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REGULAR ARTICLE

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Internodal specializations of myelinated axons in the central nervous system

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Abstract We have examined the localization of contactin-associated protein (Caspr), the *Shaker*-type potassium channels, Kv1.1 and Kv1.2, their associated β subunit, Kvβ2, and Caspr2 in the myelinated fibers of the CNS. Caspr is localized to the paranodal axonal membrane, and Kv1.1, Kv1.2, Kvβ2 and Caspr2 to the juxtaparanodal membrane. In addition to the paranodal staining, an internodal strand of Caspr staining apposes the inner mesaxon of the myelin sheath. Unlike myelinated axons in the peripheral nervous system, there was no internodal strand of Kv1.1, Kv1.2, Kvβ2, or Caspr2. Thus, the organization of the nodal, paranodal, and juxtaparanodal axonal membrane is similar in the central and peripheral nervous systems, but the lack of Kv1.1/Kv1.2/Kvβ2/Caspr2 internodal strands indicates that the oligodendrocyte myelin sheaths lack a *trans* molecular interaction with axons, an interaction that is present in Schwann cell myelin sheaths.

Keywords Myelin · Incisures · Schwann cells · Oligodendrocytes · Cell adhesion molecules · Potassium and sodium channels · Contactin-associated protein (Caspr) · Nodes · Mouse · Rat

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Introduction

Myelin is a multilamellar spiral of specialized cell membrane that ensheaths axons larger than 1 μm in diameter. Although the myelin sheaths of the central nervous system (CNS) and peripheral nervous system (PNS) differ in their cellular origins, anatomical details, and molecular constituents, they are thought to function similarly. Myelinated axons are completely covered by myelin sheaths except at the nodes of Ranvier, the small gaps (less than 1 μm in length) directly exposed to the extracellular milieu. By reducing the capacitance and/or increasing the resistance, myelin reduces current flow across the internodal axonal membrane, thereby facilitating saltatory conduction at nodes (Ritchie 1995).

The CNS myelin sheath is largely composed of compact myelin (Hudson 1990; Monuki and Lemke 1995). It is enriched in lipids, including specialized glycolipids such as galactocerebroside and sulfatide, and contains large amounts of two proteins, proteolipid protein (PLP) and myelin basic protein (MBP). The innermost aspect of the oligodendrocyte membrane (the adaxonal membrane) apposes the axon and contains myelin-associated glycoprotein (MAG; Trapp et al. 1989); the outermost membrane is enriched in myelin-oligodendrocyte glycoprotein (MOG) and the gap junction protein connexin32 (Brunner et al. 1989; Li et al. 1997; Scherer et al. 1995). The tight junction protein claudin-11, also known as oligodendrocyte-specific protein (OSP), forms the tight junctions of the inner and outer mesaxons and the so-called "radial component" of compact myelin (Gow et al. 1999; Morita et al. 1999).

The axonal membrane is organized in relation to the CNS myelin sheath (Arroyo and Scherer 2000). The nodal axonal membrane is highly enriched in voltage-gated Na^+ channels, which are linked to the spectrin cytoskeleton by $ankyrin_G$ (Bennett and Lambert 1999; Berghs et al. 2000). The paranodal axonal membrane is enriched in contactin, contactin-associated protein (Caspr; also known as paranodin), which, together with the neurofascin 155 kDa expressed in the glial endfeet,

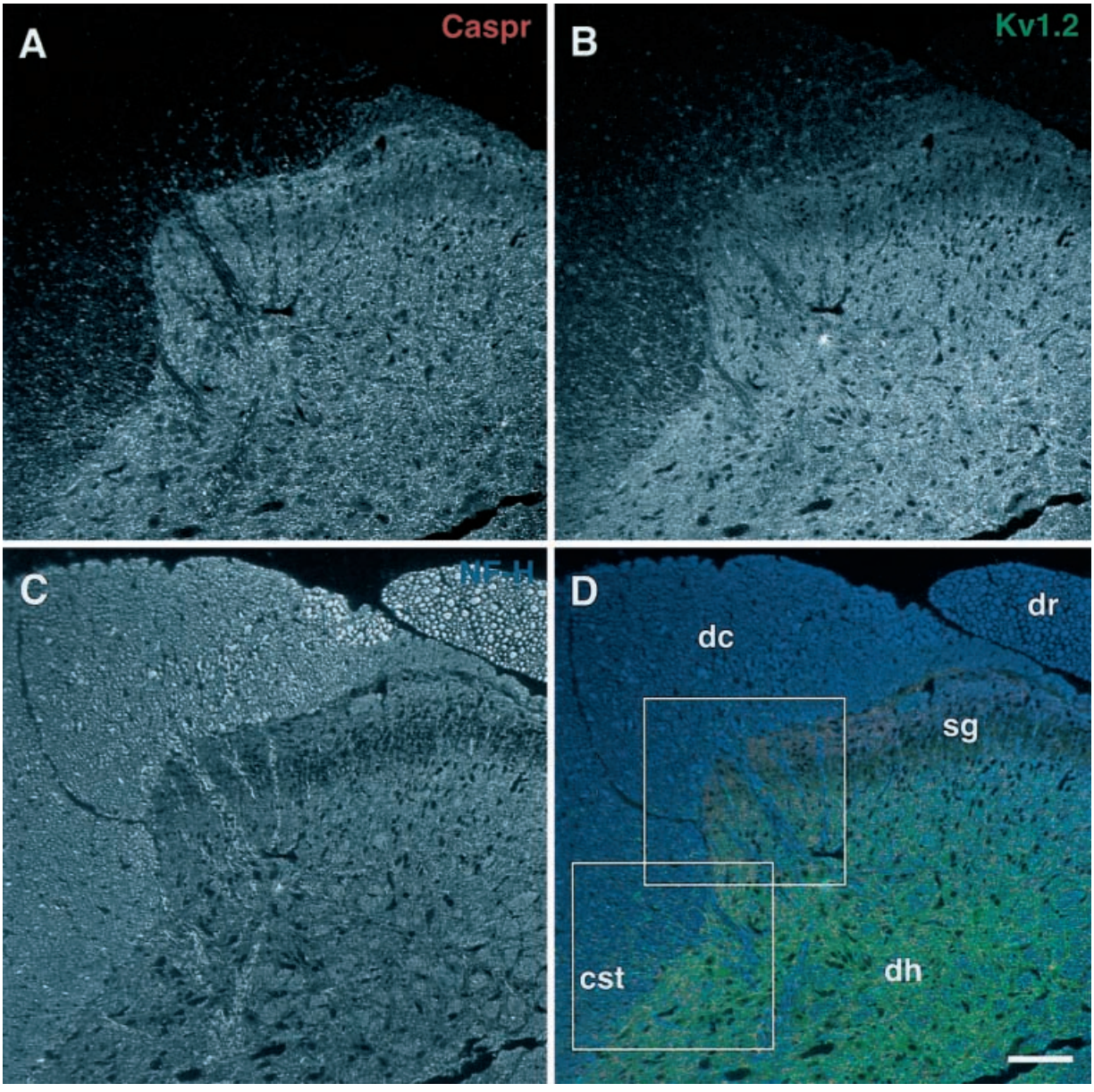

Fig. 1A–D Localization of Caspr and Kv1.2 in adult rat spinal cord. Scanning laser confocal micrographs of an adult rat spinal cord (10-µm-thick transverse section, 1 h in Zamboni's fixative), labeled with a rabbit antiserum against Caspr (**A** TRITC), a mouse monoclonal antibody against Kv1.2 (**B** FITC), and a rat monoclonal antibody against NF-H (**C** cy-5). **D** Merged image. Note the corticospinal tract (*cst*) is more heavily labeled than the rest of the dorsal columns (*dc*), and that the dorsal horn (*dh*) is heavily labeled except for the substantia gelantinosa (*sg*) (*dr* dorsal rootlet). The regions shown in Fig. 2A, C are *boxed*. *Bar* 100 µm

probably form the septate-like axoglial junctions (Einheber et al. 1997; Menegoz et al. 1997; Rios et al. 2000; Tait et al. 2000). The juxtaparanodal axonal membrane contains the *Shaker*-type potassium channels, Kv1.1 and Kv1.2, their associated β subunit, Kvβ2, and Caspr2 (Arroyo and Scherer 2000; Arroyo et al. 1999; Mi et al. 1995; Poliak et al. 1999; Rasband et al. 1998, 1999; Vabnick and Shrager 1998; Wang et al. 1993; Zhou et al. 1998). The distinct localization of these axonal proteins into nodal, paranodal, and juxtaparanodal domains provides compelling evidence that they are localized by *trans* interactions with myelinating glia cell, although the molecular basis of these interactions remains to be determined.

