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ORIGINAL PAPER

Neuronal expression of peripherin, a type III intermediate filament protein, in the mouse hindbrain

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Abstract Peripherin is a 57 kDa Type III intermediate filament protein associated with neurite extension, neuropathies such as amyotrophic lateral sclerosis, and cranial nerve and dorsal root projections. However, knowledge of peripherin expression in the CNS is limited. We have used immunoperoxidase histochemistry to characterise peripherin expression in the mouse hindbrain, including the inferior colliculus, pons, medulla and cerebellum. Peripherin immunolabelling was observed in the nerve fibres and nuclei that are associated with all cranial nerves [(CN) V–XII] in the hindbrain. Peripherin expression was prominent in the cell bodies and axons of the mesenchephalic trigeminal nucleus and the pars compacta region of nucleus ambiguus, and in the fibres that comprise the solitary tract, the descending spinal trigeminal tract and the trigeminal and facial nerves. A small proportion of peripherin positive fibres in CN VIII likely arise from cochlear type II spiral ganglion neurons. Peripherin positive fibres were also observed in the inferior cerebellar peduncle and folia in the intermediate zone of the cerebellum. Antibody specificity was confirmed by absence of labelling in hindbrain tissue from peripherin knockout mice. This study shows that in the adult mouse hindbrain, peripherin is expressed in discrete neuronal subpopulations that have sensory, motor and autonomic functions.

Keywords Sensory · Motor · Unmyelinated · Neurofilament · Trigeminal · Nucleus ambiguus · Spiral ganglion

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Introduction

The neuronal cytoskeleton contributes to the establishment of cell shape, axonal outgrowth, intracellular transport and synaptic plasticity. Intermediate filaments (IF) make up key components of the cytoskeleton, along with actin microfilaments and microtubules. Whereas the microtubules and actin microfilaments have established roles, the functions of IF are less well understood. IF proteins constitute a large gene family, but only five have been detected in mature neurons: the three neurofilament proteins (neurofilament light [NF-L], medium [NF-M] and heavy [NF-H]); α-internexin, which are type IV IF proteins; and peripherin, a type III IF protein (Lariviere and Julien 2004). The NF proteins are widely expressed throughout the nervous system, whereas α-internexin exhibits expression within defined central neuronal populations (Yuan et al. 2006). By contrast, the distribution of peripherin expression in the CNS has received little attention, although its representation

in peripheral sensory-motor neuron subpopulations is significant (Escurat et al. 1990; Gorham et al. 1990; Troy et al. 1990a; Terao et al. 2000).

Peripherin expression was first detected in mouse neuroblastoma and rat pheochromocytoma (PC12) cells (Portier et al. 1984b), where it was shown to be dependent on nerve growth factor induced differentiation of the cells. Subsequent studies have shown that peripherin is commonly found in peripheral neurons (Portier et al. 1984a; Leonard et al. 1988; Parysek et al. 1988) or at least in neurons that have some part of their axon outside the CNS (Escurat et al. 1990; Troy et al. 1990a). However, there is also growing evidence for peripherin expression in neurons that lie entirely within the CNS, including the neocortex (Rhrich-Haddout et al. 1997) and the cerebellum (Errante et al. 1998). Conclusive evidence for peripherin function in neurons has proved elusive. Early studies that looked at expression of peripherin during the development of the nervous system suggested a role for this IF in axonal outgrowth (Escurat et al. 1990; Troy et al. 1990a). Spinal cord cultures from *Xenopus* embryos showed peripherin was localised in the distal region of the extending axon, suggesting that it might be involved in growth cone organisation and thus contribute to axonal guidance (Gervasi et al. 2000; Undamatla and Szaro 2001). Peripherin also likely plays a role in neuronal regeneration following nerve injury, where an up-regulation of peripherin mRNA, protein and transport has been observed in motor neurons following axotomy of the sciatic nerve (Oblinger et al. 1989; Troy et al. 1990b; Chadan et al. 1994). Injury-induced expression of peripherin has also been observed in cerebral neurons which do not usually express the protein (Beaulieu et al. 2002). Whereas these observations suggest that peripherin may be important for neurite elongation and repair, a recently developed peripherin knockout mouse showed that peripherin is not obligatory for axogenesis (Lariviere et al. 2002). These mice had no obvious developmental abnormalities and large diameter axons developed normally, although the peripherin deficiency did result in a reduced number of small diameter, unmyelinated, sensory axons in the spinal cord. Conversely, over-expression of the peripherin protein may lead to neurotoxicity. Transgenic mice with multiple integrations of the peripherin gene with its promoter, exhibited late onset motor neuron disease which showed a similar pathology to that commonly observed in degenerating motor neurons of amyotrophic lateral sclerosis (ALS) patients (Beaulieu et al. 1999, 2000). These transgenic studies show that peripherin deficiency or over-expression had unexpected consequences on neuronal development and survival in vivo. Thus use of such mouse models holds considerable promise for resolving the function of the type III IF protein, peripherin, in neuronal physiology and pathology.

