Sodium channel $Na_v1.6$ is localized at nodes of Ranvier, dendrites, and synapses

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Voltage-gated sodium channels perform critical roles for electrical signaling in the nervous system by generating action potentials in axons and in dendrites. At least 10 genes encode sodium channels in mammals, but specific physiological roles that distinguish each of these isoforms are not known. One possibility is that each isoform is expressed in a restricted set of cell types or is targeted to a specific domain of a neuron or muscle cell. Using affinity-purified isoform-specific antibodies, we find that $Na_v1.6$ is highly concentrated at nodes of Ranvier of both sensory and motor axons in the peripheral nervous system and at nodes in the central nervous system. The specificity of this antibody was also demonstrated with the $Na_v1.6$-deficient mouse mutant strain *med*, whose nodes were negative for $Na_v1.6$ immunostaining. Both the intensity of labeling and the failure of other isoform-specific antibodies to label nodes suggest that $Na_v1.6$ is the predominant channel type in this structure. In the central nervous system, $Na_v1.6$ is localized in unmyelinated axons in the retina and cerebellum and is strongly expressed in dendrites of cortical pyramidal cells and cerebellar Purkinje cells. Ultrastructural studies indicate that labeling in dendrites is both intracellular and on dendritic shaft membranes. Remarkably, $Na_v1.6$ labeling was observed at both presynaptic and postsynaptic membranes in the cortex and cerebellum. Thus, a single sodium channel isoform is targeted to different neuronal domains and can influence both axonal conduction and synaptic responses.

The role of sodium channels in electrical conduction in axons was first described about 50 years ago, and the highest density of voltage-gated sodium channels in the nervous system occurs at the node of Ranvier, a specialization of myelinated axons that enables high speed propagation of electrical signals. The cellular mechanisms responsible for targeting and clustering of ion channels to such minute, remote domains on axons is a subject of much study and debate (1–3). More recently, the presence of sodium-dependent action potentials in dendrites has raised the possibility that sodium channels could participate in postsynaptic signal generation in these structures (4–7). In addition, sodium channels can be modulated by kinases and by G proteins (8, 9), implying that sodium channels in dendrites might contribute to synaptic plasticity.

Sodium channels, like other ion channels in the nervous system, are members of a multigene family (10), and 10 sodium channel genes have been isolated in mammals [see Goldin's article (ref. 11) for review]. Four of these genes are expressed in the central nervous system (CNS): $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, and $Na_v1.6$. A fundamental goal is to determine the significance of such diversity of isoforms. Differences in the functional properties of these channels are subtle (12–15). Nevertheless, such subtle differences could be functionally important, because small changes in voltage dependence of activation or inactivation could affect excitability. These differences would be especially significant in structures where signal processing or transduction takes place, such as in dendrites or sensory endings. Another related hypothesis proposes that each isoform contains unique structures that allow its selective localization to a specialized membrane domain, such as the node of Ranvier. Thus, each isoform would be expected to have a unique distribution in the nervous system. Evidence supporting such notions comes from studies of human and animal diseases where isoform-specific sodium channel mutations have been shown to cause tissue-specific dysfunction such as episodic periodic paralysis in skeletal muscle (16), long QT syndrome in cardiac muscle (17), and CNS disorders such as paralysis, ataxia, and dystonia (18, 19).

Other clues of isoform roles come from studies of subcellular distribution that use isoform-specific antibodies. Thus, $Na_v1.1$ and $Na_v1.3$ have been localized to the soma of CNS neurons; $Na_v1.2$ is primarily in unmyelinated axons; and both $Na_v1.1$ and $Na_v1.2$ have been reported in proximal apical dendrites (20–22). However, none of these channels ($Na_v1.1$, $Na_v1.2$, and $Na_v1.3$) nor any of the peripheral nervous system (PNS) isoforms ($Na_v1.7$, $Na_v1.8$, and Na_v2) have been localized at nodes of Ranvier. In initial studies, we found that $Na_v1.6$ was at both peripheral nerve nodes of Ranvier and in dendrites (23, 24). In this report, we characterize in some detail at both the light and electron microscopic level the distribution of $Na_v1.6$ in axons and dendrites in the PNS and CNS. Our results support an essential role for $Na_v1.6$ in electrical conduction in both myelinated and unmyelinated axons, whereas localization of $Na_v1.6$ in dendrites supports a role for this isoform in synaptic transmission and as both a presynaptic and postsynaptic sodium channel in the CNS.

Materials and Methods

We have adopted the nomenclature for voltage-gated sodium channels proposed by Goldin (11). The $Na_v1.6$ channel was originally named NaCh6 in the rat (25) and Scn8a in the mouse (26) and has also been called PN4 (14) and cer3 (27).