The internodal region of myelinated axons in the PNS is also specialized. A tripartite strand, viz., a central strand of Caspr flanked by two strands of Kv1.1 and Kv1.2, is apposed to the inner mesaxon of the myelin sheath (Arroyo et al. 1999), a configuration that has been termed “juxtamesaxonal” (Peles and Salzer 2000). In this report, we have investigated the internodal localization of Kv1.1, Kv1.2, Kvβ2, Caspr, and Caspr2 in myelinated fibers of the CNS. We confirm that Caspr is localized in an internodal strand (Einheber et al. 1997; Menegoz et

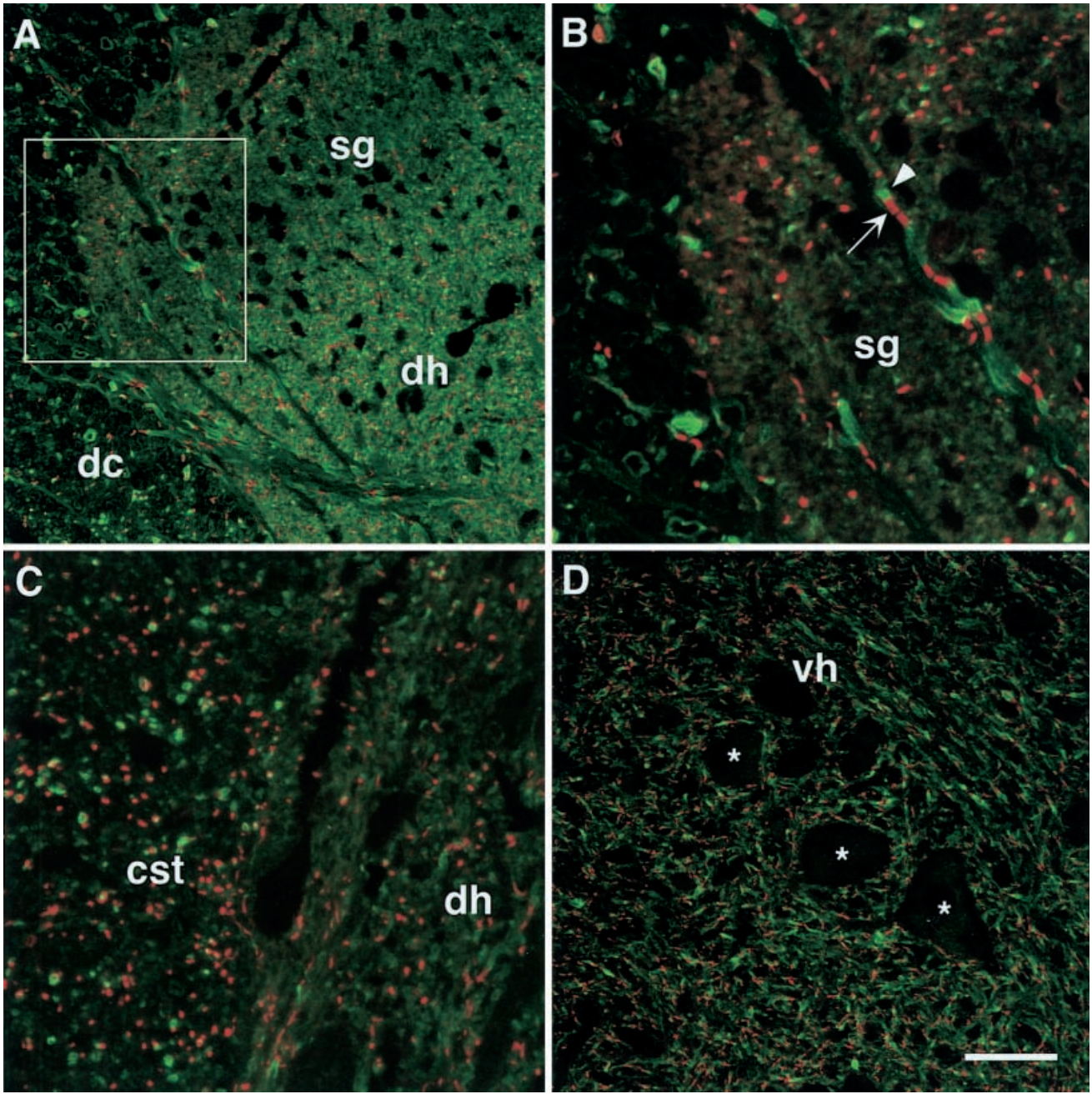

Fig. 2A–D Paranodal and juxtaparanodal proteins are more concentrated in small myelinated fibers. Scanning laser confocal micrographs of similar sections to those shown in Fig. 1; NF-H staining has been omitted. **A, B** Dorsal horn. **C** Corticospinal tract and adjacent gray matter. **D** Ventral horn (*vh*). Note that the Caspr staining and much of the Kv1.2 staining are largely comprised of puncta; at high magnification (**B, C**), many of these are revealed to be paranodes (Caspr, *arrow* in **B**) and juxtaparanodes (Kv1.1; *arrowhead* in **B**). *Asterisks* Motoneuron cell bodies; other abbrevations as in Fig. 1. *Bar* **A, D** 25 µm, **B, C** 10 µm

al. 1997), probably apposed to the inner mesaxon. In contrast, we have not been able to find internodal strands of Kv1.1, Kv1.2, Kvβ2, or Caspr2, indicating that oligodendrocytes lack the molecular means for organizing these axonal proteins.

Materials and methods

Mouse or rat sciatic spinal cords were (1) embedded unfixed in OCT and immediately frozen in a dry-ice/acetone bath, (2) briefly fixed in either freshly prepared 4% paraformaldehyde (in 0.1 M phosphate buffer pH 7.4) or Zamboni's fixative (Zamboni and de Martino 1967) and infiltrated in 20% sucrose in 0.1 M phosphate buffer (pH 7.4) overnight prior to embedding in OCT. Cryostat sections (5–10 µm thick) were cut and thaw-mounted on Super-Frost Plus glass slides, stored at –20°C, and permeabilized by immersion in acetone for 10 min, blocked at room temperature for at least 1 h in 5% fish-skin gelatin containing 0.5% Triton X-100 in phosphate-buffered saline, and incubated 24–48 hours at 4°C with various combinations of primary antibodies. We used rabbit antisera against rat Kv1.1 and Kv1.2 (diluted 1:300; Alomone Labs, Jerusalem, Israel), Caspr (diluted 1:1000; Einheber et al. 1997; Peles et al. 1997), Caspr2 (diluted 1:100; Poliak et al. 1999), MAG (diluted 1:300; Pedraza et al. 1990), claudin-11/OSP (dilut-