Previous studies have characterised CNS peripherin expression in rat (Brody et al. 1989; Escurat et al. 1990). However, data on peripherin expression in the mature mouse CNS, the preferred model for genetic manipulation of peripherin expression, is limited. The present study was designed to address this issue by establishing the expression profile of peripherin in the mouse hindbrain.

Materials and methods

Animals

Eleven C57/BL6 mice aged between postnatal days 21–28 (P21–P28) and six 129/BL6 mice, aged at 13 and 28 weeks were used for peripherin immunolabelling studies. Three of the 129/BL6 mice were peripherin-null mutants (Lariviere et al. 2002) used to test for antibody specificity. The mice were euthanized by intraperitoneal injection of sodium pentobarbitol (90 mg/kg, Vibrac Laboratories, New Zealand). All procedures in the study were approved by the University of Auckland Animal Ethics Committee.

Immunohistochemistry

Brain tissue was fixed by transcardial perfusion with normal saline containing heparin (0.9% NaCl; 1 mg/ml $NaNO_2$ and 0.02% heparin), followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The dissected brain tissue was then post-fixed overnight at room temperature. Consequently, the brain tissue, from the inferior colliculus to the spinal cord, was rinsed in phosphate buffered saline (0.1 M, pH 7.4; PBS) and placed in 10% sucrose in PBS for 8 h, then 30% sucrose solution for 2–3 days for cryoprotection. Tissue was mounted with Tissue-Tek optimal cutting temperature compound (O.C.T.; Miles, Diagnostics Division, Elkhart, IN, USA), snap frozen and sectioned at 50 μm (Cryocut 1800, Reichert-Jung, Germany). Sections were incubated for 30 min in a 50% methanol, 1% hydrogen peroxide (H_2O_2) solution to quench endogenous peroxidase activity. Subsequently the sections were incubated in a blocking/permeabilising solution (10% normal goat serum [NGS, Vector Laboratories, Burlingane, CA, USA] and 1% Triton X-100 in PBS) for 2 h at room temperature. Tissue was then incubated in the primary antibody solution [peripherin polyclonal rabbit antiserum (PII/SE411) at 1:1,500 dilution in 0.1 M PBS with 5% NGS and 0.1% Triton X-100] overnight at 4°C. The peripherin antibody was a gift from Dr. Annie Wolff, Division de Biochimie, Universite Pierre et Marie Curie, Paris, France. This polyclonal antibody was raised in rabbit and specifically targets a peptide corresponding to residues 432–461 of rat peripherin, which has the following amino acid sequence:

IETRDGEKVVTESQKEQHSELDKSSIHSY. This antibody has previously been fully characterised (Djabali et al. 1991). The following day, sections were washed in PBS, then incubated for 40 min in biotinylated secondary antibody solution [biotinylated goat anti-rabbit IgG (Vector Laboratories) at 1:500 dilution in 0.1 M PBS containing 5% normal goat serum and 0.1% Triton X-100] and then in avidin–biotin–HRP complex (Vector Laboratories) for a further 40 min. Peripherin immunoreactivity was visualised by incubation in 3,3′-diaminobenzidine (DAB) with nickel chloride (Vector Laboratories) for 5 min and sections were subsequently washed and then mounted on glass slides using Citifluor (Agar Scientific, UK).

Brightfield images were obtained using a Zeiss Axioskop microscope (Axioskop2 mot plus; Zeiss, Germany) with Nomarski differential interference contrast optics, and a digital camera (AxioCam HRc, Zeiss).

The location of sections was estimated using a mouse brain stereotaxic atlas (Franklin and Paxinos 1997) and are identified relative to 'Bregma' (i.e. the anatomical landmark denoting the intersection of the sagittal and coronal sutures of the skull). The Bregma positions are shown in all figures at the bottom of the panel a except Fig. 3.