Site-Directed Antibody Generation. A 19-residue peptide was synthesized by Macromolecular Resources (Ft. Collins, CO) and corresponded to amino acids 460–477 (CSEDAIEEE-GEDGVGSPRS) of the rat channel (25). An amino-terminal cysteine was added to this peptide for linkage to keyhole limpet hemocyanin for immunizations and to permit coupling to a column for affinity purification. Antisera were raised in New Zealand White rabbits, and antibodies were purified with peptide affinity chromatography as described (1).

Immunoblotting and Immunohistochemistry. Animal use followed guidelines established by the National Institutes of Health and the University of Colorado Health Sciences Center Animal Care Committee. Mice and rats were anesthetized with phenobarbital and perfused through the heart with 4% (wt/vol) paraformal-

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Abbreviations: CNS, central nervous system; PNS, peripheral nervous system; GFAP, glial fibrillary acidic protein.

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dehyde, and brains were cryostat sectioned (15 μm). Proteins from rat brain membrane preparations were separated by PAGE, transferred to nitrocellulose membranes, and probed on immunoblots with anti-$Na_v1.6$ antibody or with a "pan-specific" antibody directed against a conserved epitope that recognizes all vertebrate Na_v1 isoforms (28). Sciatic nerves were removed from freshly killed mice, fixed for 30 min in 4% (wt/vol) paraformaldehyde, and teased mechanically to separate axons. Primary antibodies were incubated overnight at 4°C. Primary antibodies and dilutions used were anti-$Na_v1.6$ (1:100), anti-caspr (mAb clone 275; ref. 29; 1:300), anti-SV2 (gift of R. Kelly, Univ. of California, San Francisco; 1:100), and anti-GFAP (Roche Molecular Biochemicals; 1:500; GFAP, glial fibrillary acidic protein). Secondary antibodies used for anti-$Na_v1.6$ visualization were Cy3, RRX (Jackson ImmunoResearch), or Alexa 568 (Molecular Probes) anti-rabbit conjugates, whereas the other mouse mAbs were detected with either Alexa 488 or biotinylated anti-mouse/fluorescein streptavidin (Jackson ImmunoResearch). Sections were mounted in a photobleaching protective medium (Vectashield, Vector Laboratories) and viewed on a Nikon PCM-2000 laser scanning confocal microscope with digital images collected at a series of focal planes. Other images were collected with a DeltaVision digital deconvolution system (Applied Precision, Issaquah, WA); a series of images at 0.2-μm intervals was collected and deblurred analytically.

To determine the level of nonspecific staining, control tissue was incubated with primary antibody that had been blocked by preincubation for 3 h with a large molar excess ($\approx$100-fold) of the corresponding peptide before incubation of the antibody with the tissue. Images comparing blocked and unblocked antibody labeling were acquired and digitally processed identically. Final image processing was done with ALDUS PHOTOSTYLER or ADOBE PHOTOSHOP (version 5.0), and figures were composed with CORELDRAW (version 7).

Electron Microscopy. Vibratome sections (50 μm) of cerebral cortex and cerebellum were cut from a female rat brain fixed with 4% (wt/vol) paraformaldehyde + 15% (vol/vol) saturated picric acid in phosphate buffer + sucrose. After rinsing in PBS + 0.08 M sucrose (SS), sections were incubated in a blocking buffer [SS + 4% (wt/vol) BSA + 0.05% NaN_3 + 1.5% (vol/vol) normal goat serum], followed by incubation in blocking buffer + anti-$Na_v1.6$ antibody with or without the antigenic peptide at room temperature on a gyrotary shaker for 2.5 days. The sections were rinsed in SS, incubated in blocking buffer + 1/200 biotin-goat anti-rabbit IgG (Vectastain Elite ABC Kit, Vector Laboratories) at room temperature for 2 h, rinsed in SS, incubated in SS + 2% (wt/vol) BSA + ABC reagent (Vectastain Elite ABC Kit) for 2 h, and rinsed in SS. The bound antibody was visualized by incubating sections for 1.25 min in diaminobenzidine + H_2O_2 + nickel in buffer (Vectastain Elite ABC Kit), followed by rinsing in SS. Sections were postfixed in 1% OsO_4 in PBS + sucrose, rinsed in SS, dehydrated in a graded series of ethanol, and followed by propylene oxide and then Spurr's resin. Sections were flat embedded, polymerized, thin-sectioned on a Reichert Ultracut E, placed on grids, and observed in a Philips North America (Mahwah, NJ) CM 10 EM without further counterstaining.