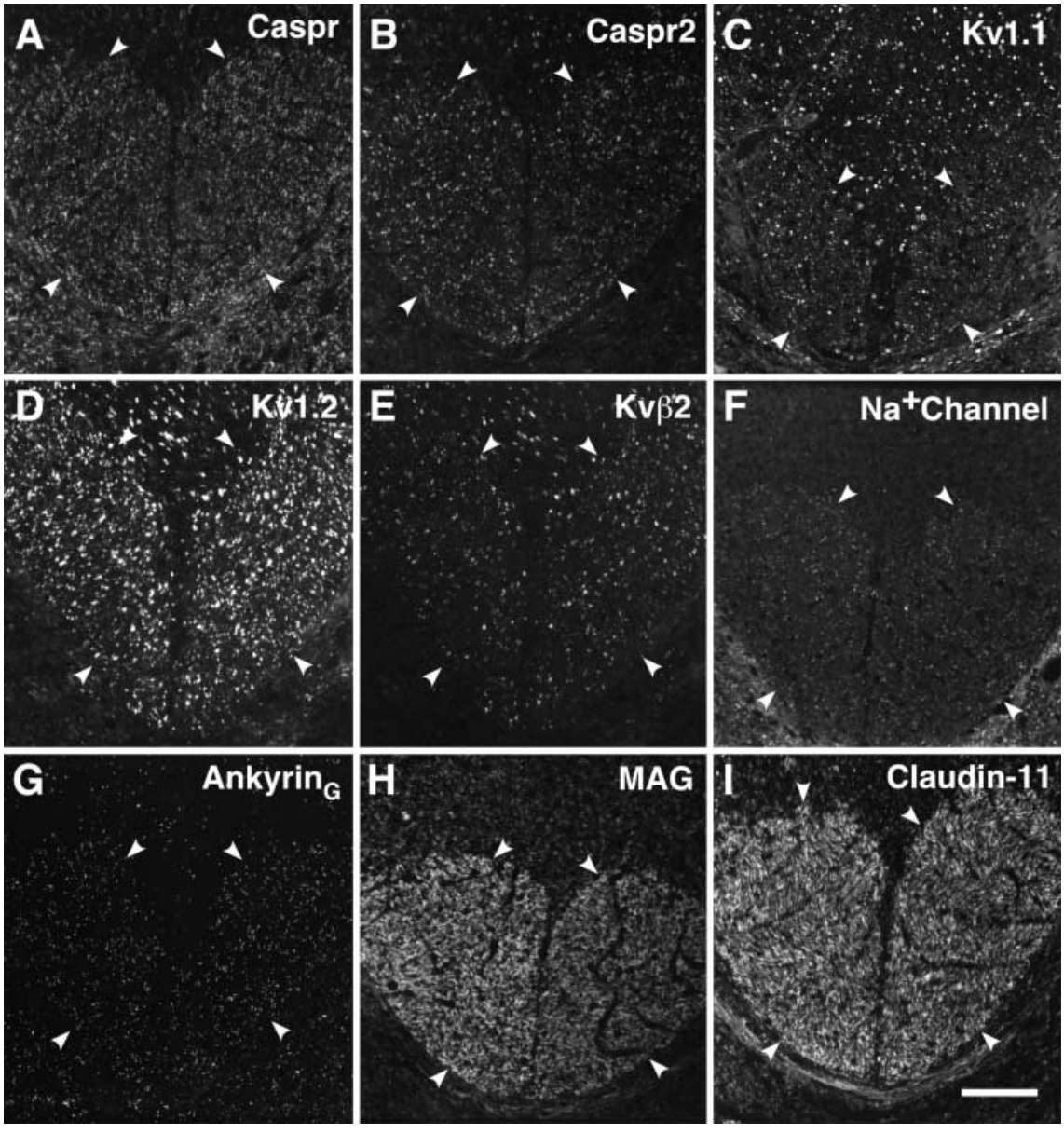

Fig. 3A–I Caspr, Caspr2, Kv1.1, Kv1.2, Kvβ2, Na^+ channel, $ankyrin_G$, MAG, and claudin-11/OSP localization in the spinal cord. Micrographs of adult rat spinal cord: transverse sections immunolabeled for Caspr (**A** rabbit antiserum), Caspr2 (**B** rabbit antiserum), Kv1.1 (**C** mouse monoclonal), Kv1.2 (**D** mouse monoclonal), Kvβ2 (**E** mouse monoclonal), Na^+ channels (**F** mouse monoclonal), $ankyrin_G$ (**G** mouse monoclonal), MAG (**H** mouse monoclonal), and claudin-11/OSP (**I** rabbit antiserum). Note that the CST (*arrowheads*) is more heavily labeled than either of the overlying dorsal columns. *Bar* 50 μm

ed 1:500–1000; Gow et al. 1999; Morita et al. 1999), and nodal Na^+ channels (diluted 1:200; Vabnick et al. 1997), and in various combinations with mouse monoclonal antibodies against rat Caspr (diluted 1:50; Poliak et al. 1999), MAG (513, diluted 1:100; Boehringer Mannheim), MOG (Y11, diluted 1:10; Piddlesden et al. 1991), nodal Na^+ channels (diluted 1:50; Sigma), $ankyrin_G$ (diluted 1:50; Zymed, South San Francisco, Calif.), Kv1.1 and Kv1.2 (diluted 1:100; Upstate Biotechnology, Lake Placid, N.Y.), Kvβ2 (diluted 1:100; Rhodes et al. 1997), and a rat monoclonal antibody against neurofilament heavy (NF-H; Ta51, diluted 1:10; Lee et al. 1982). We have previously shown that the antibodies against Kv1.1 and Kv1.2 do not stain myelinated axons in Kv1.1- and Kv.1.2-null mice, respectively, thereby demonstrating their specificity (Arroyo et al. 1999). After being incubated with the primary antibodies, the slides were washed, incubated with the appropriate fluorescein-, rhodamine-, and cyanine-5 (cy-5)-conjugated donkey anti-rabbit and/or anti-mouse cross-affinity purified secondary antibodies (diluted 1:100; Jackson ImmunoResearch Laboratories, West Grove, Pa.), and mounted with Vectashield (Vector Laboratories, Burlingame, Calif.). Specimens were examined by epifluorescence with tetramethylrhodamine isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC) optics on a Leica DMR light microscope and photographed on Kodak film (ASA 400) or by means of a Leica TCS laser scanning confocal microscope followed by image manipulation with Adobe Photoshop.

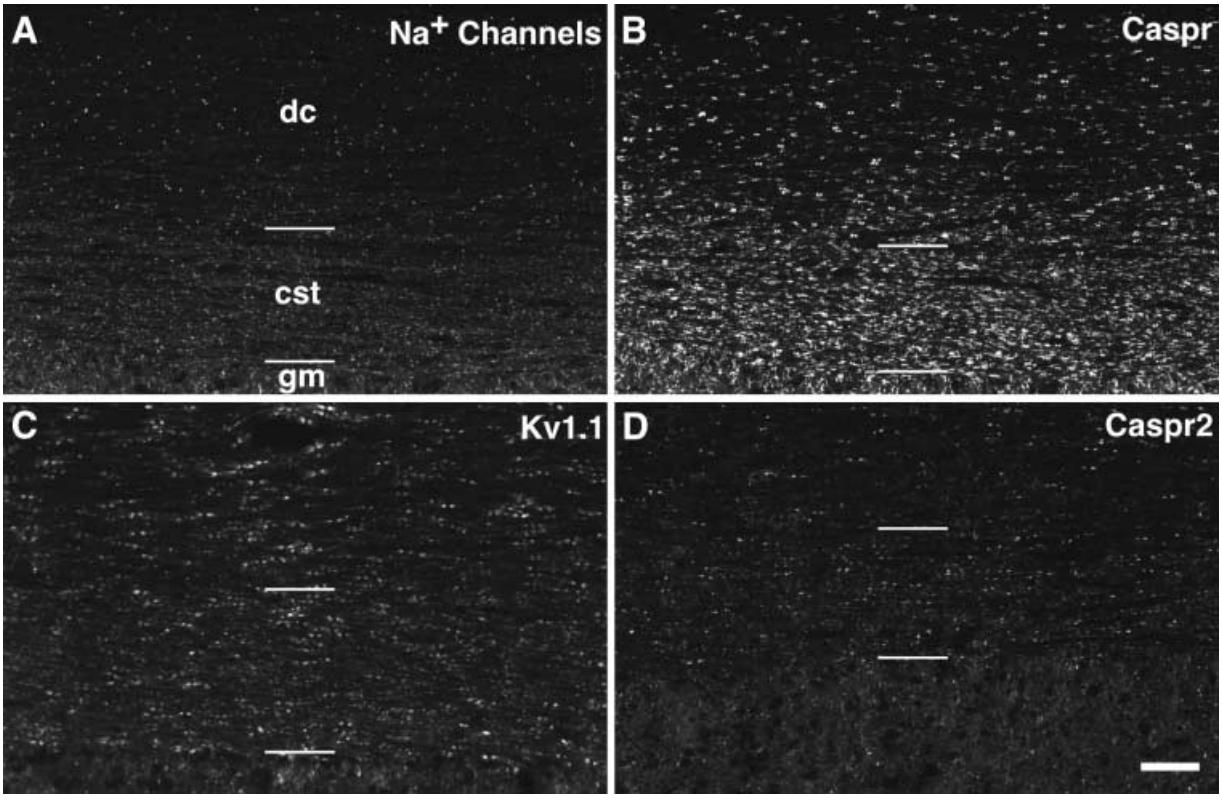

Fig. 4A–D Na^+ channels, Caspr, Kv1.1, and Caspr2 localization in the spinal cord. Micrographs of parasagittal sections of adult rat spinal cords (1 h in Zamboni's fixative), immunolabeled for Na^+ channels (**A** mouse monoclonal), Caspr (**B** mouse monoclonal), Kv1.1 (**C** mouse monoclonal), Caspr2 (**D** rabbit antiserum). Note that the corticospinal tract (*cst*) is more heavily labeled than the rest of the dorsal columns (*dc*), and nodal (**A**), paranodal (**B**), and juxtaparanodal (**C, D**) staining accounts for the bulk of the immunoreactivity. *Bar* 50 μm