Results

The majority of the data we present here is from the C57/BL6 mouse tissue and shows pronounced peripherin immunolabelling within specific regions of the hindbrain. Fibre tracts containing sensory and motor fibres of peripheral origin were generally labelled, such as the fibre tracts of V, VII, VIII, IX, X and XII (e.g. see Figs. 1a, d, 2b, 5b, 6b). Sections from the 129/BL6 peripherin-null mice confirm the specificity of the SE411 antibody and show very low background staining (see Fig. 1c). Viability of this tissue for immunohistochemistry was confirmed by immunostaining for $P2X_2$ protein (data not shown) and the staining we observed was comparable with that reported for the rat hindbrain (Kanjhan et al. 1999). The wild-type 129/BL6 adult mice tissue showed identical distribution of peripherin immunolabelling (data not shown) to that described for C57/BL6 mice, used here for illustration of our findings.

Trigeminal nerve and associated structures

Peripherin immunolabelling was observed in a number of nuclei and fibre tracts related to the trigeminal nerve. These were, from rostral-caudal, the principal sensory and motor trigeminal nuclei (Pr 5VL in Fig. 1a, d; Pr 5DM in Fig. 2b; and Mo5 in Fig. 1a, d) the mesencephalic trigeminal nucleus (Me5 in Figs. 1a, 1b, 2b, 2c) and the spinal trigeminal nuclei (Sp5OVL in Fig 4b; DMSp5 in Fig 4b, 5b; Sp5I in Fig 5b, 6b). Peripherin immunolabelling in the ventrolateral principal sensory trigeminal nucleus was confined to nerve fibres and no labelling was observed in the neuronal somata (Pr 5VL in Fig. 1d). Similar labelling was observed in the other parts of this nucleus and in the spinal trigeminal nuclei. This labelling pattern indicates that only the afferent axons expressed peripherin, whereas the second order neurons within these nuclei did not. The strong peripherin immunostaining in the trigeminal (CN V) sensory nerve root (s5 in Fig. 1) and the

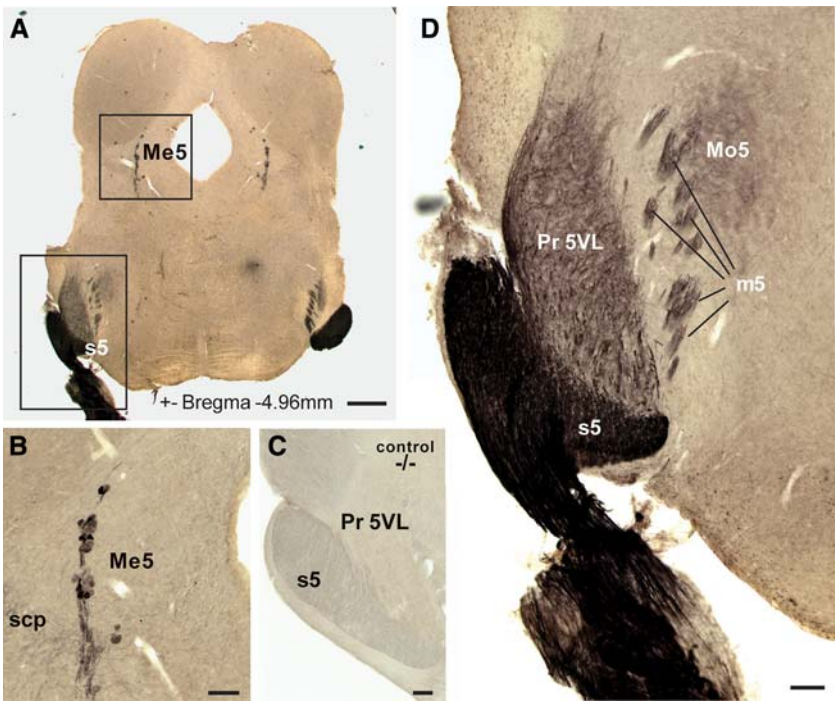

Fig. 1 Peripherin immunostaining in a transverse section of the pons. **a** Transverse section shows strong peripherin immunoreactivity in the mesencephalic trigeminal nucleus (*Me5*, detailed in **b**) and the trigeminal sensory nerve root (*s5*, detailed in **d**). **b** Cell bodies and nerve fibres in Me5 showed peripherin immunostaining. **c** Tissue from peripherin null mice shows only low level background staining in an equivalent section that shows s5 and the principal sensory nucleus (*Pr 5VL*) of the trigeminal nerve. **d** Immunoreactivity is observed in the region of the motor nucleus (*Mo5*), Pr 5VL and in the axons that comprise the sensory and motor nerve roots of the trigeminal nerve (*s5* and *m5*, respectively). *Scale bars* **a** = 1 mm, **b**–**d** = 100 μm