Results

We generated rabbit polyclonal antibodies directed against a synthetic peptide corresponding to an epitope in a large intracellular domain of $Na_v1.6$, a sodium channel isoform widely expressed in the CNS and PNS (23–27). Immunoblots of rat brain membrane proteins were probed with either a pan-specific antibody (Fig. 1, pan lane) or the anti-$Na_v1.6$ antibody (Fig. 1, 6 lane). The apparent molecular size of $Na_v1.6$ was smaller than that of the general population of brain sodium channels labeled

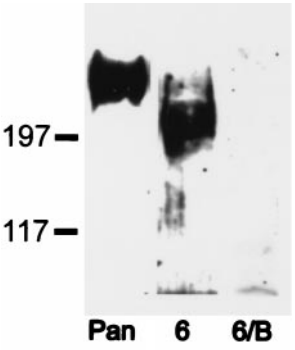

Fig. 1. Immunoblot of rat brain membranes. A sodium channel pan-specific antibody labeled a major band at $\approx$250 kDa (pan lane). An anti-$Na_v1.6$ antibody labeled a band at $\approx$225 kDa (6 lane). $Na_v1.6$ labeling was abolished by preincubation of the antibody with the antigenic peptide (6/B lane).

by a pan-specific antibody (1), which is consistent with the predicted molecular mass from the amino acid sequence and the fact that it contains fewer glycosylation sites compared with the brain channels $Na_v1.1$, $Na_v1.2$, and $Na_v1.3$. Further exposure of the pan lane revealed a fainter band at 225 kDa (data not shown), presumably corresponding to the less abundant subpopulation of $Na_v1.6$. The $Na_v1.6$ signal at $\approx$225 kDa was abolished by preincubation of the antibody with the antigenic peptide (blocked antibody; Fig. 1, 6/B lane).

Immunofluorescent labeling of rat sciatic nerve with anti-$Na_v1.6$ antibody showed intense labeling of nodes of Ranvier (Fig. 2*A*) that were identified by (*i*) their morphology and (*ii*) colabeling with antibodies against caspr (contactin associated protein; refs. 30 and 31; Fig. 2*A*) or with antibodies against myelin-associated glycoprotein (data not shown), both of which are proteins found in the paranodal regions that flank the node. The labeling seemed to be associated with the nodal axolemma, as could be seen from the ring of nodal staining when the fiber axis was obliquely oriented to the view axis (Fig. 2 *A* and *C*, *Insets*). We next considered whether $Na_v1.6$ was to be found at nodes of Ranvier throughout the nervous system. For example, was $Na_v1.6$ at the nodes of both sensory and motor axons in the PNS? Was it to be found in CNS nodes? To address the former question we first looked at $Na_v1.6$ staining in dorsal and ventral spinal roots. There was abundant labeling of nodes in both dorsal and ventral roots (data not shown), indicating that $Na_v1.6$ is the nodal channel in both sensory and motor myelinated axons. Was $Na_v1.6$ present at nodes of all myelinated axons, or was it in subsets of sensory and motor myelinated axons? We examined this question in two ways. First, nodes were identified morphologically with brightfield optics and subsequently examined for labeling for $Na_v1.6$. Of 104 nodes examined, all had $Na_v1.6$ labeling. Second, every node labeled by the anti-caspr antibody was also labeled for $Na_v1.6$ (856 nodes examined). Thus, we conclude that all nodes of Ranvier in peripheral nerves contain $Na_v1.6$. Moreover, the intensity of labeling compared with that of a pan-specific antibody suggests that it is the major nodal channel. Lastly, we saw no evidence of $Na_v1.6$ staining in unmyelinated fiber bundles in sciatic nerve under conditions when such bundles were brightly stained both by pan-specific and $Na_v1.7$ (PN1) isoform-specific antibodies (A. G. Koszoswki and S.R.L., unpublished observations).

As additional tests of the antibody specificity, we labeled sciatic nerves of normal (wild-type) mice (Fig. 2*B1*), *med*^{J} mice homozygous for a mutation in $Na_v1.6$ that is a null phenotype (Fig. 2*B2*), and normal mice with the blocked antibody (Fig. 2*B3*). The *med*^{J} mice have a mutation at an RNA splice site that results in abnormal splicing and a truncated, nonfunctional gene