Results

Abundant expression of nodal, paranodal, and juxtaparanodal proteins in the gray matter

To determine the localization of Na^+ channels, $ankyrin_G$, Caspr, Kv1.1, Kv1.2, Kvβ2, and Caspr2 in the CNS, we examined cryosections of adult rat spinal cords labeled with antibodies against these proteins. The spinal cord is advantageous for this analysis because it contains myelinated fibers of various calibers, including some of the largest in the CNS, and because it has a well known architecture. Sections were made from spinal cords fixed for various periods of time in either 4% paraformaldehyde or Zamboni's fixative, and without fixation; we usually preferred fixation in Zamboni's for 1 h, as it preserved structural details, while still giving strong staining.

Our initial examination of transverse sections revealed an apparent paradox: the gray matter was more heavily labeled for these proteins than the surrounding white matter, even though these proteins are localized, at least in part, to the nodal region of myelinated fibers. This is shown for Caspr and Kv1.2 in Figs. 1 and 2, which show a transverse section containing the corticospinal tract (CST), the dorsal columns, and the dorsal horn. One clue to the resolution of this paradox came from the observation that the CST was more heavily labeled than the rest of the dorsal columns; this was true for Na^+ channels, $ankyrin_G$, Caspr, Kv1.1, Kv1.2, Kvβ2, and Caspr2, and for other constituents of the myelin sheath, viz., claudin-11/OSP, MAG (Fig. 3), and MOG (data not shown). This makes sense because the dorsal columns have larger myelinated fibers than does the CST (Chung and Coggeshall 1987); smaller myelinated fibers have thinner myelin sheaths and shorter internodal lengths (Hildebrand 1972; Murray and Blakemore 1980), making the nodal, paranodal, and juxtaparanodal proteins more conspicuous in the CST. Examining longitudinal sections confirmed that nodes were much more common in the CST, and that most of the immunoreactivity was localized to discrete puncta corresponding to nodal, paranodal, and juxtaparanodal regions, as shown in Fig. 4. MAG, claudin-11/OSP, and MOG were also relatively more abundant in the CST compared with the rest of the dorsal column, whereas the intensity of PLP and MBP staining had the opposite pattern (data not shown), consistent with the idea that the CST contains abundant, tightly packed, small myelinated fibers (Figs. 1, 2, 3, 4). In addition, the overall level of immunoreactivity for nodal, paranodal, and juxtaparanodal proteins was enhanced in the CST (Figs. 1, 2, 3, 4), perhaps because of the diffuse staining of the non-myelinat-

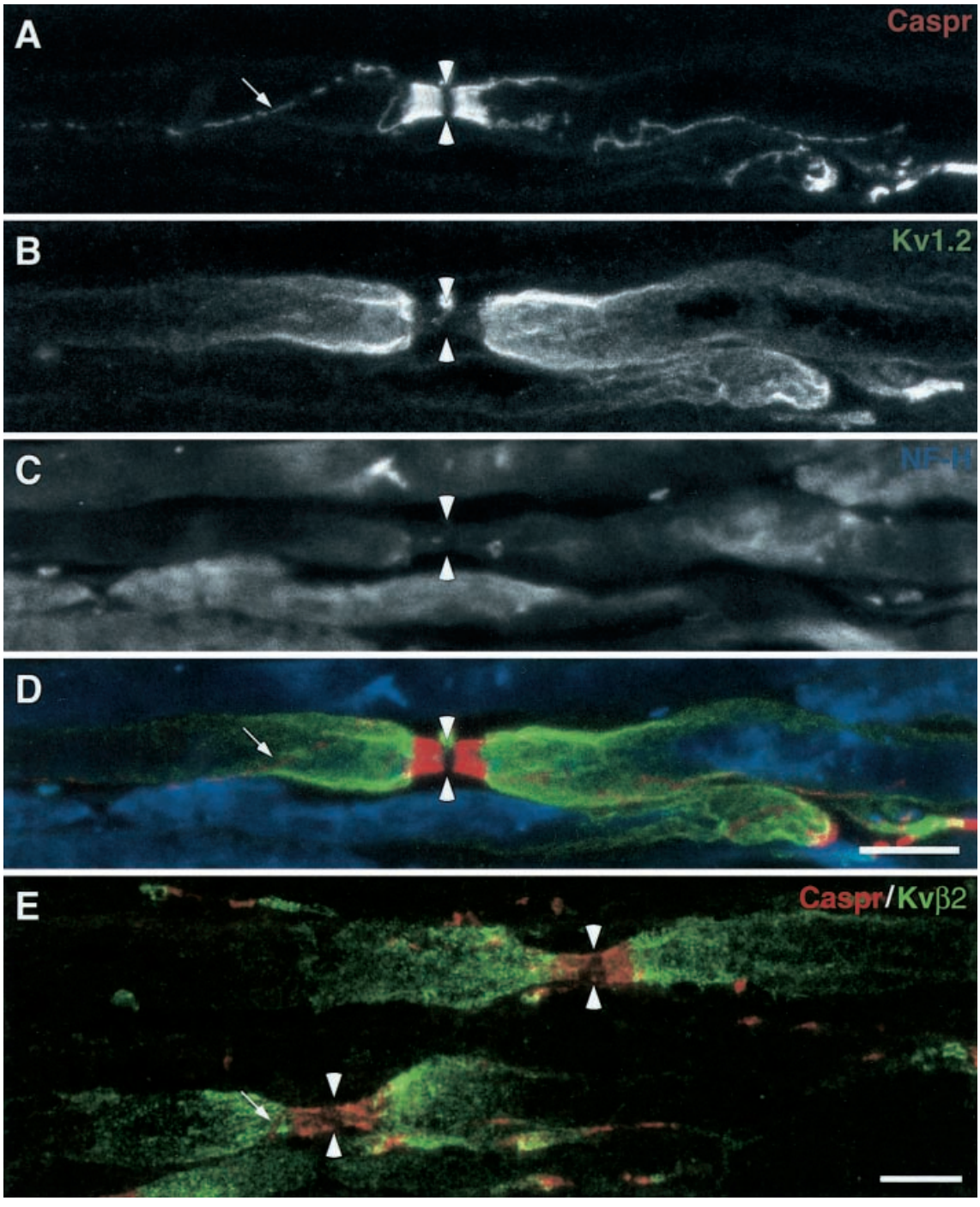

Fig. 5A–E Juxtaparanodal and internodal localization of Caspr. Scanning laser confocal micrographs of an adult rat spinal cord (10-μm-thick longitudinal section; **A–D** 1 h in Zamboni's fixative, **E** unfixed), labeled with a rabbit antiserum against Caspr (**A, E** TRITC), a mouse monoclonal antibody against Kv1.2 (**B** FITC) or KVβ2 (**E** FITC), and a rat monoclonal antibody against NF-H (**C** cy-5). **D** Merged image of **A–C** (*arrowheads* nodes of Ranvier, *arrows* juxtaparanodal/internodal line of Caspr). *Bars* 10 μm

ed axons (Davis et al. 1996), which are also abundant in the CST (Chung and Coggeshall 1987).

