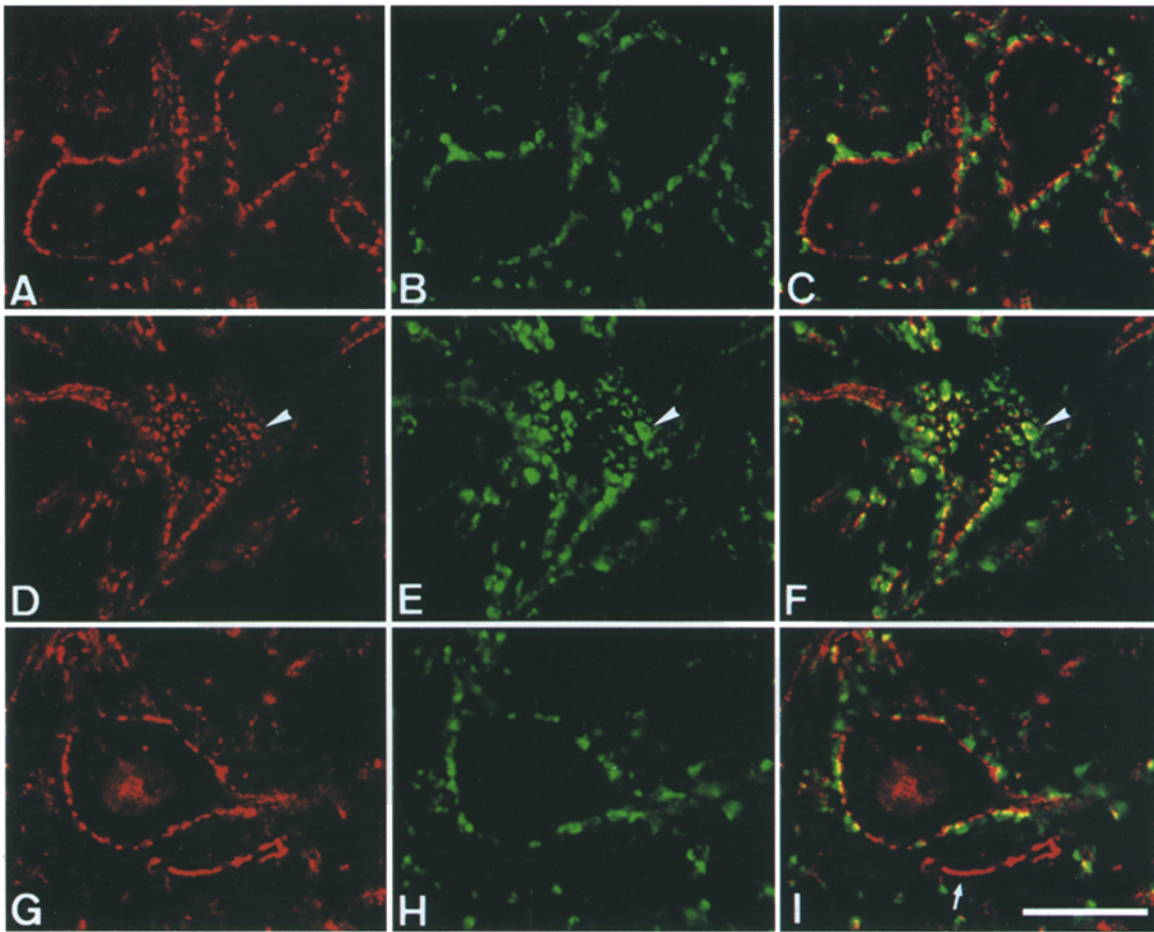
For closer observations of the colocalization of synaptophysin and  $\alpha$ N-catenin, the cerebellar nuclei were double-immunostained for these proteins, and then subjected to confocal microscopic analysis. At the lateral surface of neuronal bodies or dendrites, catenin signals were observed as sharp, punctate signals along their borders (Fig. 2 A). Synaptophysin was also detected as patchy signals surrounding the cell bodies and dendrites (Fig. 2 B), which may represent presynaptic boutons. Double-staining images for the catenins and synaptophysin clearly showed that their signals overlapped each other (Fig. 2 C). When focus was adjusted to the top surface of cell bodies, we could observe the overall morphology of these structures. Each  $\alpha$ N-catenin signal exhibited a small dot with irregular morphology (Fig. 2 D). Synaptophysin signals were larger and often circular (Fig. 2 E), and a single synaptophysin-positive zone tended to contain multiple, separated signals of  $\alpha$ N-catenin (Fig. 2 F). A similar distribution pattern was observed for  $\beta$ -catenin (Fig. 2, G–I). We also examined primary cultures of hippocampal neurons. Synaptophysin was detected as dotted signals on their cell body and dendrites.  $\alpha$ N-catenin was more broadly distributed on the neuronal surfaces; however, its strong signals were colocalized with those of synaptophysin (Fig. 1, I and J).

### Immunoelectron Microscopic Localization of $\alpha$ N- and $\beta$ -Catenin

To determine the subcellular location of the above cadherin-associated proteins, we performed immunoelectron microscopy. We first examined cerebellar nuclei to locate  $\alpha$ N-catenin, using the peroxidase staining method, and found that it was concentrated in synaptic junctional areas (Fig. 3 A). For more accurate determination of the catenin localization, we used the preembedding immunogold labeling method. By means of this staining, we detected  $\alpha$ N- and  $\beta$ -catenin signals in synaptic junctions as clusters distributed in a symmetrical fashion underneath the pre- and postsynaptic plasma membranes (Fig. 3, B–D). These catenin signals were often located next to the transmitter release zones that could be identified by their association with synaptic vesicle clusters (Fig. 3, B and D). The catenins were found either at both sides of the transmitter release zone or at a single side. Some of the catenin signals were isolated from the transmitter release zones (Fig. 3 C). Behind these catenin signals, symmetrical paramembranous dense materials were observed. The typical length of the catenin-positive structures was 70 to 200 nm. When histological sections of cerebellar nuclei were carefully examined, junctions with symmetrical cytoplasmic plaques,



**Figure 1.** Codistribution of  $\alpha$ N-catenin and synaptophysin in adult brain sections and in cultured neurons. All the panels show double-immunofluorescence staining for  $\alpha$ N-catenin (A, C, E, G, and I) and synaptophysin (B, D, F, H, and J). (A and B) Cerebellum at low magnification. (C and D) Part of the cerebellar cortex. Strong signals in the granular layer represent cerebellar glomeruli. Note the colocalization of  $\alpha$ N-catenin and synaptophysin on Purkinje cell perikarya (arrowheads). (E and F) CA3 region in hippocampus.  $\alpha$ N-catenin signals are observed as small dots in the stratum radiatum. (G and H) Part of the cerebral cortex. (I and J) A hippocampal neuron cultured in vitro. (Arrowheads) Coincident signals of  $\alpha$ N-catenin and synaptophysin. E18 rat hippocampal neurons were cultured on glial cell monolayers for 14 d before use, as described by Baughman et al. (1991) with modifications. CN, cerebellar nucleus; m, molecular layer; p, Purkinje cell layer; g, granular layer; SR, stratum radiatum; SP, stratum pyramidale. Bars: (A and B) 100  $\mu$ m; (C–I) 20  $\mu$ m.