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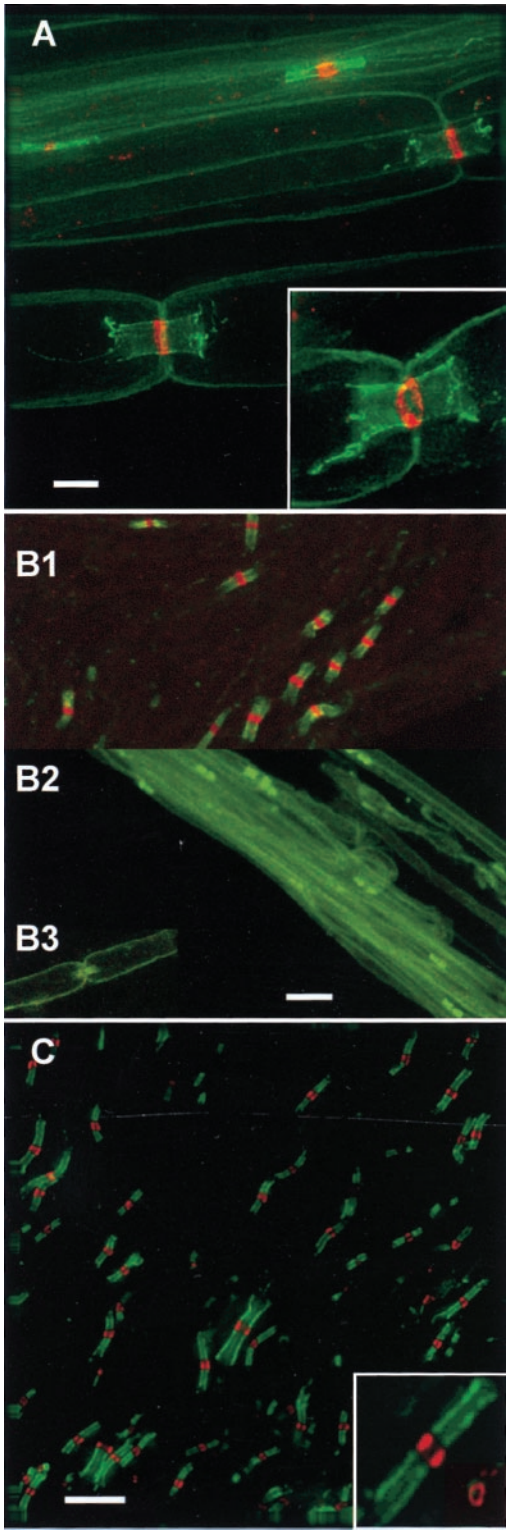

Fig. 2. Node of Ranvier labeling in the PNS (rat and mouse sciatic) and CNS (rat optic nerve). Immunofluorescence with anti-$Na_v1.6$ antibody (red) and anti-caspr (green). Anti-caspr labeled the paranodal region on either side of the nodal labeling by anti-$Na_v1.6$. (*A*) Rat sciatic nerve. All nodes labeled by caspr were labeled for $Na_v1.6$. (*A Inset*) Another node viewed obliquely such that the circumferential labeling of axonal membrane can be seen. (*B*) Mouse sciatic nerve. Labeling controls for $Na_v1.6$ and caspr. (*B1*) Wild-type mouse labeled for $Na_v1.6$ (red) at nodes shown by caspr labeling (green). (*B2*) Mutant mouse (*med*^{J}) lacking $Na_v1.6$ had no labeling for $Na_v1.6$ at nodes. (*B3*) One node illustrating that preincubation of the anti-$Na_v1.6$ antibody with the peptide eliminated nodal labeling in the wild-type mouse. (*C*) Optic nerve axons. Optical sections (0.2 μm apart; $n = 25$) were deconvolved and projected onto a single plane. Every node was labeled for $Na_v1.6$ (caspr labeling in the figure without $Na_v1.6$ labeling was at the top or bottom of the series of sections). (*C Inset*) Two nodes, one viewed longitudinally and one viewed transversely such that the nodal labeling appears circular. (Bars = 5 μm for *A* and *C*, 3.3 μm for *A Inset* and *C Inset*, 6 μm for B1, 10 μm for B2, and 13 μm for B3.)

product (32). These mice die at 21–28 days after birth. No labeling with anti-$Na_v1.6$ antibody was observed at nodes of these mice (Fig. 2*B2*). Labeling in the normal mouse sciatic was abolished at every node when the antibody was preincubated with the antigenic peptide (Fig. 2*B3*).

Because $Na_v1.6$ is widely expressed in the CNS, we next investigated the distribution of $Na_v1.6$ at nodes of Ranvier in CNS axons. Immunofluorescent labeling of sections of retina and optic nerve revealed labeling of ganglion cell unmyelinated axons within the retina (not shown) and labeling of nodes of Ranvier in the optic nerve (Fig. 2*C*). Of 387 nodes examined, every node in the optic nerve that labeled with the anti-caspr antibody also labeled for $Na_v1.6$. Thus, retinal ganglion cell axons are labeled in both the unmyelinated segment of the axons (within the retina) and at nodes of Ranvier in the myelinated regions (in the optic nerve).