We similarly analyzed the gray matter itself. At high magnification, much of the immunoreactivity for Caspr, Kv1.1, Kv1.2, Kvβ2, and Caspr2 was localized in small puncta, just as in the white matter. In many cases, pairs of puncta were seen; these were paranodes and juxtaparanodes of small myelinated fibers (examples are shown in Fig. 2 for Caspr and Kv1.2). To confirm that the gray matter contained abundant small, thinly myelinated fibers, we examined 0.5-μm-thick epoxy sections of adult rat and mouse spinal cords stained with toluidine blue and found that this was indeed the case (data not shown). Furthermore, compared with MBP and PLP, the level of MAG, MOG, and claudin-11/OSP staining was relatively higher in the gray matter than in the white matter and ap-

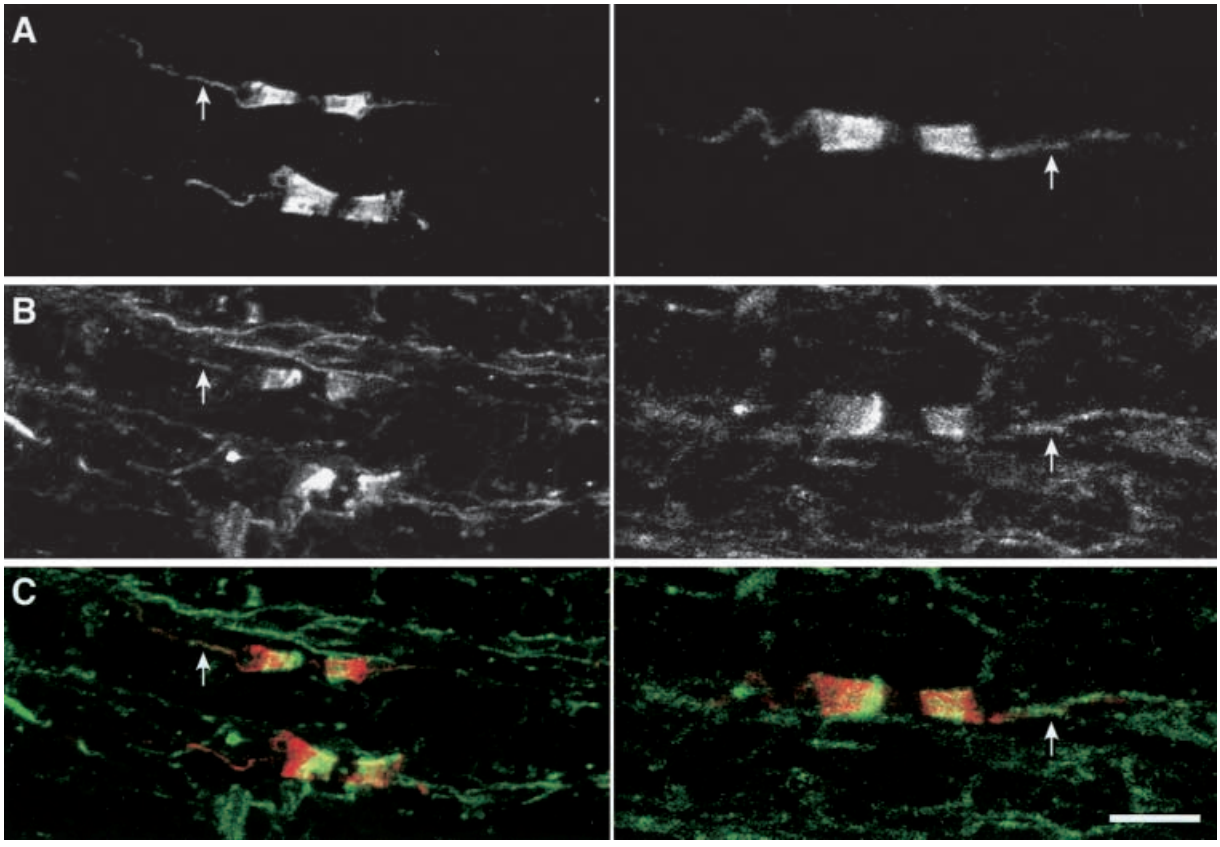

Fig. 6A–C Juxtaparanodal and internodal staining of Caspr. Micrographs of adult rat spinal cord myelinated fibers (10-µm-thick longitudinal section, 1 h in Zamboni's fixative), labeled for Caspr (**A** mouse antiserum, TRITC) and claudin-11/OSP (**B** rabbit antiserum, FITC). Note the colocalization of Caspr and claudin-11/OSP in the internodal region (*arrows*). **C** Merged image. Bar 5 µm

peared to be associated with these small, thinly myelinated axons (data not shown). Thus, although other neuronal and/or glial structures contain some Caspr, Kv1.1, Kv1.2, Kvβ2, or Caspr2, the punctate pattern of their immunoreactivity at least in the spinal cord can be accounted for by the abundant, thinly myelinated fibers.

Internodal staining of Caspr

To examine the largest myelinated fibers in more detail, we examined longitudinal sections of the ventral funiculus. As shown in Figs. 5, 6, 7, 8, 9, Caspr immunoreactivity was well developed in the paranodal region of such fibers (Einheber et al. 1997; Menegoz et al. 1997). We also noted that some large myelinated fibers had a thin line of Caspr immunoreactivity that spiraled in the juxtaparanodal region (Figs. 5, 6A, B). Double-labeling for Caspr and either Kv1.1 or Kv1.2 occasionally revealed a corresponding void in the region of this spiral (Fig. 6A, B), as we previously noted for myelinated PNS axons (Arroyo et al. 1999).

In some fibers, we could trace a thin line of Caspr staining from the paranode into the juxtaparanodal and even the internodal region (Figs. 5, 6). This staining was less robust than that in the PNS and could not be demonstrated in most myelinated fibers, in spite of the use of several ways to fix, section, and permeabilize the tissue. In the PNS, we have previously shown that this internodal staining of Caspr is apposed to the inner mesaxon of myelinating Schwann cells (Arroyo et al. 1999), as originally suggested by Menegoz et al. (1997). To determine whether the internodal Caspr staining the CNS is also "juxtamesaxonal" (Peles and Salzer 2000), we double-labeled sections with a monoclonal antibody against Caspr and a rabbit antisera against claudin-11/OSP, which labels the tight junctions in the inner mesaxon (Gow et al. 1999, Morita et al. 1999). The monoclonal antibody against Caspr seldom labeled the internodal strand of Caspr, and we only found a few examples of the co-localization of Caspr and claudin-11/OSP in the internodal region (Fig. 6). In the paranodal region, Caspr and claudin-11/OSP were typically co-localized, with a band of claudin-11/OSP surrounding Caspr (Fig. 6). We also noted internodal "rings" of claudin-11/OSP staining in the myelin sheaths of large myelinated fibers, putatively identified as CNS incisures by Gow and colleagues (1999), but we did not see corresponding rings of Caspr staining (data not shown).