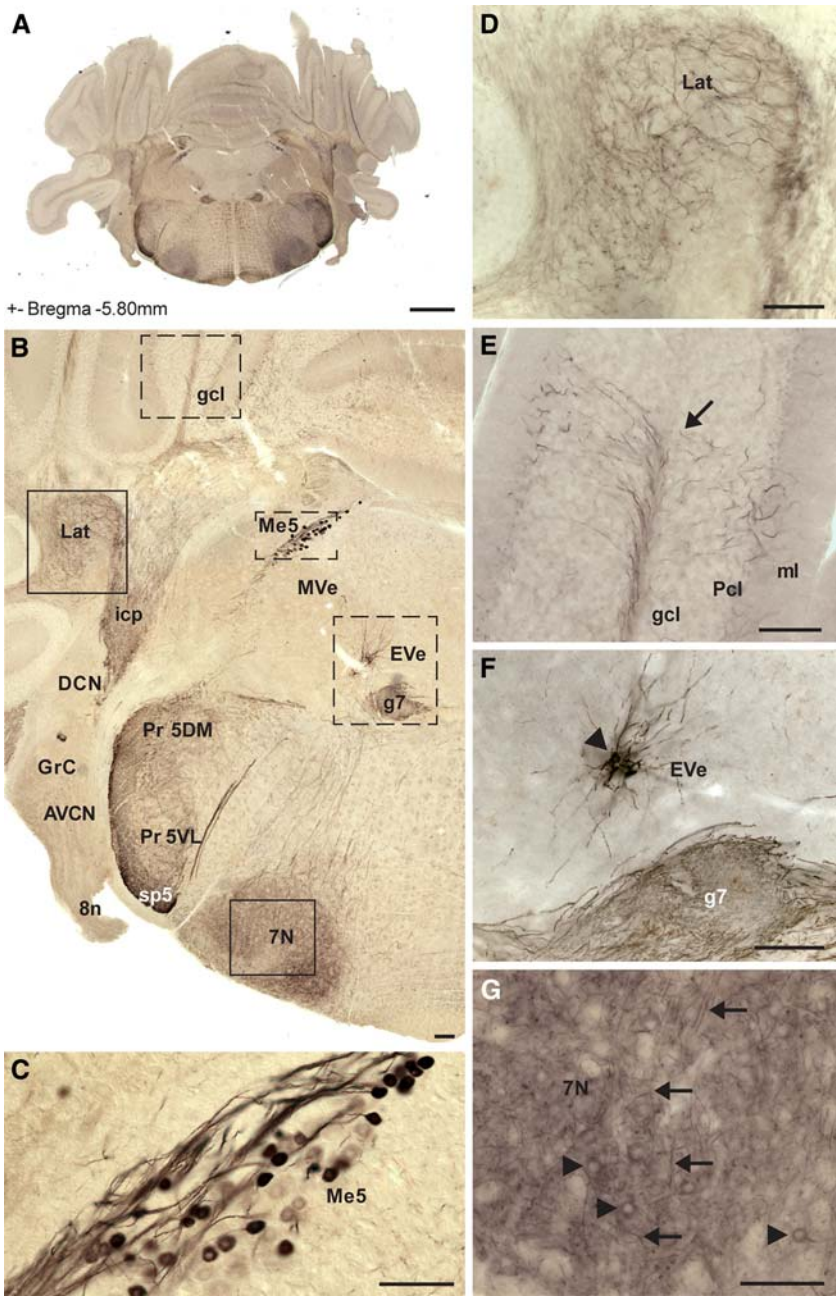

Fig. 2 Peripherin expression in a transverse section of the pons and cerebellum. **a** Low power photomicrograph of the mouse pons and cerebellum shows localisation of peripherin immunoreactivity that is presented at higher magnification in the following panels. **b** Dense peripherin immunoreactivity is observed in the mesencephalic trigeminal nucleus (*Me5*, detailed in **c**), dorsomedial (*Pr5DM*) and ventrolateral (*Pr5VL*) principle sensory trigeminal nuclei, and the descending spinal trigeminal tract (*sp5*). Peripherin immunolabelled fibres are found in the inferior cerebellar peduncle (*icp*), the lateral (dentate) cerebellar nucleus (*Lat*, detailed in **d**) and innervating folia in the intermediate zone of the cerebellum, detail of which is shown in **e**. Peripherin is also expressed in the nucleus of origin of vestibular efferent fibres (*EVe*, detailed in **f**), the genus of the facial nerve (*g7*, detailed in **f**) and the facial nucleus (*7N*, detailed in **g**). Note there is no peripherin immunolabelling in the medial vestibular nucleus (*MeV*). *Dashed boxes* indicate that the panels which show detail of these regions come from a different section. 8n, vestibulocochlear nerve root; *DCN* dorsal cochlear nucleus; *GrC* granule cell lamina. **c** The *Me5* shows peripherin immunolablleing within the cell bodies and axons arising from these neurons. **d** Nerve fibres, but not cell bodies within the lateral deep cerebellar nucleus show peripherin immunostaining. **e** Transverse section through a folium lying in the medial region of the cerebellar hemisphere shows that peripherin expressing nerve fibres (*arrow*) project through the granule cell layer (*gcl*) and Purkinje cell layer (*Pcl*), into the deeper regions of the molecular layer (*ml*). **f** High levels of peripherin expression are observed in neuronal cell bodies (*arrowhead*) that give rise to the efferent vestibular fibres (*EVe*) and in the axons that form the genu of the facial nerve (*g7*). **g** Cell bodies within the facial nucleus (*arrowheads*) as well as their axonal projections (*arrows*), show peripherin immunoreactivity. *Scale bars* **a** = 1 mm, **b**–**f** = 100 μm