Dendrites of several classes of neurons in the CNS contain voltage-gated sodium channels, and these dendrites can generate sodium-dependent action potentials (for review, see Magee *et al.*, ref. 7). Therefore, we examined pyramidal cells of the neocortex and Purkinje cells of the cerebellum. Dendritic labeling was observed in both cell types. Apical and basal dendrites of cortical pyramidal cells were strongly labeled (Fig. 3*A*). Cell bodies were also labeled. Much of the label in the soma and dendrites appeared to be cytoplasmic. Coimmunostaining for SV2, a synaptic vesicle protein, produced punctate labeling that outlined cell bodies and dendrites, and there was little overlap of SV2 and $Na_v1.6$ labeling. In the cerebellum, Purkinje cell dendrites were strongly labeled for $Na_v1.6$ (Fig. 3*B*), much of which appeared to be intracellular. There was a homogenous, fine, punctate pattern of $Na_v1.6$ labeling in the molecular layer (Fig. 3*B*, m). Labeling of $Na_v1.6$ in the granule cell layer (Fig. 3*B*, gr) was weaker than that in the molecular layer. $Na_v1.6$ is also expressed in glia (23, 25). Coimmunostaining for the glial marker GFAP showed that radial glial processes in the molecular layer were labeled for both GFAP and $Na_v1.6$ but that GFAP-positive astrocytes in the granule cell layer did not contain $Na_v1.6$. Thus, not all GFAP-positive glia were labeled for $Na_v1.6$. Colabeling of SV2 and $Na_v1.6$ in the cerebellum was similar to that seen in the neocortex (data not shown).

The subcellular distribution was investigated further with electron microscopy. In the cerebral cortex, $Na_v1.6$ was at both presynaptic and postsynaptic membranes (Fig. 4 *A* and *B*, arrows) and in membranes of axons (Fig. 4*A*, a). Preincubation of the antibody with the antigenic peptide blocked all labeling (Fig. 4*D*). Some dendritic processes were heavily labeled (Fig. 4*B*). The dendritic labeling was on both nonsynaptic membranes (arrowhead) and associated with microtubules. The microtubule labeling is consistent with vesicles containing $Na_v1.6$ being transported, but vesicular labeling remains to be demonstrated. The intradendritic labeling is also consistent with the immunofluorescence illustrated in Fig. 3. Labeling of the molecular layer of the cerebellar cortex occurred at presynaptic and postsynaptic membranes of parallel fibers and Purkinje cell dendritic spines (Fig. 4*C*, arrow). Labeling was also observed on nonsynaptic membranes of parallel fiber axons (Fig. 4*C*, p), which are unmyelinated axons of granule cells. Parallel fiber labeling had a patchy appearance in regions that were not presynaptic, raising

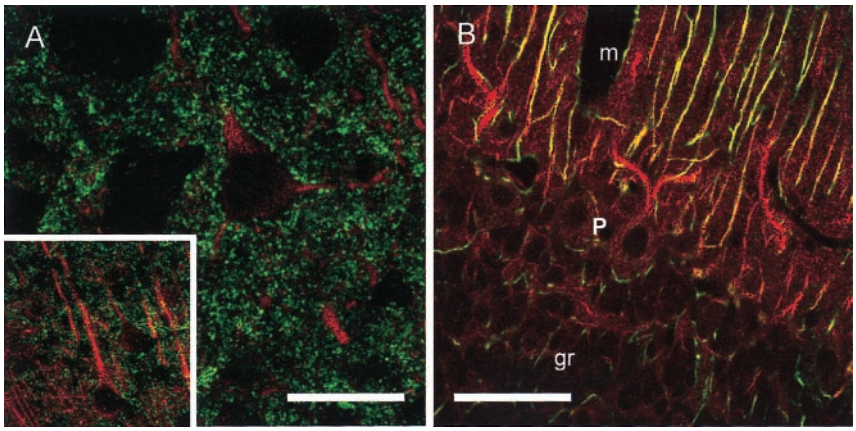

Fig. 3. Immunofluorescence labeling of cerebral cortex for $Na_v1.6$ and synaptic vesicles and of cerebellar cortex for $Na_v1.6$ and glial cells. Single confocal sections. (*A*) Cerebral cortex labeled for $Na_v1.6$ (red) and SV2 (green). Apical dendrites of neurons were labeled for $Na_v1.6$ within the dendrite. SV2 labeling was throughout the neuropil and outlined the cell bodies and large dendrites of the neurons. (*Inset*) Deconvolved image of cortical neurons (lower magnification than in *A*). (*B*) Cerebellar cortex labeled for $Na_v1.6$ (red) and GFAP (green). $Na_v1.6$ labeling was intense in apical dendrites of Purkinje cells in the vertically oriented GFAP-positive radial glial cell processes (yellow) and was diffusely present in the molecular layer (m). Glial cell processes that were GFAP-positive in the granule cell layer (gr) were not labeled for $Na_v1.6$. Purkinje cell bodies (P layer) were lightly labeled in the cytoplasm. (Bars = 20 μm for *A*, 75 μm for *Inset*, and 50 μm for *B*.)

the possibility that $Na_v1.6$ is clustered even in the purely conductive regions of unmyelinated axons.

Discussion

The $Na_v1.6$ sodium channel isoform has a remarkable subcellular distribution. It is at many locations expected for sodium channels from classical studies (myelinated axons in the PNS and CNS and unmyelinated axons in the CNS) and from recent studies (dendrites). It is also found in presynaptic and postsynaptic membranes in neocortex and cerebellum. These results underscore the importance of understanding how this isoform is targeted and modulated. The fact that $Na_v1.6$ resides at multiple locations suggests that this isoform interacts with different proteins to produce the diversity of sites at which it is localized.