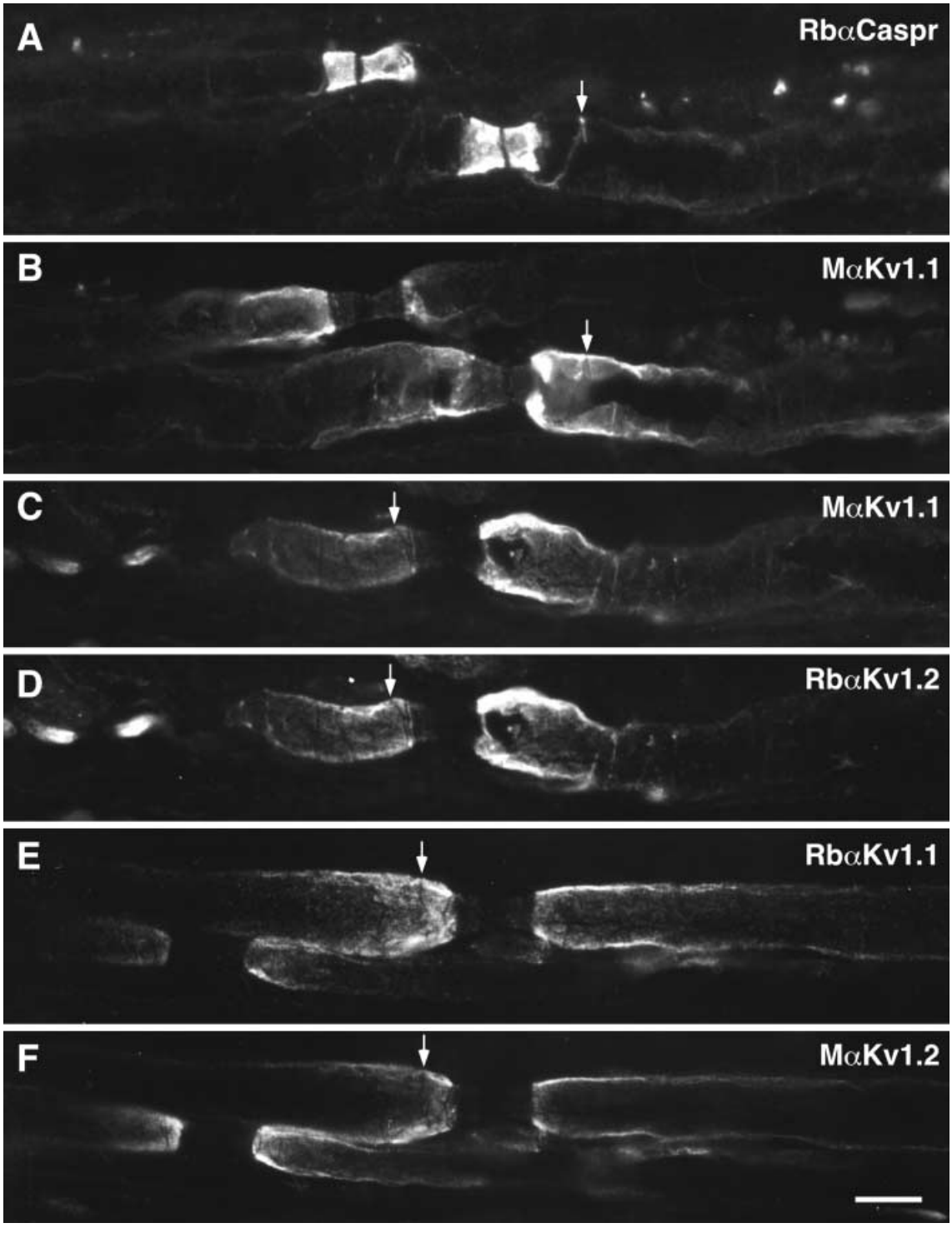

Fig. 7A–F Myelinated CNS fibers lack internodal Kv1.1 and Kv1.2. Micrographs of longitudinal sections of adult rat spinal cord (1 h in Zamboni's fixative), labeled for Caspr (**A**), Kv1.1 (**B, C, E**), and Kv1.2 (**D, F**). **A, B** Note the void of juxtaparanodal Kv1.1 staining where Caspr is localized (*arrows*). **C–F** Note a similar void of both Kv1.1 and Kv1.2 where Caspr staining is presumed to be located (*arrows*). *Bar* 10 μm

No internodal strand of Kv1.1, Kv1.2, Kvβ2, or Caspr2 in the CNS

Kv1.1 and Kv1.2 are co-localized in the juxtaparanodal region of myelinated fibers in the PNS and CNS (Mi et al. 1995; Poliak et al. 1999; Rasband et al. 1998, 1999; Rhodes et al. 1996; Wang et al. 1993). In addition, in the PNS, these potassium channels are located internodally as a double line flanking a single line of Caspr; this tri-

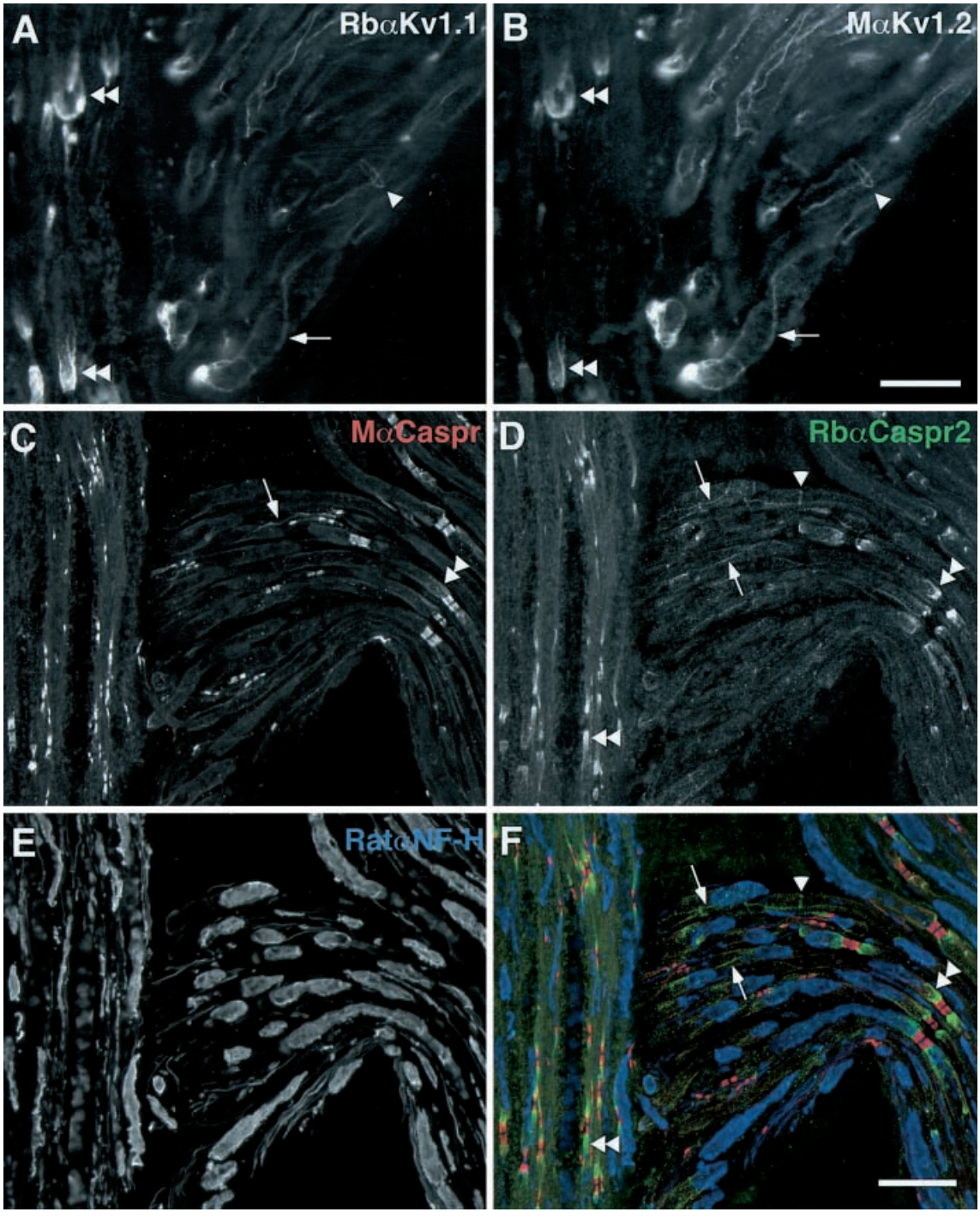

Fig. 8A–F The juxtaparanodal and internodal localization of Kv1.1, Kv1.2, and Caspr2. Micrographs (**A, B**) and laser scanning confocal images (**C–F**) of adult rat spinal cord (10-µm-thick longitudinal sections, 1 h in Zamboni's fixative) at the ventral root entry zone. Note that the juxtaparanodes (*double arrowheads*) of myelinated fibers in the spinal cord and the ventral rootlets are labeled, but that the juxtamesaxonal (*arrows*) and "juxtaincisural" (*single arrowheads*) staining of Kv1.1, Kv1.2, Caspr, and Caspr2 is restricted to the ventral rootlets. *Bar* 25 µm

partite line apposes the inner mesaxon and the incisures (Arroyo et al. 1999). We double-labeled sections of rat spinal cord with a combination of either a rabbit antiserum against Kv1.1 and a mouse monoclonal antibody against Kv1.2 or a rabbit antiserum against Kv1.2 and a mouse monoclonal antibody against Kv1.1. Both combinations gave similar results: juxtaparanodal staining was readily seen, but we could not find strands of juxtamesaxonal staining (Fig. 7). The lack of juxtamesaxonal staining in the CNS was dramatically documented in sections that included the ventral root entry zone, the posi-