descending spinal trigeminal tract (sp5 in Figs. 2b, 4b, 5b, 6b) confirms that the first order afferent nerve fibres express peripherin within the sensory nuclei. The mesencephalic nucleus contains the cell bodies of the sensory neurons that are involved in proprioception and innervate the muscles of mastication and the periodontal ligaments. These afferent neurons showed peripherin expression in both their axons and their cell bodies (Me5 in Figs. 1b, 2c). Most of the motor neurons were immunolabelled in the trigeminal motor nucleus (Mo5 in Fig. 1d). This nucleus showed diffuse peripherin immunostaining, while the axons that arise from this nucleus and form the motor root of CN V (m5 in Fig. 1d) showed relatively intense staining.

Facial nerve and nucleus

Peripherin immunolabelling was observed in the cell bodies and axons that make up the facial nerve. We show immunostaining in the cell bodies that lie in the nucleus of CN VII (7N in Figs. 2b, 2g, 4b). These neural somata give rise to axons which exhibit relatively stronger peripherin immunostaining and extend in a dorsal direction toward the abducens nucleus, where they form a loop [genu of the facial nerve (g7 in Fig. 2b, 2f)] and finally coalesce to form the facial nerve.

Cerebellum

Peripherin expression was observed in fibres that constitute the inferior cerebellar peduncle (icp in Figs. 2b, 4b) and innervate folia within the cerebellum (Fig. 2b top dashed box labelled 'gcl' which is displayed in Fig. 2e). These fibres were not observed across all folia and were generally only seen in the intermediate zone of the cerebellum (as seen in Fig. 2b), which comprises the ventrolateral regions of the vermis and the folia of the medial region of the cerebellar hemispheres. Fibres that comprise the inferior cerebellar peduncle arise from cell somata located in Clarke's column in the spinal cord, the external cuneate nucleus and the inferior olive in the caudal region of the medulla, and in the vestibular ganglion and the vestibular nuclei in the pons. However, we did not observe peripherin immunolabelling in the vestibular nuclei (e.g. MVe in Figs. 2b, 4b and SpVe in Figs. 4b, 5b), the inferior olive (ventromedial region of the medulla in Fig. 6b marked with*), or the external cuneate nucleus (ECu in Fig. 6b).

Fibre labelling was also evident in the lateral (dentate) deep cerebellar nucleus (Lat labelled box in Fig. 2b, which is displayed in Fig. 2d). These fibres likely represent axons which arise from the neurons within this nucleus and project to the thalamus via the superior cerebellar peduncle. Limited peripherin immunoreactive fibres were observed in the superior cerebellar peduncle (scp in Fig. 1b).

Vestibulocochlear nerve and associated nuclei

Dense peripherin immunoreactivity was observed in the cell bodies and dendrites that lie in the nucleus of origin of the vestibular efferent fibres (EVe in Fig. 2b, f). The axonal projections of these efferent neurons were not labelled in our sections, however peripherin immunolabelling in these neurons and their axonal projections was observed in a study in guinea pig (Leonard and Kevetter 2006). We did not observe peripherin immunoreactivity in any of the vestibular nuclei (MVe in Figs. 2b, 4b, 5b and SpVe in Figs. 4b, 5b).