We conclude that $Na_v1.6$ is the predominant sodium channel at nodes of Ranvier in myelinated axons of the PNS for the following reasons. (*i*) $Na_v1.6$ is the only sodium channel to be identified at any node. (*ii*) Every node that we examined in the PNS was labeled for $Na_v1.6$. (*iii*) Although not rigorously quantitated, the intensity of labeling was comparable to that of the pan-specific antibody. Is $Na_v1.6$ the only sodium channel at peripheral nodes? It is possible that the epitopes chosen for the other sodium channel isoform antibodies are masked by proteins that associate with the channels at the node. However, antigen retrieval methods have thus far failed to reveal peripheral nodal staining of $Na_v1.1$, 1.2, or 1.7 isoforms, whereas $Na_v1.7$ antibodies vigorously stain PNS unmyelinated fibers (A. G. Koszowski and S.R.L., unpublished observations). Action potentials could be recorded from peripheral nerve of mice lacking $Na_v1.6$ (33), and pan-specific sodium channel antibodies label these nodes (data not shown), implying that other isoforms either are normally present early in development or are targeted to nodes to compensate for the loss of $Na_v1.6$. If compensation does occur, it is apparently inadequate for normal nervous system function, because these mice die 3–4 weeks after birth.

CNS nodes of Ranvier were also labeled for $Na_v1.6$. Every node in the optic nerve labeled by the anti-caspr antibody was also labeled for $Na_v1.6$. Is $Na_v1.6$ the only Na channel at central nodes? $Na_v1.2$ is normally not localized in myelinated axons. However, in the hypomyelination mutant mouse *Shiverer*, $Na_v1.2$ is found in axon tracts that are normally myelinated (21). Sodium channel labeling with pan-specific antibody showed that few nodes are present, and many aggregates were abnormal (34). It is not known whether $Na_v1.2$ is at these nodal-like aggregates or is more uniformly distributed as it is in unmyelinated axons.

Our results in the PNS and CNS imply that in the mature animal, every node contains $Na_v1.6$. The demonstration that $Na_v1.6$ is the predominant nodal channel now allows one to test for the requirements for targeting and immobilization at the node. Currently, there is significant evidence that supports

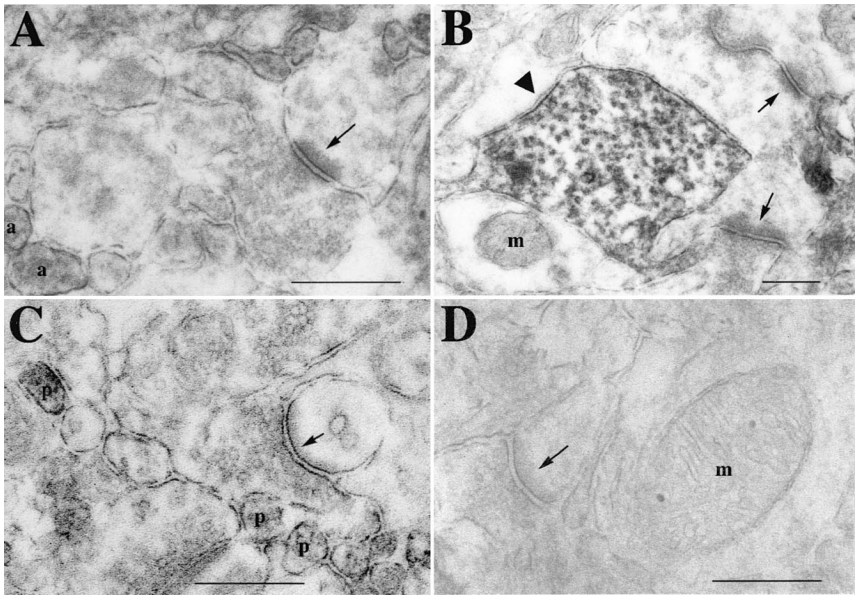

Fig. 4. Axonal and dendritic labeling of $Na_v1.6$ in cerebral and cerebellar cortex. (*A*) Labeling of presynaptic and postsynaptic membranes (arrow) in cerebral cortex. Plasma membranes of axons (a) were also labeled. (*B*) Synaptic membrane labeling (arrows) as well as membrane (arrowhead) and microtubule-associated labeling in a dendrite of cerebral cortex. Plasma membrane labeling of the dendrite was independent of a synapse, and adjacent membrane (arrowhead) was unlabeled, indicating that labeling of adjacent membranes at synapses was not due to diffusion of reaction product. A mitochondrion (m) was unlabeled. (*C*) Molecular layer of the cerebellar cortex. Presynaptic and postsynaptic labeling between a parallel fiber (with synaptic vesicles) and a dendritic spine (arrow) of a Purkinje cell. Parallel fiber axon profiles (p) were also labeled. (*D*) Cerebral cortex showed no labeling when the antibody was preincubated with the antigenic peptide. A synapse (arrow) and mitochondrion (m) were not labeled. (Bars = 0.4 μm for *A*, *C*, and *D* and 0.3 μm for *B*.)