**Figure 2.** Confocal images of immunofluorescence staining for catenins and synaptophysin in an adult cerebellar nucleus. (A–F) Staining for  $\alpha$ N-catenin (red) and synaptophysin (green). (G–I) Staining for  $\beta$ -catenin (red) and synaptophysin (green). (A, B, D, E, G, and H) Images of each single component. (C, F, and I) Double-staining images. In D–F, focus was adjusted to the top surface of a neuronal body, while in the other sets of panels, focus was adjusted to the lateral surface of neuronal bodies. Catenins show punctate, dotted signals. Each presynaptic bouton, depicted by a single patch of synaptophysin signal, often contains multiple signals of  $\alpha$ N-catenin (arrowheads). Blood vessels are positive for  $\beta$ -catenin (arrow). Bar, 20  $\mu$ m.

not directly associated with synaptic vesicles, were observed (Fig. 3 E, *asterisks*). These structures may correspond to the catenin-associated junctions. We did not find any differences in distribution between  $\alpha$ N- and  $\beta$ -catenin.

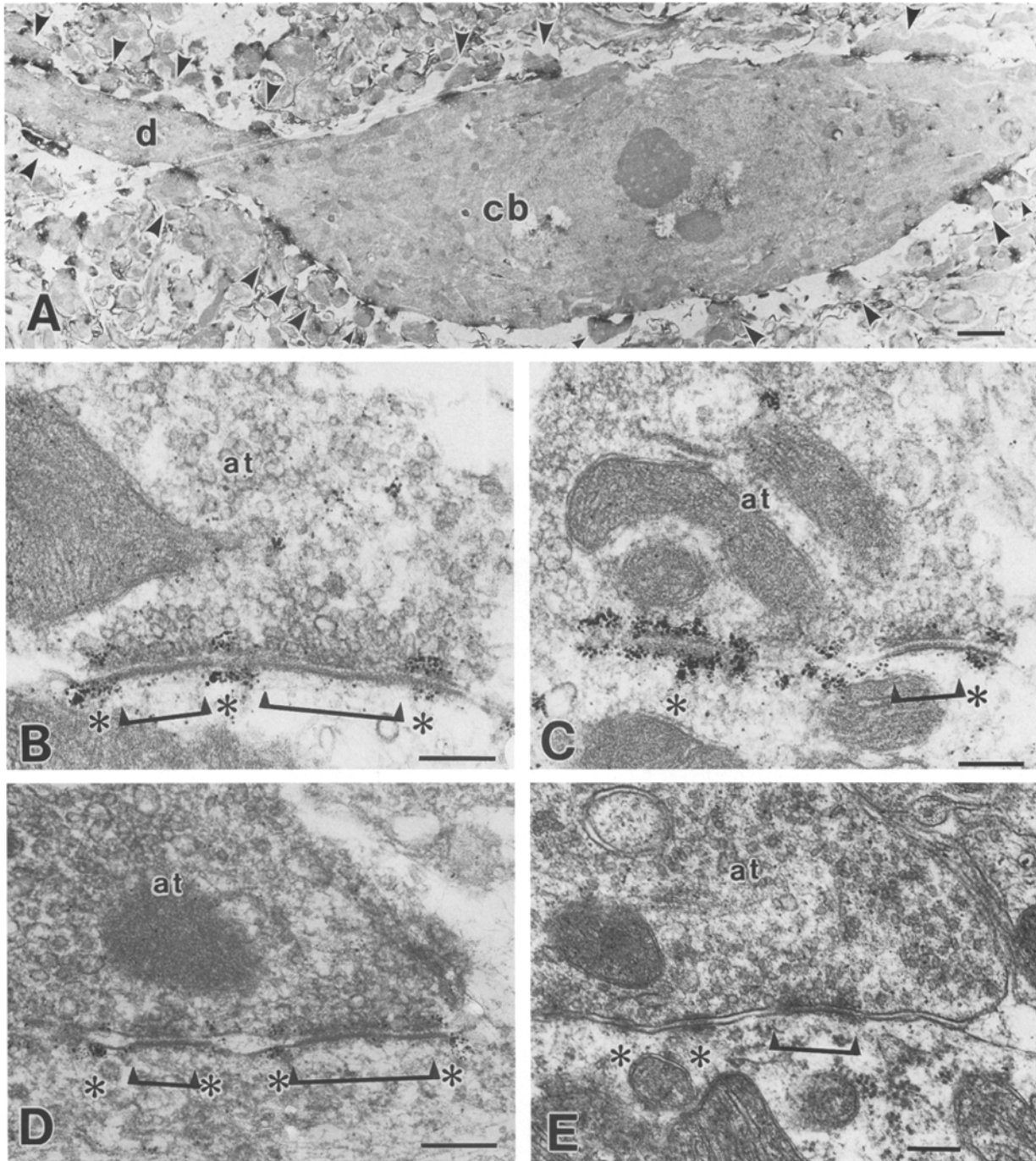
We next studied the distribution of  $\alpha$ N- and  $\beta$ -catenin in two other regions of the adult brain, the cerebral and cerebellar cortices. In the cerebral cortex, at least three types of synapses are present, axo-spinous (Fig. 4 A), axo-dendritic (Fig. 4 B), and axo-somatic synapses. The axo-spinous synapses often have asymmetrical transmitter release zones (Gray's type I). In such synapses,  $\alpha$ N-catenin- or  $\beta$ -catenin-positive junctions sharply bordered the release sites (Fig. 4, C and D). Consistently, in conventional histological sections, we could detect junctions clearly segregated from the transmitter release zones (Fig. 4 A, *asterisks*). In axo-dendritic and axo-somatic synapses, which have symmetrical junctions (Gray's type II),  $\alpha$ N-catenin signals tended to overlap synaptic vesicle clusters; i.e., their segregation from the transmitter release zones appeared to be incomplete (Fig. 4, E and F). In histological sections, we could observe junctions with symmetrical cytoplasmic plaques

within the domain of transmitter release zones (Fig. 4 B, *asterisk*); these could be catenin-associated junctions.

In the granular layer of the cerebellar cortex, the mossy fiber terminals constitute major synapses. In contrast with the above observations, we detected few catenin signals in these synapses (Fig. 4 G). Instead, we found an accumulation of catenins in the junctions between granule cell dendrites, which had originally been designated as attachment plaques or desmoid junctions (Gray, 1961; Altman, 1972). These structures are likely identical to the junctions that were recently found to have M-cadherin (Rose et al., 1995). The parallel fiber-Purkinje synapses in the molecular layer also showed few catenin signals (Fig. 4 H). This result is consistent with the above light microscopical observations. Thus, some types of synapses lack catenins.