Peripherin immunolabelled nerve fibres associated with the cochlear portion of CN VIII innervated a number of regions in the cochlear nucleus. These were: the anteroventral cochlear nucleus (AVCN arrows in Fig. 3b, e, f), the dorsal cochlear nucleus (DCN, white arrow in Fig. 3d), the superficial granule cell layer (SGL in Figs. 3b, c) and the granule cell lamina (GrC, black arrow in Fig. 3d) that surround and separate the DCN and AVCN respectively (Mugnaini et al. 1980). These peripherin-positive fibres were sparsely distributed and only a small number were observed in each transverse section. The peripherin expressing axons showed numerous swellings within the DCN and the AVCN (Figs. 3d, e, f). No peripherin immunolabelled fibres were observed in the posteroventral cochlear nucleus (PVCN in Fig. 4b). These peripherin-positive fibres appear to be the axonal projections of the primary auditory neurons originating in the cochlea, since they are observed innervating the AVCN near the nerve root of CN VIII (Fig. 3e, inset arrows). Specifically, these fibres most likely arise from the unmyelinated cochlear type II spiral ganglion neurons (SGNII) (Berglund and Ryugo 1987; Ryugo et al. 1991), which express peripherin (Hafidi 1998), innervate the AVCN and the DCN, but not the PVCN (Brown et al. 1988) and make up less than 10% of the primary auditory neuronal population. Peripherin expressing fibres observed innervating the superficial granule cell layer and the granule cell lamina are also most likely SGNII axons, which are well known to have terminations within these regions and where postsynaptic targets of SGNII have been determined (Brown and Ledwith 1990; Berglund and Brown 1994; Hurd et al. 1999; Benson and Brown 2004).

Glossopharyngeal, vagal and hypoglossal nerve and associated nuclei

Intense peripherin immunolabelling was found in the nerve fibres that comprise CN IX, X and XII and in structures associated with these fibre projections (denoted by 9n for CN IX in Fig. 5b; and 10n for CN X and 12n for CN XII in Fig. 6b). The nerve fibres that comprise the solitary tract (sol) were peripherin positive and the nucleus of the