essential roles for Schwann cell–axonal contact and Schwann cell elongation for channel clustering and spacing during nodal formation in the PNS (1). However, other studies suggest that in both the PNS and CNS node-like channel, clusters can occur independently of direct glial contact, likely involving a secreted glial factor (2). These issues may be addressed by focusing on domains of $Na_v1.6$ that interact with extracellular or intracellular molecules and the manner in which such interactions are controlled during nodal formation.

Unmyelinated axons of ganglion cells in the retina and of parallel fibers in the cerebellum were labeled by the $Na_v1.6$ antibody. Thus, in the CNS (but not the PNS) $Na_v1.6$ is not only the nodal channel but is also used for conduction in unmyelinated axons. $Na_v1.2$ has been localized to unmyelinated axons in the hippocampus and is in the molecular layer of the cerebellum (20, 22). Thus, it is likely that parallel fibers in the cerebellum contain both $Na_v1.2$ and $Na_v1.6$. It is not clear why two isoforms coexist in the same unmyelinated axons. The possibility that one is used strictly for conduction and the other is associated only with synaptic membranes does not fit with our observation that $Na_v1.6$ is at both conducting and synaptic sites. Labeling of $Na_v1.6$ in unmyelinated axons in the cerebellum had a patchy appearance at the ultrastructural level, suggesting that $Na_v1.6$ may be clustered. Similar clusters have been observed in PNS unmyelinated axons stained with an anti-$Na_v1.7$ antibody (A. G. Koszowski and S.R.L., unpublished observations). Clustering may occur, because sodium channels associate with a complex of anchoring and modulating proteins in all axons.

Intracellular labeling associated with microtubules in dendrites was prominent. We tentatively interpret this labeling as being associated with vesicles that contain sodium channels either in transit to distal dendrites or constituting a pool of mobilizable sodium channels. A large fraction (≈70%) of glutamate receptors has been shown to be intracellular, especially in dendrites (35, 36). These intracellular glutamate receptors constitute a pool that is recruited to dendritic membrane and spines after tetanic stimulation of the neuron (36). It is possible that the intracellular pool of $Na_v1.6$ can also be recruited to active synapses and may be a factor in synaptic plasticity.

Some plasma membrane labeling was on dendritic shaft membrane that was not associated with a synapse. Sodium channels were predicted to be at this location from electrophysiological studies (4–7). These $Na_v1.6$ channels could alter synaptic integration in dendrites, e.g., by supporting orthodromic or antidromic propagation of dendritic action potentials.

An unexpected and striking finding was that $Na_v1.6$ is localized in the CNS in presynaptic and postsynaptic membranes, sites where sodium channels have not been observed previously. $Na_v1.6$ was localized to presynaptic membranes of synapses in the cerebral and cerebellar cortices. Colocalization of synaptic vesicle protein and $Na_v1.6$ was not evident at the light microscopic level, perhaps because the synaptic vesicle protein is much more abundant than $Na_v1.6$ in the terminal. The presence of $Na_v1.6$ in presynaptic membranes suggests that it may be important for local regulation of synaptic vesicle release. The localization of $Na_v1.6$ in postsynaptic membrane is analogous to the high density of $Na_v1.4$ in the muscle postsynaptic membrane (37) where the channels reduce the threshold for action potential initiation. Thus, $Na_v1.6$ may directly affect synaptic strength at both the presynaptic and postsynaptic membranes.