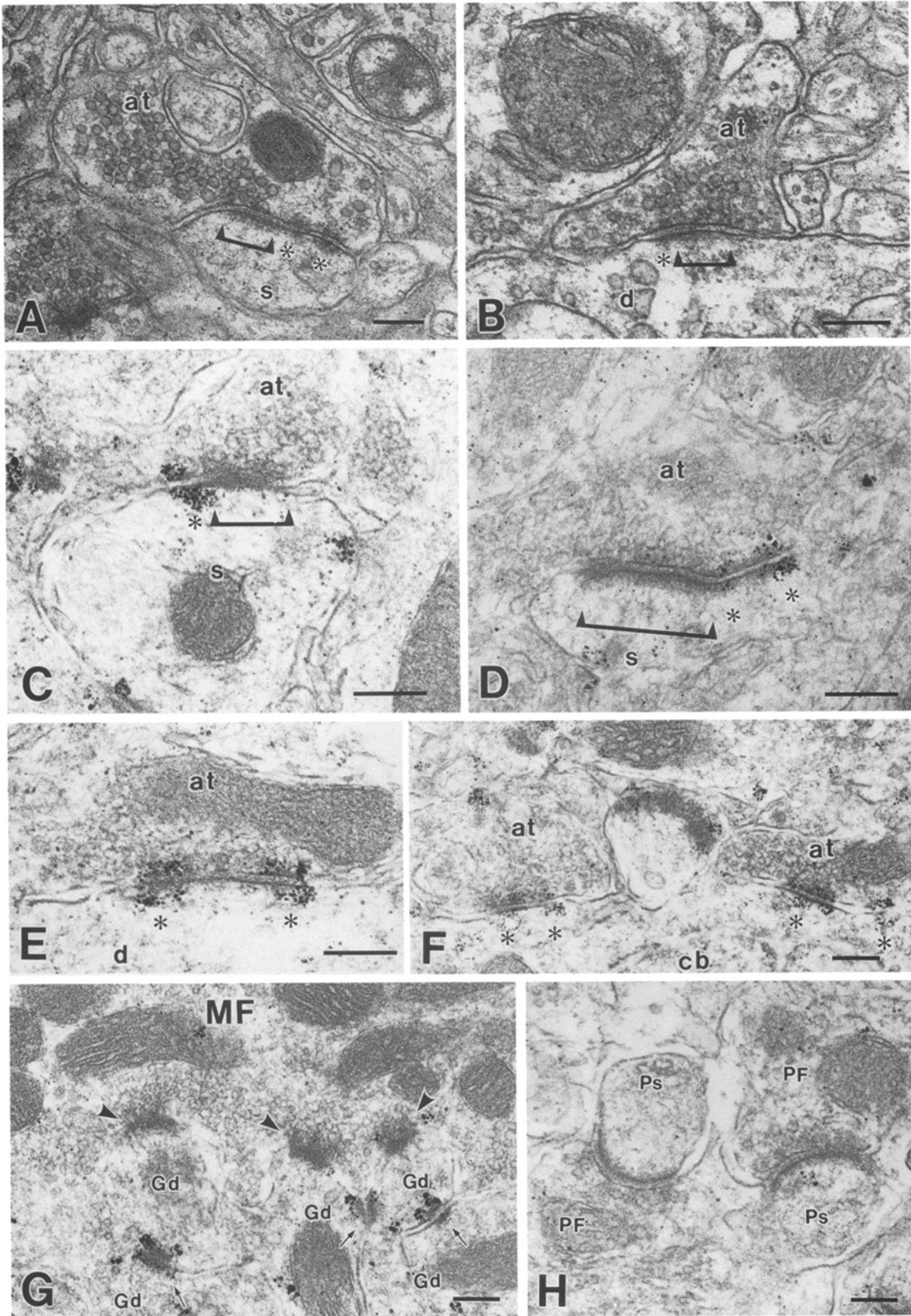
#### *Catenin Expression in Developing Synaptic Junctions*

We next analyzed  $\alpha$ N-catenin expression in developing synapses, using the cerebellum of postnatal mice at P7 to P14 stages. Immunofluorescence staining for  $\alpha$ N-catenin



**Figure 3.** Immunoelectron microscopic localization of  $\alpha$ N- and  $\beta$ -catenin in an adult cerebellar nucleus. (A) Immunoperoxidase staining for  $\alpha$ N-catenin. Almost all synaptic sites (*arrowheads*) are positive. (B–D) Immunogold staining for  $\alpha$ N-catenin (B and C) and  $\beta$ -catenin (D). (E) Conventional histological section. Bars indicate transmitter release zones associated with synaptic vesicles. Asterisks mark catenin-positive zones in B–D, and symmetrical junctions not associated with synaptic vesicles in E. *at*, axon terminal; *cb*, cell body; *d*, dendrite. Bars: (A) 2  $\mu$ m; (B–E) 200 nm.

**Figure 4.** Immunoelectron microscopic localization of  $\alpha$ N- and  $\beta$ -catenin in adult cerebral and cerebellar cortices. (A and B) Conventional histological sections for axo-spinous (A) and axo-dendritic (B) synapses with asymmetrical and symmetrical transmitter release zones indicated by bars, respectively. Asterisks indicate junctions with symmetrical cytoplasmic plaques. (C–H) Immunogold staining for  $\alpha$ N-catenin (C and E–H) and  $\beta$ -catenin (D). (C–F) Cerebellar cortex. Signals are detected in various types of synapses with asymmetrical (C and D) and symmetrical (E and F) transmitter release zones. (G and H) Cerebellar cortex. In the granular layer (G), synapses between mossy fibers and granule cell dendrites (*arrowheads*) are negative, whereas symmetrical junctions between granule cell dendrites are positive (*arrows*). In the molecular layer (H), parallel fiber–Purkinje synapses are negative. *at*, axon terminal; *d*, dendrite; *s*, spine; *cb*, cell body; *Gd*, granule cell dendrite; *PF*, parallel fiber terminal; *Ps*, Purkinje cell spine. Bars, 200 nm.



of these samples showed this molecule to be present throughout the cerebellum (Fig. 5, A, C, and E). Even the molecular layer, which had only a low amount of catenin in the adult, was strongly positive at these developmental stages. Axon fascicles in the white matter were also positive.

For ultrastructural analysis, we first chose P10 cerebellar nuclei. In these samples, we could observe many contacts between afferent nerve endings and the cell body or dendrites. Many of these contacts, however, did not yet have well-developed transmitter release zones; i.e., they lacked accumulation of synaptic vesicles at specific sites. In these immature synaptic contacts, there were many symmetrical intercellular junctions (Fig. 6 A, *asterisks*), as observed in many other brain regions (Adinolfi, 1972; Hinds and Hinds, 1976; Macgraw and McLaughlin, 1980; for review

see Vaughn, 1989; Jacobson, 1991). Immunostaining for  $\alpha$ N-catenin showed that these junctions had abundant  $\alpha$ N-catenin signals (Fig. 6 B). The  $\alpha$ N-catenin-bearing junctions were larger and more frequently observed in those immature nuclei than in adult samples. Notably, we detected  $\alpha$ N-catenin-positive junctions even in mossy fiber terminals and parallel fiber-Purkinje synapses (Fig. 6, C and D), which had been absent in their adult samples (see above). All these findings indicate that immature synaptic contacts are dominated by  $\alpha$ N-catenin-positive symmetrical junctions.