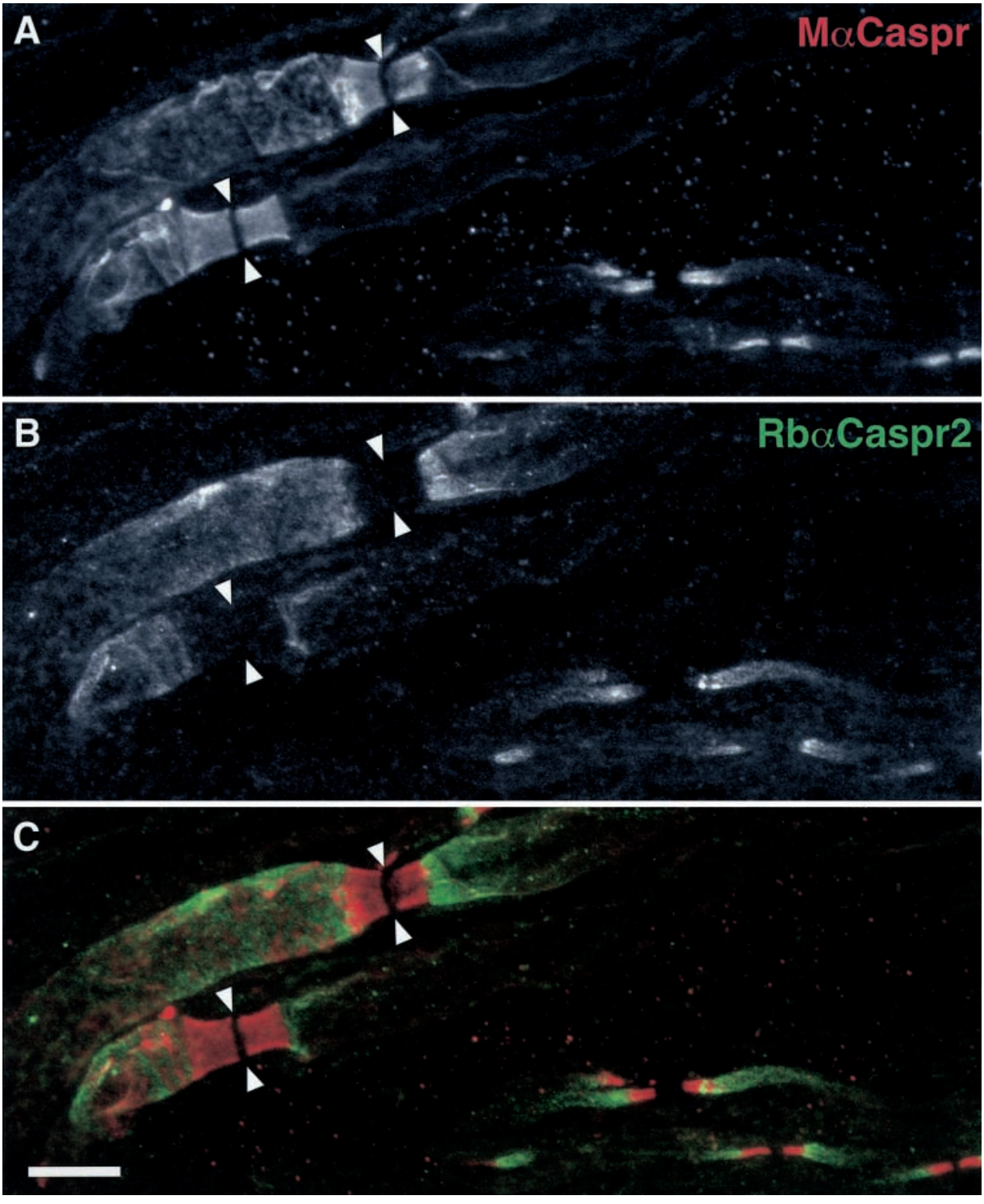

Fig. 9A–C Caspr and Caspr2 localization in the ventral root entry zone. Laser scanning confocal images of adult rat spinal cord (10-µm-thick longitudinal sections; 1 h in Zamboni's fixative) at the ventral root entry zone (*arrowheads* two nodes). The CNS/oligodendrocyte myelin sheath lies *left* of these nodes; the PNS/Schwann cell myelin sheath lies *right*. Note the diffuse juxtaparanodal Caspr and Caspr2 staining under the CNS/oligodendrocyte myelin sheath, compared with the normal pattern of Caspr and Caspr2 staining in the spinal cord *below*. *Bar* 10 µm

tion of an abrupt transition from CNS to PNS myelin; for individual fibers, this occurs at a single node (Fraher 1992). At the root entry zone, Kv1.1 and Kv1.2 internodal staining was seen under PNS/Schwann cell myelin sheaths, but not under CNS/oligodendrocyte myelin sheaths (Fig. 8A, B).

We also examined the localization of Kvβ2, which binds to the cytoplasmic domains of Kv1.1 and Kv1.2. Kvβ2 is known to be co-localized with these channels in

the juxtaparanodal region (Gulbis et al. 1999; Rhodes et al. 1996, 1997) in the CNS and the PNS and is co-localized with these channels in the juxtamesaxonal and juxtaincisural regions in the PNS (Arroyo et al. 1999). To determine whether this was also the case in the CNS, we double-labeled sections of rat spinal cord with a monoclonal antibody against Kvβ2 and a rabbit antiserum against Caspr. In longitudinal sections, Kvβ2 appeared to be localized to the juxtaparanodal region of most, if not all, myelinated fibers (Fig. 5E), but we did not see juxtamesaxonal staining. Because Kvβ2 staining was abolished by fixation, and the internodal localization of Caspr was difficult to demonstrate in unfixed tissue, we cannot completely exclude the possibility of internodal Kvβ2 staining. However, since Kvβ2 is co-localized with Kv1.1 and Kv1.2 everywhere else in myelinated axons, this possibility seems unlikely.

Caspr2 is a recently described protein that is highly homologous to Caspr and is localized to the juxtaparanodal axonal membrane (Poliak et al. 1999). Using empirically optimized conditions (1 h Zamboni's fixative), we found that essentially all juxtaparanodes could be stained (Figs. 8, 9), in contrast to the previous report that only some juxtaparanodes express Caspr2 (Poliak et al. 1999). In the ventral root entry zone, there was juxtamesaxonal and juxtaincisural staining the PNS, but not in the CNS (Fig. 8), a pattern that we had observed for Kv1.1 and Kv1.2 (see above). Finally, we noted a unique distribution of Caspr, and of Caspr2, Kv1.1, and Kv1.2, under the last CNS myelin sheath in the root entry zone: there was diffuse Caspr staining in the juxtaparanodal region, and this overlapped, at least in part, with an enlarged juxtaparanodal staining of Caspr2, Kv1.1, and Kv1.2 (Fig. 9).

Discussion

In both the PNS and the CNS, the organization of the axonal membrane of myelinated axons is intricately related to their myelin sheaths. Voltage-dependent Na^+ channels and $ankyrin_G$ are localized to nodes, Caspr to paranodes, and Kv1.1, Kv1.2, Kvβ2, and Caspr2 to juxtaparanodes (Arroyo and Scherer 2000). In this paper, we have examined the internodal localization of these proteins in myelinated CNS axons. In contrast to the their organization in PNS myelinated axons, we have found that Kv1.1, Kv1.2, Kvβ2, and Caspr2 are not localized in a juxtamesaxonal manner, as schematized in Fig. 10. Caspr, on the other hand, does appear to be localized in relation to the inner mesaxon, as it is in the PNS. These data document for the first time that the internodal organization of membrane-associated axonal proteins is organized in a different manner in the CNS from the PNS (Arroyo and Scherer 2000; Peles and Salzer 2000).