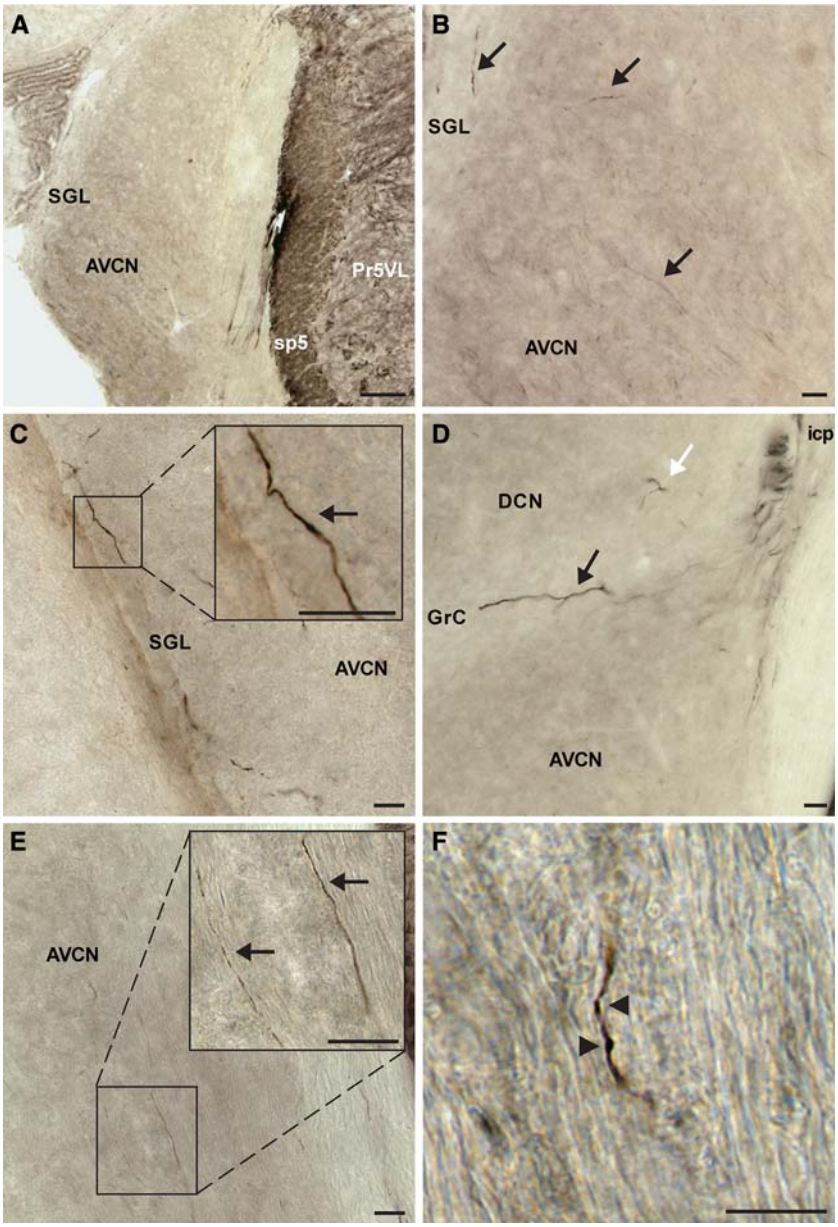

Fig. 3 Photomicrographs of transverse sections of the cochlear nucleus show peripherin immunoreactive nerve fibres innervating various regions within this nucleus. **a** Photomicrograph of the lateral region of the pons shows the superficial granule cell layer (*SGL*) and anteroventral cochlear nucleus (*AVCN*) that comprise the cochlear nucleus, as well as the sp5 and the Pr 5VL. Peripherin immunolabelled fibres that innervate this region are detailed in the following panels. **b**, **c** A small number of peripherin immunoreactive nerve fibres (*arrows*) innervate the AVCN and the SGL. *Inset* in **c** shows a fibre at high power and demonstrates the strong peripherin expression. **d** Peripherin immunoreactive fibres observed in the dorsal cochlear nucleus (*DCN*) (*white arrow*) and the granule cell lamina (*GrC*) (*black arrow*). **e** Ventral region of the AVCN, close to the nerve root of CN VI-II, shows peripherin labelled nerve fibres in the sagittal plane. *Inset* displays these fibres (*arrows*) at higher power and shows that they are amongst the bundles of nerve fibres that enter the AVCN. **f** Neurites displaying peripherin immunoreactivity frequently exhibited swellings (*black arrowheads*). *Scale bars* **a** = 100 μm, **b**–**e** = 20 μm, **f** = 10 μm

solitary tract (Sol) displayed dense innervation by these fibres (sol and Sol in Figs. 4b, 5b, 6b, c). The sensory fibres that innervate the rostral regions of this nucleus are carried by CN VII and IX and likely represent the taste afferents. Innervation of this nucleus by peripherin expressing CN IX nerve fibres (9n) is shown in Fig. 5b. The caudal region of the nucleus of the solitary tract is more densely innervated by peripherin immunolabelled nerve fibres (Sol in Fig. 6b, c). Afferent input into this region of the nucleus is by the viscerosensory neurons, whose axons are contained within CN IX and X. There was strong peripherin expression in the nucleus ambiguus pars compacta rostral to obex (Amb in Fig. 5b, which is detailed in Fig. 5c). Cell somata and the axons of these motor neurons show intense peripherin expression (Fig. 5c). Figure 5b indicates these axons constitute part of CN IX (9n labels). There is no expression in the caudal region of this nucleus (Fig. 6a, b), where motor neurons provide output via CN X and XI. The dorsal motor nucleus of the vagus (10N in Fig. 6c) and the hypoglossal nucleus (12N in Fig. 6d) show peripherin expression in cell somata and in the nerve fibres that give rise to preganglionic parasympathetic fibres of CN X (10n) and the efferent fibres of XII (12n), which innervate the tongue (Fig. 6b).

Discussion

This is the first study to provide a detailed description of peripherin expression in the mature mouse hindbrain. We have shown that peripherin is expressed in a broad range of