### Localization of N-Cadherin in Synaptic Junctions

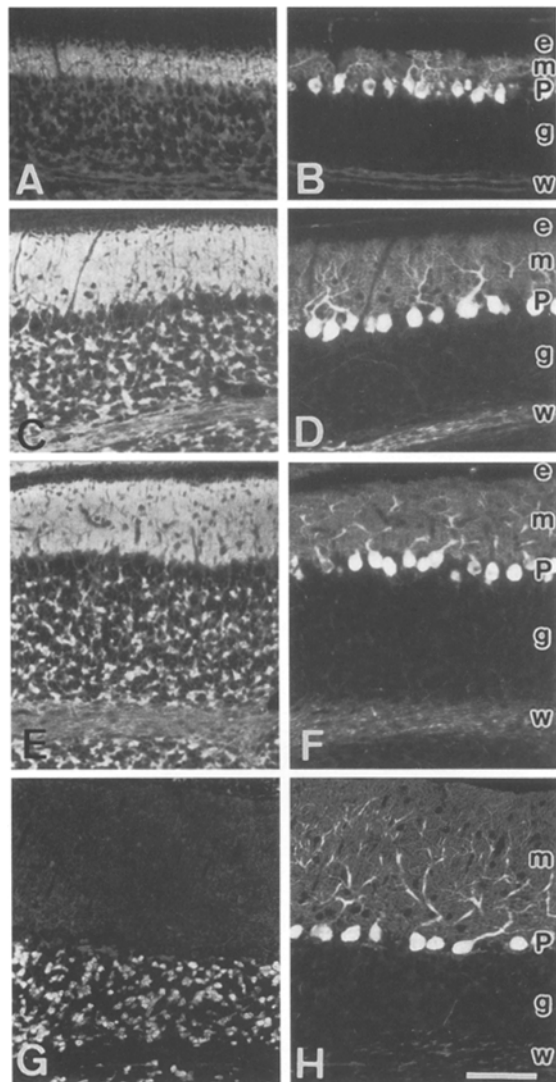
Catenins localized at cell-cell junctions, in general, are associated with certain cadherins. In the above catenin-positive portions of the synapse, some electron-dense materials were visible in the interspace of the plasma membranes. To test whether cadherins indeed localize in synaptic junctions, we studied the distribution of N-cadherin in chick brains, since antibodies suitable for immunoelectron microscopy are available for this particular cadherin.

In the chick brain, N-cadherin is expressed in various nuclei associated with the visual system as well as in the optic tectum (Redies et al., 1993). Our in situ hybridization for N-cadherin mRNA of newly hatched chicken midbrain detected intense, discrete signals in the nucleus pretectalis (PT)<sup>1</sup> (Fig. 7 A). This nucleus is known to receive projections from the tectum, mainly originating from the layer stratum griseum centrale (sgc) (Hunt and Künzel, 1976), which also exhibited N-cadherin expression (Fig. 7 B). Then, we immunostained these regions with anti-N-cadherin antibodies and found that the PT reacted with the antibodies but was surrounded by negative tissues (Fig. 7 C). Its peripheral region was most strongly stained, with a gradual decrease in signal intensity toward the central portion. This pattern is reminiscent of that of the tectal projection to the PT (Hunt and Künzel, 1976), suggesting that the N-cadherin-positive regions are the sites for receiving afferent axons from tectal cells. When  $\alpha$ N-catenin was stained, its distribution in the PT resembled that of the N-cadherin staining (Fig. 7 D). However,  $\alpha$ N-catenin was not restricted to this nucleus, as expected from its broad distribution.

The PT was subjected to immunoelectron microscopy to determine the localization of N-cadherin and  $\alpha$ N-catenin. As found in the mouse brain,  $\alpha$ N-catenin was detected in a symmetrical structure next to the transmitter release zone (Fig. 7 F). N-cadherin signals emanated from a similar site of the synapse, except that they were present in the intercellular space (Fig. 7 E). These N-cadherin signals seemed to demarcate the transmitter release zones. To confirm the molecular interaction between N-cadherin and catenins, we immunoprecipitated  $\alpha$ N-catenin from a lysate of newly hatched chick brains. The immunoprecipitate contained not only  $\beta$ -catenin but also N-cadherin (Fig. 8).

### Discussion

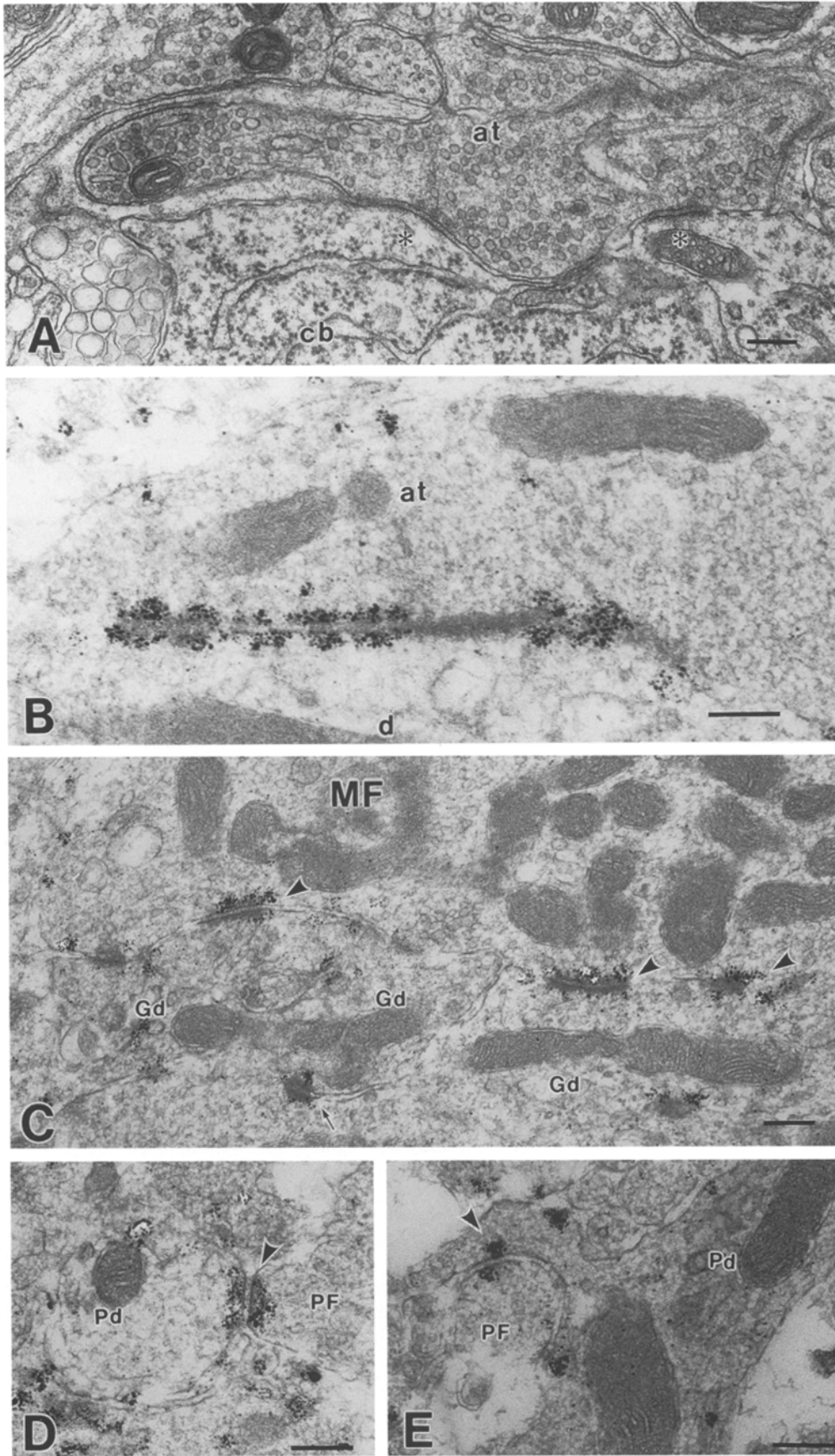
We demonstrated that two classes of cadherin-associated