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1. Dugandzija-Novakovic, S., Koszowski, A. G., Levinson, S. R. & Shrager, P. (1995) *J. Neurosci.* **15,** 492–503.
2. Kaplan, M. R., Meyer-Franke, A., Lambert, S., Bennett, V., Duncan, I. D., Levinson, S. R. & Barres, B. A. (1997) *Nature (London)* **386,** 724–728.
3. Salzer, J. L. (1999) *Neuron* **23,** 627–629.
4. Huguenard, J. R., Hamill, O. P. & Prince, D. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2473–2477.
5. Regehr, W. G. & Armstrong, C. M. (1994) *Curr. Biol.* **4,** 436–439.
6. Stuart, G., Spruston, N., Sakmann, B. & Hausser, M. (1997) *Trends Neurosci.* **20,** 125–131.
7. Magee, J., Hoffman, D., Colbert, C. & Johnston, D. (1998) *Annu. Rev. Physiol.* **60,** 327–346.
8. Schreibmayer, W. (1999) *Cell. Physiol. Biochem.* **9,** 187–200.
9. Ma, J. Y., Catterall, W. A. & Scheuer, T. (1997) *Neuron* **19,** 443–452.
10. Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H. & Numa, S. (1986) *Nature (London)* **320,** 188–192.
11. Goldin, A. L. (1999) *Ann. N.Y. Acad. Sci.* **868,** 38–50.
12. Noda, M., Ikeda, T., Suzuki, H., Takeshima, H., Takahashi, T., Kuno, M. & Numa, S. (1986) *Nature (London)* **322,** 826–828.
13. Auld, V. J., Goldin, A. L., Krafte, D. S., Marshall, J., Dunn, J. M., Catterall, W. A., Lester, H. A., Davidson, N. & Dunn, R. J. (1988) *Neuron* **1,** 449–461.
14. Dietrich, P. S., McGivern, J. G., Delgado, S. G., Koch, B. D., Eglen, R. M., Hunter, J. C. & Sangameswaran, L. (1998) *J. Neurochem.* **70,** 2262–2272.
15. Raman, I. M., Sprunger, L. K., Meisler, M. H. & Bean, B. P. (1997) *Neuron* **19,** 881–891.
16. Cannon, S. C. (1997) *Neuromuscul. Disord.* **7,** 241–249.
17. Wang, Q., Shen, J., Splawski, I., Atkinson, D., Li, Z., Robinson, J. L., Moss, A. J., Towbin, J. A. & Keating, M. T. (1995) *Cell* **80,** 805–811.
18. Kohrman, D. C., Smith, M. R., Goldin, A. L., Harris, J. & Meisler, M. H. (1996) *J. Neurosci.* **16,** 5993–5999.
19. Sprunger, L. K., Escayg, A., Tallaksen-Greene, S., Albin, R. L. & Meisler, M. H. (1999) *Hum. Mol. Genet.* **8,** 471–479.
20. Westenbroek, R. E., Merrick, D. K. & Catterall, W. A. (1989) *Neuron* **3,** 695–704.
21. Westenbroek, R. E., Noebels, J. L. & Catterall, W. A. (1992) *J. Neurosci.* **12,** 2259–2267.
22. Gong, B., Rhodes, K. J., Bekele-Arcuri, Z. & Trimmer, J. S. (1999) *J. Comp. Neurol.* **412,** 342–352.
23. Krzemien, D. M., Schaller, K. L., Levinson, S. R. & Caldwell, J. H. (2000) *J. Comp. Neurol.*, **420,** 70–83.
24. Schaller, K. L. & Caldwell, J. H. (2000) *J. Comp. Neurol.*, **420,** 84–97.
25. Schaller, K. L., Krzemien, D. M., Yarowsky, P. J., Krueger, B. K. & Caldwell, J. H. (1995) *J. Neurosci.* **15,** 3231–3242.
26. Burgess, D. L., Kohrman, D. C., Galt, J., Plummer, N. W., Jones, J. M., Spear, B. & Meisler, M. H. (1995) *Nat. Genet.* **10,** 461–465.
27. de Miera, E. S., Rudy, B., Sugimori, M. & Llinas, R. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 7059–7064.
28. Toledo-Aral, J. J., Moss, B. L., He, Z. J., Koszowski, A. G., Whisenand, T., Levinson, S. R., Wolf, J. J., Silos-Santiago, I., Halegoua, S. & Mandel, G. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 1527–1532.
29. Poliak, S., Gollan, L., Martinez, R., Custer, A., Einheber, S., Salzer, J. L., Trimmer, J. S., Shrager, P. & Peles, E. (1999) *Neuron* **24,** 1037–1047.
30. Einheber, S., Zanazzi, G., Ching, W., Scherer, S., Milner, T. A., Peles, E. & Salzer, J. L. (1997) *J. Cell Biol.* **139,** 1495–1506.
31. Peles, E., Nativ, M., Lustig, M., Grumet, M., Schilling, J., Martinez, R., Plowman, G. D. & Schlessinger, J. (1997) *EMBO J.* **16,** 978–988.
32. Kohrman, D. C., Harris, J. B. & Meisler, M. H. (1996) *J. Biol. Chem.* **271,** 17576–17581.
33. Duchen, L. W. & Stefani, E. (1971) *J. Physiol. (London)* **212,** 535–548.
34. Rasband, M. N., Peles, E., Trimmer, J. S., Levinson, S. R., Lux, S. E. & Shrager, P. (1999) *J. Neurosci.* **19,** 7516–7528.
35. Rubio, M. E. & Wenthold, R. J. (1999) *J. Neurosci.* **19,** 5549–5562.
36. Shi, S. H., Hayashi, Y., Petralia, R. S., Zaman, S. H., Wenthold, R. J., Svoboda, K. & Malinow, R. (1999) *Science* **284,** 1811–1816.
37. Caldwell, J. H., Campbell, D. T. & Beam, K. G. (1986) *J. Gen. Physiol.* **87,** 907–932.