Abundant myelinated fibers in gray matter

We were surprised by the relatively high expression of Na^+ channels, $ankyrin_G$, Caspr, Kv1.1, Kv1.2, Kvβ2, and Caspr2 in the gray matter. Our analysis indicated that the punctate immunostaining in the gray matter could be attributed to the numerous thinly myelinated fibers. We confirmed this to be the case, as immunostaining for MAG and claudin-11/OSP also revealed robust staining in the gray matter, as previously reported for MOG (Coffey and McDermott 1997). Nevertheless, Kv1.1 and Kv1.2 are localized in synapses (Rhodes et al. 1996, 1997; Wang et al. 1994), suggesting that some of the "diffuse" immunoreactivity is probably localized in non-axonal structures.

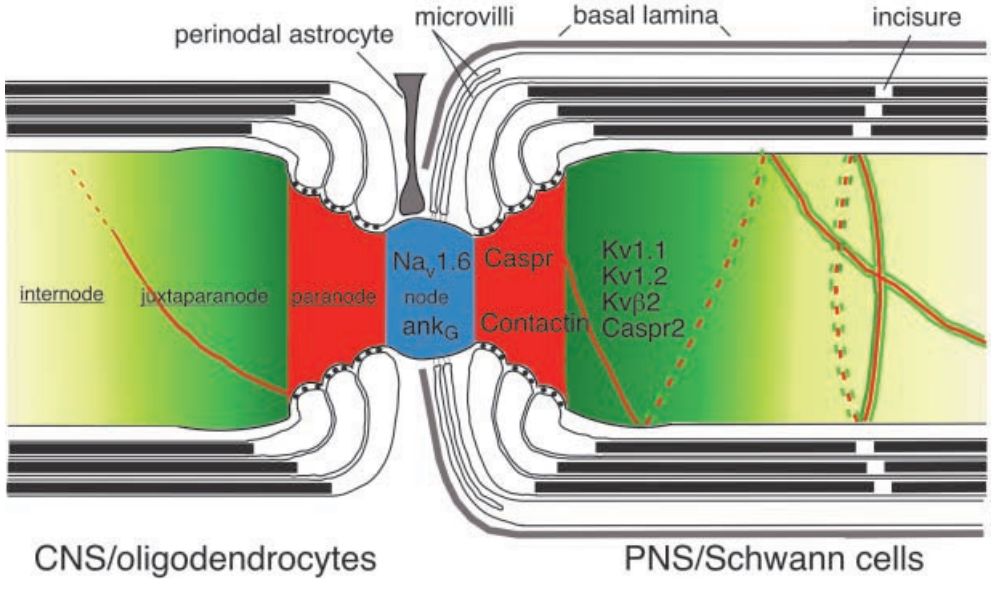

Fig. 10 CNS and PNS internodes. A schematic drawing that illustrates the differences in the organization of the internodal axonal membrane in the CNS and PNS. $Ankyrin_G$ and $Na_v1.6$ are localized to nodes; Caspr and contactin to paranodes; Kv1.1, Kv1.2, Kvβ2, and Caspr2 to juxtaparanodes. In the PNS, a single strand of Caspr and contactin extends from the paranode into the juxtaparanodal and internodal membrane and is flanked by a double line of juxtaparanodal proteins (Kv1.1, Kv1.2, Kvβ2, and Caspr2). In the CNS, in contrast, a single line of Caspr and contactin extends from the paranodal region but is not flanked by these juxtaparanodal proteins in the internodal region

The localization of axonal proteins in relation to CNS myelin sheaths

As in the gray matter, the bulk of Caspr, Kv1.1, Kv1.2, Kvβ2, and Caspr2 staining in the white matter was associated with myelinated axons. As previously described, Caspr was mainly localized in the paranode, and Kv1.1, Kv1.2, Kvβ2, and Caspr2 were mainly found in the juxtaparanodal region (Mi et al. 1995; Poliak et al. 1999; Rasband et al. 1998, 1999; Rhodes et al. 1997; Vabnick and Shrager 1998; Wang et al. 1993; Zhou et al. 1998). The co-localization of these juxtaparanodal proteins is mediated by protein-protein interactions, as Kv1.1 form tetramers with Kv1.2 and these also contain Kvβ2 (Gulbis et al. 1999; Hopkins et al. 1994), and Caspr2 co-immunoprecipitates with Kv1.2 (Poliak et al. 1999). A protein with multiple PDZ domains has been postulated to bind to the PDZ-binding domains of Caspr2, Kv1.1, and Kv1.2 (Peles and Salzer 2000).

The internodal localization of Caspr in the CNS appeared to be related to the inner mesaxon, as previously suggested by Menegoz et al. (1997), and previously shown in the PNS (Arroyo et al. 1999). However, we did not observe circumferential rings of internodal Caspr staining in the CNS, unlike the PNS in which these rings appose the innermost aspect of incisures (Arroyo et al. 1999). The reason for this difference may be related to the rarity of incisures in the CNS (Blakemore 1969; Hildebrand 1971), or indeed, a fundamental difference in their molecular organization. Incisures in the PNS contain gap junctions, tight junctions, and adherens junctions (and the molecular components thereof); whether CNS incisures contain these components remains to be determined.

We have not found internodal strands of Kv1.1, Kv1.2, Kvβ2, or Caspr2 in the CNS. In the PNS, these molecules are co-localized in a pair of strands that flank a single strand of Caspr; this tripartite strand apposes the inner mesaxon and innermost aspect of incisures (Arroyo and Scherer 2000; Arroyo et al. 1999). This difference between the internodal organization of these proteins is related to the cellular origin of the myelin sheath and is not an intrinsic property of the axon itself, as best substantiated by our observation of myelinated fibers in the ventral root entry zone.

Do myelin sheaths organize axonal proteins?

The intricate organization of Na^+ channels, ankyrin$_G$, Caspr, Kv1.1, Kv1.2, Kvβ2, and Caspr2 in relation to the myelin sheath is strong evidence that there are *trans* interactions between myelinating glial cells and their axons (Arroyo et al. 1999). Whereas it seems more plausible that myelin sheaths organize the axonal membrane than vice versa, nodal proteins can become localized in regularly spaced, focal aggregates, even in the absence of glial cells in retinal ganglion cells (Kaplan et al. 1997), but not in sensory neurons (Ching et al. 1999). Several other observations favor a central role of the myelinating cells in organizing the axonal membrane. When axons are acutely demyelinated in the PNS, these proteins become progressively less-well localized over a few days but relocalize to their appropriate positions if remyelination occurs (Duyandzija-Novakovic et al. 1995; Novakovic et al. 1996, 1998). Similarly, in mice and rats with a variety of genetic perturbations that result in deficient myelination of either the CNS or the PNS, Na^+ channels, ankyrin$_G$, Caspr, Kv1.1, Kv1.2, and Kvβ2 are highly localized if, and only if, myelin sheaths are formed (Noebels et al. 1991; Rasband et al. 1999; Wang 1995). These results indicate that axonal membranes are locally organized by single myelinating glial cells.

The way in which glial cells arrange these axonal proteins is unknown. At PNS nodes, cell adhesion molecules in Schwann cell microvilli have been postulated to organize the nodal axolemma (Bennett and Lambert 1999); the so-called “perinodal astrocytes” may function similarly in the CNS (Black and Waxman 1988; Butt et al. 1999). The co-localization of contactin, Caspr, and neurofascin 155 kDa at the paranodes of CNS and PNS myelinated fibers indicates that these proteins help to form the transverse bands of axo-glial junctions (Arroyo and Scherer 2000; Peles and Salzer 2000). The juxtaparanodal and internodal axonal and glial membranes are also specialized (Arroyo and Scherer 2000), but no ultrastructural connections join these membranes. Nevertheless, the colocalization of the Schwann cell inner mesaxon and the axonal proteins Caspr, Caspr2, Kv1.1, Kv1.2, and Kvβ2 provides reasonable evidence to assert that the inner/adaxonal Schwann cell membrane has trans-interactions with the apposed axonal membrane (Arroyo et al. 1999). The absence of internodal strands of Kv1.1 and Kv1.2 staining in myelinated CNS axons, in contrast, indicates that oligodendrocye myelin sheaths do not have the same trans-interactions.

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