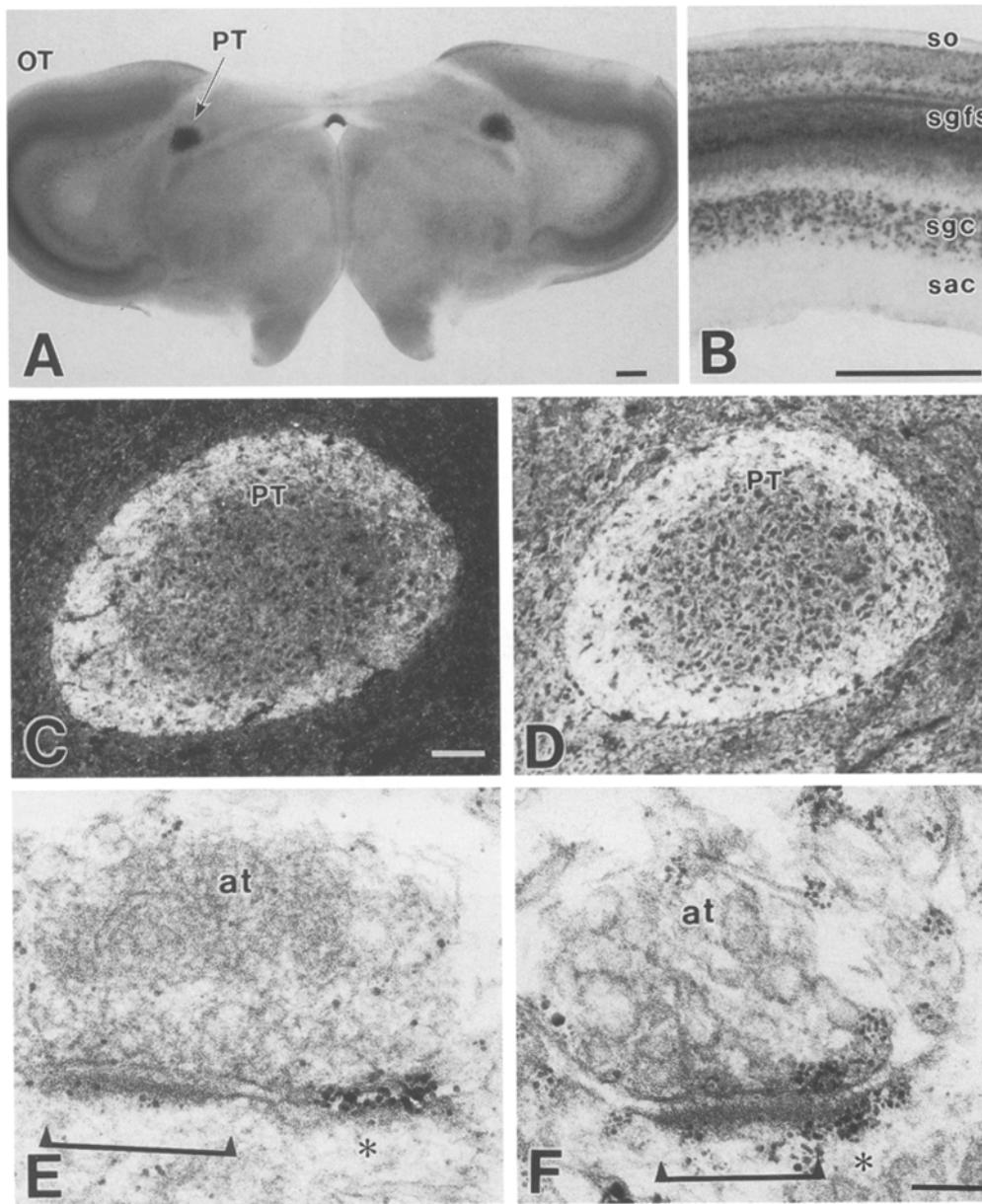
**Figure 5.** Developmental change in  $\alpha$ N-catenin expression in the cerebellar cortex. All sets of photographs show double-immunofluorescence staining for  $\alpha$ N-catenin (*left*) and calbindin-D, a Purkinje cell marker (*right*). (A and B) P7. (C and D) P10. (E and F) P14. (G and H) Adult. At these developing stages, the molecular layer (*m*) and white matter (*w*) are positive for  $\alpha$ N-catenin expression. *e*, external granular layer; *g*, inner granular layer; *P*, Purkinje cell layer. Bar, 100  $\mu$ m.

1. Abbreviations used in this paper: PT, pretectalis; sgc, stratum griseum centrale.





**Figure 6.** Immunoelectron microscopic localization of  $\alpha$ N-catenin in developing brains. (A) Histological section of a P10 cerebellar nucleus. An axon terminal is in contact with the cell body of a neuron through symmetrical intercellular junctions. Synaptic vesicles have not clustered yet at particular junctional sites. (B–E) Immunogold staining for  $\alpha$ N-catenin. (B) P10 cerebellar nucleus. Note the accumulation of  $\alpha$ N-catenin signals along a wide stretch of cell–cell contact sites. (C) P10 cerebellar glomerulus.  $\alpha$ N-catenin signals are detected in immature mossy fiber synapses (arrowheads) as well as in junctions between granule cell dendrites (arrow). (D and E) P7 parallel fiber–Purkinje synapses. Symmetrical junctions between parallel fiber terminals and Purkinje dendrites (arrowheads) have  $\alpha$ N-catenin signals. at, axon terminal; cb, cell body; d, dendrite; MF, mossy fiber terminal; Gd, granule cell dendrite; Pd, Purkinje cell dendrite; PF, parallel fiber terminal. Bars, 200 nm.

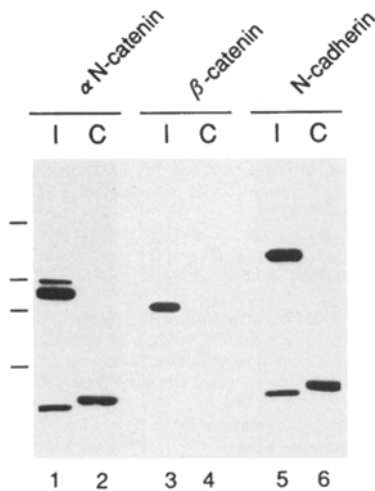


**Figure 7.** N-cadherin expression in the chick visual system. (A) In situ hybridization for N-cadherin mRNA in a P2 chick midbrain section. (B) Larger magnification of the tectal portion. Various layers of the tectum and nucleus pretectalis (PT) express N-cadherin mRNA. (C and D) Immunofluorescence staining for N-cadherin (C) and  $\alpha$ N-catenin (D) proteins. (E and F) Immunogold staining for N-cadherin (E) and  $\alpha$ N-catenin (F) proteins. OT, optic tectum; so, stratum opticum; sgfs, stratum griseum et fibrosum superficiale; sgc, stratum griseum centrale; sac, stratum album centrale; at, axon terminal. Bars: (A and B) 500  $\mu$ m; (C and D) 100  $\mu$ m; (E and F) 200 nm.

proteins,  $\alpha$ N-catenin and  $\beta$ -catenin, are localized in synaptic junctions, defining a specific subdomain structure of the junctions. The major partners for the catenins at cell-cell junctions are cadherins. In cells without any cadherins, catenins are never concentrated in cell-cell contact sites (Kemler, 1993; Nakagawa and Takeichi, 1995). Thus, it is most likely that the observed catenin distribution represents that of cadherin/catenin complexes. This notion is supported by three other observations: first, N-cadherin was localized in synaptic junctions. Second, N-cadherin and catenins formed molecular complexes. Third, the catenin distribution in synapses was always symmetrical, implying that the catenins associate with certain molecules with homophilic binding activity. Our recent studies showed that many cadherin subtypes are expressed in the brain; for example, cerebellar nuclei and Purkinje cells express cadherin-8 and -11, and the cerebral cortex expresses cadherin-6 in addition to those cadherins (Suzuki, S.C., T.

Inoue, and M. Takeichi, manuscript in preparation). These cadherins could be molecular partners for the catenins localized in synapses. However, we cannot rule out the possibility that some unidentified adhesion molecules bind to a fraction of the synaptic catenins. Even if this is the case, they should be a class of homophilic adhesion molecules, as suggested by the symmetrical distribution of catenins. Recently, catenins were found to associate with APC, a tumor suppressor gene product (Rubinfeld et al., 1993; Su et al., 1993), and some APC molecules are colocalized with synaptotagmin in hippocampal neurons (Matsumine et al., 1996). It remains to be investigated, however, whether these APC molecules associate with synaptic junctions.

Early ultrastructural studies showed that the synaptic junctional areas contain at least two types of adhesion structure. One is the transmitter release zone associated with synaptic vesicles. The other is a symmetrical junction called puncta adhaerentia or punctum adherens (Peters



**Figure 8.** Molecular association between N-cadherin and catenins in the chick brain.  $\alpha$ N-catenin was immunoprecipitated with NCAT2 from a lysate of P2 chicken brains. The immunoprecipitate (I) was analyzed for  $\alpha$ N-catenin (lane 1),  $\beta$ -catenin (lane 3), and N-cadherin (lane 5). As a control (C), DCAT1 was used as primary antibody (lanes 2, 4, and 6). Lower bands represent primary antibody–secondary antibody complexes. Molecular mass markers for 200, 116, 97, and 66 kD are shown.

et al., 1976, 1991), defined by the two criteria that they have symmetric paramembranous dense materials and have no association with synaptic vesicles. The catenin-bearing junctions apparently belong to the latter category of junctions. However, it remains to be investigated whether the catenin-bearing junctions are indeed identical to the ones morphologically described. It is even possible that those junctions represent a new group of adhesion structures in synapses, which have been missed in histological analyses. In epithelia, the cadherin/ $\beta$ -catenin/ $\alpha$ -catenin complexes form the zonula adherens, which is also called the adherens junction, intermediate junction, and belt desmosome. The synaptic catenin-bearing junctions can be regarded as a punctuated form of the adherens junction, which can be referred to as synaptic adherens junction. Thus, the synaptic junctions comprise molecularly distinct subdomains, i.e., transmitter release zone and catenin/cadherin-bearing adherens junction. A recent study using a peroxidase-staining protocol showed that N-cadherin is localized at asymmetric synapses throughout their cleft space in the embryonic chick tectum (Yamagata et al., 1995). This is consistent with our findings in the point that N-cadherin is present in synaptic junctions, but inconsistent as to its precise location. We found that peroxidase reaction products diffuse to some extent, which would give an inaccurate indication of the actual location of the antigen.

The adherens-type junctions were found in most regions of the nervous system, including cerebral cortex (Špacák, 1985; Peters et al., 1990), cerebellum (Peters et al., 1991), thalamus (Špacák and Lieberman, 1974; Ilinsky and Kultas-Ilinsky, 1990), subthalamic nucleus (Chang et al., 1983), cochlear nucleus (Wouterlood et al., 1984), and spinal cord (Vaughn, 1989; Peters et al., 1991). Nevertheless, some regions lack this type of junction. According to Špacák (1985), there are at least two types of spines in the

cerebral cortex, thin (T) and mushroom-shaped (M) types. The T-type has simple (circular or oval) synapses, and the M-type has complex (annulate or horseshoe-shaped) ones. Most of the complex synapses have puncta adhaerentia, whereas the simple ones do not. We found little catenin immunoreactivity in parallel fiber–Purkinje synapses, which have no apparent puncta adhaerentia morphologically. Those early studies, together with our present findings, indicate that the adherens-type junctions are not a ubiquitous component of the mature synapses.

In postnatal brains, however, the situation was different. Developing future synaptic contacts had abundant catenin-based junctions. Even the parallel fiber–Purkinje synapses showed  $\alpha$ N-catenin signals at early stages. This finding suggests that the synaptic adherens junctions play a general and important role in early phases of synaptogenesis. The cadherin/catenin system is known to be crucial for the stable association of epithelial cells. Without the activity of this adhesion system, cells fall apart, and other adhesion systems, including tight junction and desmosome, cannot form normally (Gumbiner and Simons, 1986; Gumbiner et al., 1988; Meyer et al., 1992; Watabe et al., 1994), indicating that cadherins are central for the organization of cell–cell adhesion structures. This principle could also be the case for interneuronal adhesions; i.e., cadherin-mediated initial contacts could be a prerequisite for the development of synaptic junctions. As synapses mature, the role of the adherens junctions could decrease, and, instead, that for the transmitter release zone could increase to warrant synaptic signal transmission. The presence of catenins in many adult synapses, however, suggests that these molecules are still required by them. Synapses are thought to be dynamic adhesion structures, as implied by their functional plasticity. Cadherins/catenins perhaps contribute to the formation and maintenance of synaptic junctions throughout life, at least in certain neurons.

It should be noted that a number of different cadherin subtypes with distinct adhesion specificity are differentially expressed in postnatal brains (Suzuki, S.C., T. Inoue, and M. Takeichi, manuscript in preparation). Because of their region-specific distribution and binding specificity, it is likely that each cadherin subtype is used for the specific association of limited sets of neurons and that catenins ubiquitously support the cadherin function. N-cadherin is one of such neuronal cadherins, which is expressed by restricted components of the chicken visual circuit (Redies et al., 1993). Our present observation showed that N-cadherin is specifically localized at tectal and PT junctions in the midbrain stem, supporting the above idea.

Cadherins/catenins are localized not only in synaptic junctions but also along axonal fibers, especially at embryonic stages (Uchida et al., 1994; Redies et al., 1993). E-cadherin was found to be located at axon–axon contacts of unmyelinated sensory fibers (Uchiyama et al., 1994), suggesting their roles in axonal fasciculation. Interestingly, most of the catenin signals observed along axonal fibers disappear after their maturation; catenin signals were hardly detected in axon fascicles of the adult brain. Perhaps myelinated fibers do not require this adhesion system, which is then confined to their axon terminals. We also detected catenins in nonsynaptic junctions between granule cell dendrites in the cerebellum. The cadherin/

catenins seem to play multiple roles in neuronal connections.

Finally, how do the two subdomains of the synaptic junction, transmitter release zone and adherens junction, molecularly relate to each other? Their close positional relations imply that some molecular linkers might be present between the two structures, and it is even possible that they share some molecular constituents. The two subdomains could be initially an intermingled structure, but later segregated. To test such ideas, it is most important to further characterize the molecular components of each junctional structure.

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#### References

Aberle, H., S. Butz, J. Stappert, H. Weissig, R. Kemler, and H. Hoschuetzky. 1994. Assembly of the cadherin-catenin complex in vitro with recombinant proteins. *J. Cell Sci.* 107:3655-3663.

Adinolfi, A.M. 1972. Morphogenesis of synaptic junctions in layers I and II of the somatic sensory cortex. *Exp. Neurol.* 34:372-382.

Altman, J. 1972. Postnatal development of the cerebellar cortex in the rat III. Maturation of the components of the granular layer. *J. Comp. Neurol.* 145: 465-514.

Baughman, R.W., J.E. Huettner, K.E. Jones, and A.A. Khan. 1991. Cell culture of neocortex and basal forebrain from postnatal rats. In *Culturing Nerve Cells*. G. Banker and K. Goslin, editors. The MIT Press, Cambridge, MA. 227-249.

Beesley, P.W., R. Mummery, and J. Tibaldi. 1995. N-cadherin is a major glycoprotein component of isolated rat forebrain postsynaptic densities. *J. Neurochem.* 64:2288-2294.

Boller, K., D. Vestweber, and R. Kemler. 1985. Cell-adhesion molecule uvomorulin is localized in the intermediate junctions of adult intestinal epithelial cells. *J. Cell Biol.* 100:327-332.

Burky, R.W., D.D. Vandre, and D.M. Hayes. 1992. Silver enhancement of gold antibody probes in pre-embedding electron microscopic immunocytochemistry. *J. Histochem. Cytochem.* 40:1849-1856.

Chang, H.T., H. Kita, and S.T. Kitai. 1983. The fine structure of the rat subthalamic nucleus: an electron microscopic study. *J. Comp. Neurol.* 221:113-123.

Colonnier, M. 1968. Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. *Brain Res.* 9: 268-287.

Fannon, A.M., D.L. Sherman, G. Ilyina-Gragerova, P.J. Brophy, V.L. Friedrich, Jr., and D.R. Colman. 1995. Novel E-cadherin-mediated adhesion in peripheral nerve: Schwann cell architecture is stabilized by autotypic adherens junctions. *J. Cell Biol.* 129:189-202.

Gautam, M., P.G. Noakes, L. Moscoso, F. Rupp, R.H. Scheller, J.P. Merlie, and J.R. Sanes. 1996. Defective neuromuscular synaptogenesis in agrin-deficient mice. *Cell.* 85:525-535.

Gray, E.G. 1959. Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *J. Anat.* 93:420-433.

Gray, E.G. 1961. The granule cells, mossy synapses, and Purkinje spine synapses of the cerebellum: light and electron microscope observations. *J. Anat.* 96:309-316.

Gumbiner, B., and K. Simons. 1986. A functional assay for proteins involved in establishing an epithelial occluding barrier: identification of uvomorulin-like polypeptide. *J. Cell Biol.* 102:457-468.

Gumbiner, B., B. Stevenson, and A. Grimaldi. 1988. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. *J. Cell Biol.* 107:1575-1587.

Hall, A.W., and J.R. Sanes. 1993. Synaptic structure and development: the neuromuscular junction. *Cell* 72/*Neuron*. 10 (Suppl.):99-121.

Hatta, K., and M. Takeichi. 1986. Expression of N-cadherin adhesion molecule

associated with early morphogenetic events in chick development. *Nature (Lond.)*. 320:447-449.

Hinds, J.W., and P.L. Hinds. 1976. Synapse formation in the mouse olfactory bulb. II. Morphogenesis. *J. Comp. Neurol.* 169:41-62.

Hirano, S., and M. Takeichi. 1994. Differential expression of  $\alpha$ N-catenin and N-cadherin during early development of chicken embryos. *Int. J. Dev. Biol.* 38:379-384.

Hirano, S., N. Kimoto, Y. Shimoyama, S. Hirohashi, and M. Takeichi. 1992. Identification of neural  $\alpha$ -catenin as a key regulator of cadherin function and multicellular organization. *Cell.* 70:283-301.

Hunt, S.P. and H. Künzel. 1976. Observation on the projections and intrinsic organization of the pigeon optic tectum: an autoradiographic study based on anterograde and retrograde, axonal and dendritic flow. *J. Comp. Neurol.* 170:153-172.

Ilnsky, I.A., and K. Kultas-Ilnsky. 1990. Fine structure of the magnocellular subdivision of the ventral anterior thalamic nucleus (VAsmc) of *Macaca mulatta*. I. Cell type and synaptology. *J. Comp. Neurol.* 294:455-478.

Jacobson, M. 1991. *Developmental Neurobiology*. Third edition. Plenum Press, New York/London. 223-283.

Jessel, T.M., and E.R. Kandel. 1993. Synaptic transmission: a bidirectional and self-modifiable form of cell-cell communication. *Cell* 72/*Neuron*. 10 (Suppl.): 1-30.

Jongens, T.A., L.D. Ackerman, J.R. Swedlow, L.Y. Jan, and Y.N. Jan. 1994. Germ cell-less encodes a cell type-specific nuclear pore-associated protein and functions early in the germ-cell specification pathway of *Drosophila*. *Genes & Dev.* 8:2123-2136.

Jou, T.S., D.B. Stewart, J. Stappert, W.J. Nelson, and J.A. Marrs. 1995. Genetic and biochemical dissection of protein linkages in the cadherin-catenin complex. *Proc. Natl. Acad. Sci. USA.* 92:5067-5071.

Kemler, R. 1993. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends Genet.* 9:317-321.

Kuenzel, W.J., and M. Masson. 1988. *A Stereotaxic Atlas of the Brain of the Chick (Gallus domesticus)*. The Johns Hopkins University Press, Baltimore/London.

Matsumine, A., A. Ogai, T. Senda, N. Okumura, K. Satoh, G.-H. Baeg, T. Kawahara, S. Kobayashi, M. Okada, K. Toyoshima, et al. 1996. Binding of APC to the human homolog of the *Drosophila* discs large tumor suppressor protein. *Science (Wash. DC)*. 272:1020-1023.

McGraw, C.F., and B.J. McLaughlin. 1980. Fine structural studies of synaptogenesis in the superficial layers of the chick optic tectum. *J. Neurocytol.* 9:79-93.

Meyer, R.A., D.W. Laird, J.-P. Revel, and R.G. Johnson. 1992. Inhibition of gap junction and adherens junction assembly by connexin and A-CAM antibodies. *J. Cell Biol.* 119:179-189.

Nagafuchi, A., and S. Tsukita. 1994. The loss of the expression of a catenin, the 102 kD cadherin associated protein, in central nervous tissues during development. *Dev. Growth Differ.* 36:59-71.

Nakagawa, S., and M. Takeichi. 1995. Neural crest cell-cell adhesion controlled by sequential and subpopulation specific expression of novel cadherins. *Development (Camb.)*. 121:1321-1332.

Navone, F., R. Jahn, G.D. Gioia, H. Stukenbrok, P. Greengard, and P.D. Camilli. 1986. Protein p38: an integral membrane protein specific for small vesicles of neurons and neuroendocrine cells. *J. Cell Biol.* 103:2511-2527.

Noakes, P.G., M. Gautam, J. Mudd, J.R. Sanes, and J.P. Merlie. 1995. Aberrant differentiation of neuromuscular junctions in mice lacking s-laminin/laminin  $\beta$ 2. *Nature (Lond.)*. 374:258-262.

Oda, H., T. Uemura, Y. Harada, Y. Iwai, and M. Takeichi. 1994. A *Drosophila* homolog of cadherin associated with *Armadillo* and essential for embryonic cell-cell adhesion. *Dev. Biol.* 165:716-726.

Peters, A., S.L. Palay, and H. Webster. 1976. *The Fine Structure of the Nervous System: The Neurons and Their Supporting Cells*. Saunders, Philadelphia.

Peters, A., C. Sethares, and M. Harriman. 1990. Different kinds of axon terminals forming symmetric synapses with the cell bodies and initial axon segments of layer II/III pyramidal cells. II. Synaptic junctions. *J. Comp. Neurol.* 19:584-600.

Peters, A., S.L. Palay, and H. Webster. 1991. *The Fine Structure of the Nervous System: The Neurons and Their Supporting Cells*. Third edition. Oxford University Press, New York/Oxford. 1-494.

Redies, C. 1995. Cadherin expression in the developing vertebrate CNS: from neuromere to brain nuclei and neural circuits. *Exp. Cell Res.* 220:243-256.

Redies, C., K. Engelhart, and M. Takeichi. 1993. Differential expression of N- and R-cadherin in functional neuronal system and other structures of the developing chicken brain. *J. Comp. Neurol.* 333:398-416.

Rose, O., C. Grund, S. Reinhard, A. Starzinski-Powitz, and W.W. Franke. 1995. Contactus adherens, a special type of plaque-bearing adhering junction containing M-cadherin, in the granule cell layer of the cerebellar glomerulus. *Proc. Natl. Acad. Sci. USA.* 92:6022-6026.

Rubinfeld, B., B. Souza, I. Albert, O. Müller, S.H. Chamberlain, F.R. Marsiartz, S. Munemitsu, and P. Polakis. 1993. Association of the APC gene product with  $\beta$ -catenin. *Science (Wash. DC)*. 262:1731-1734.

Shibamoto, S., M. Hayakawa, K. Takeuchi, T. Hori, K. Miyazawa, N. Kitamura, K.R. Johnson, M.J. Wheelock, N. Matsuyoshi, M. Takeichi, et al. 1995. Association of p120, a tyrosine kinase substrate, with E-cadherin/catenin complexes. *J. Cell Biol.* 128:949-957.

Shimamura, K., S. Hirano, A.P. McMahon, and M. Takeichi. 1994. Wnt-1-dependent regulation of local E-cadherin and  $\alpha$ N-catenin expression in the embry-

- onic mouse brain. *Development (Camb.)*. 120:2225–2234.
- Špacák, J. 1985. Relationship between synaptic junctions, puncta adhaerentia and spine apparatus at neocortical axo-spinous synapses. A serial section study. *Anat. Embryol.* 173:129–135
- Špacák, J., and A.R. Lieberman. 1974. Ultrastructure and three-dimensional organization of synaptic glomeruli in rat somatosensory thalamus. *J. Anat.* 117: 487–516
- Su, L., B. Vogelstein, and K.W. Kinzler. 1993. Association of the APC tumor suppressor protein with catenins. *Science (Wash. DC)*. 262:1734–1737.
- Südhof, T. C. 1995. The synaptic vesicle cycle: a cascade of protein-protein interactions. *Nature (Lond.)*. 375:645–653.
- Takeichi, M. 1988. The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. *Development (Camb.)*. 102:639–655.
- Takeichi, M. 1995. Morphogenetic roles of classic cadherins. *Curr. Opin. Cell Biol.* 7:619–627.
- Uchida, N., K. Shimamura, S. Miyatani, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, and M. Takeichi. 1994. Mouse  $\alpha$ N-catenin: two isoforms, specific expression in the nervous system, and chromosomal localization of the gene. *Dev. Biol.* 163:75–85.
- Uchiyama, N., M. Hasegawa, T. Yamashita, J. Yamashita, K. Shimamura, and M. Takeichi. 1994. Immunoelectron microscopic localization of E-cadherin in dorsal root ganglia, dorsal root and dorsal horn of postnatal mice. *J. Neurocytol.* 23:460–468.
- Vaughn, J.E. 1989. Review: fine structure of synaptogenesis in the vertebrate central nervous system. *Synapse*. 3:255–285.
- Volk, T., and B. Geiger. 1986. A-CAM: a 135-kD receptor of intercellular adherens junctions. I. Immunoelectron microscopic localization and biochemical studies. *J. Cell Biol.* 103:1441–1450.
- Watabe, M., A. Nagafuchi, S. Tsukita, and M. Takeichi. 1994. Induction of polarized cell-cell association and retardation of growth by activation of the E-cadherin–catenin adhesion system in a dispersed carcinoma line. *J. Cell Biol.* 127:247–256.
- Wouterlood, F.G., E. Mugnaini, K.K. Osen, and A. Dahl. 1984. Stellate neurons in rat dorsal cochlear nucleus studied with combined Golgi impregnations and electron microscopy: synaptic connections and mutual coupling by gap junctions. *J. Neurocytol.* 13:639–664.
- Yamagata, M., J.P. Herman, and J.R. Sanes. 1995. Lamina-specific expression of adhesion molecules in developing chick optic tectum. *J. Neurosci.* 15: 4556–4571.