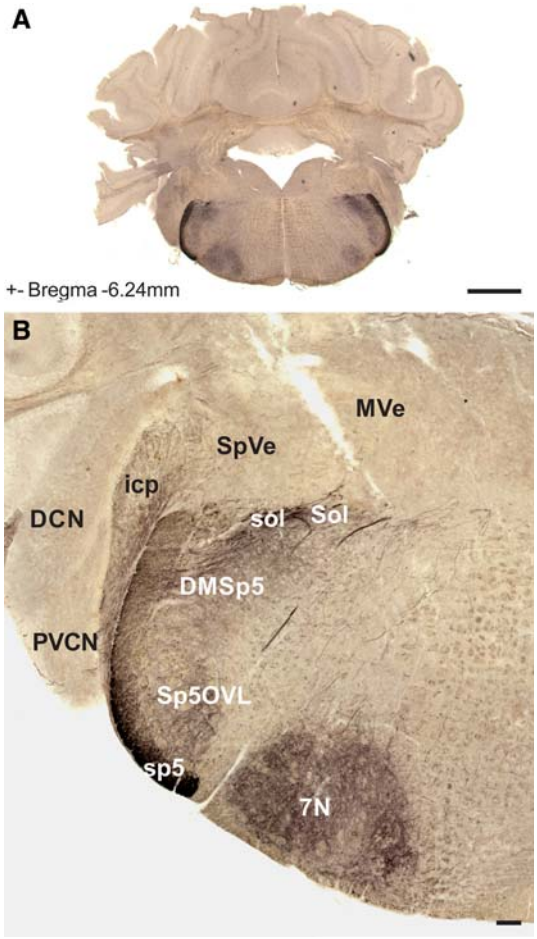

Fig. 4 Distribution of peripherin expression in transverse sections of mouse medulla and cerebellum. **a** Low power photomicrograph shows localisation of peripherin immunostaining that is presented from a different section at a higher magnification in the following panel. **b** Peripherin immunoreactivity is seen in the nerve fibres within the dorsomedial (*DMSp5*) and ventrolateral (*Sp5OVL*) spinal trigeminal nuclei, and in the descending sp5. The rostral region of the solitary tract (*sol*) and its associated nucleus (*Sol*) contains peripherin positive nerve fibres, as does the icp. The cell bodies and axons of the motor neurons within the facial nucleus (*7N*) also show peripherin expression. No peripherin immunolabelled fibres are observed in the posteroventral cochlear nucleus (*PVCN*), the dorsal cochlear nucleus (*DCN*), or in the medial and spinal vestibular nucleus (*MVe* and *SpVe*) at this level of the medulla. *Scale bars* **a** = 1 mm, **b** = 100 μm

neuronal sub-populations within the pons, medulla and cerebellum. The peripherin antibody (SE411/PII) used here was thoroughly validated in a previous study where the interaction between nuclear lamin B and peripherin was examined in rat and mouse neural tissue (Djabali et al. 1991), as well as subsequent peripherin immunolabelling studies in rat (Rhrich-Haddout et al. 1997; Terao et al. 2000). In this investigation of the mouse hindbrain, immunolabelling was confined to discrete neuronal populations and the lack of staining in sections from peripherin knockout mice indicated that the primary antibody was selectively labelling the type III intermediate filament protein peripherin.

The majority of the peripherin expression we observed was in the cranial nerves and their associated nuclei. High levels of immunoreactivity occurred in the nerve fibres that comprise the sensory and motor portion of CN V, IX and X as well in motor nerve fibres that form CN VII and XII, and the sensory fibres that form part of CN VIII. In the neuronal sub-populations where the nuclei originate in the pons and medulla, we observed that peripherin was mainly localised within the axons of the neurons and generally lower levels of immunoreactivity were observed in the neuronal cell bodies. This observation was evident in the motor nuclei of CN V, VII, X and XII. The peripherin expression we observed in these cranial nerves and nuclei agrees with the schematic description of immunolabelling in the adult rat CNS, which used an antibody that was raised against a 57 kDa neural intermediate filament protein, later determined to be peripherin (Brody et al. 1989). Ontogenic studies in rat (Escurat et al. 1990) and mouse (Troy et al. 1990a) used immunohistochemistry to examine the changes in peripherin expression during the development of the embryo. In addition to identifying peripherin expression in the dorsal root ganglion (DRG), ventral spinal motor roots and autonomic ganglia, expression was confirmed in central cranial nerve projections and the olfactory bulb (Escurat et al. 1990).

In the present study, peripherin immunolabelling was observed in the nerve fibres in CN VIII that innervate the cochlear nucleus. The labelling of fibres in the AVCN, DCN, superficial granule cell layer and granule cell lamina (as noted above) likely arise from the cochlear SGNII (Berglund and Ryugo 1987; Brown et al. 1988; Brown and Ledwith 1990; Ryugo et al. 1991; Berglund and Brown 1994; Hurd et al. 1999; Benson and Brown 2004). SGNII, with cell bodies located within Rosenthal's canal in the cochlea, are known to express peripherin in both rat (Hafidi 1998) and mouse (Schimmang et al. 2003). Species differences exist, as peripherin-positive cell bodies observed in the rat cochlear nuclei (Hafidi 1998), were not evident in the mouse tissue in our study. Innervation from non-auditory structures has been reported (Itoh et al. 1987; Weinberg and Rustioni 1987; Shore et al. 1991; Wright and Ryugo 1996; Shore et al. 2000; Haenggeli et al. 2005). However, it is unknown whether or not such fibres express peripherin. We cannot discount the possibility that a portion of the peripherin immunolabelled fibres we observe in this region are from this source.

In the cerebellum, we show intense peripherin immunolabelling in the inferior cerebellar peduncle and in fibres that innervate the proximal two thirds of the molecular layer that lies in the folia in the intermediate zone of the cerebellum. A previous immunohistochemical study (Troy

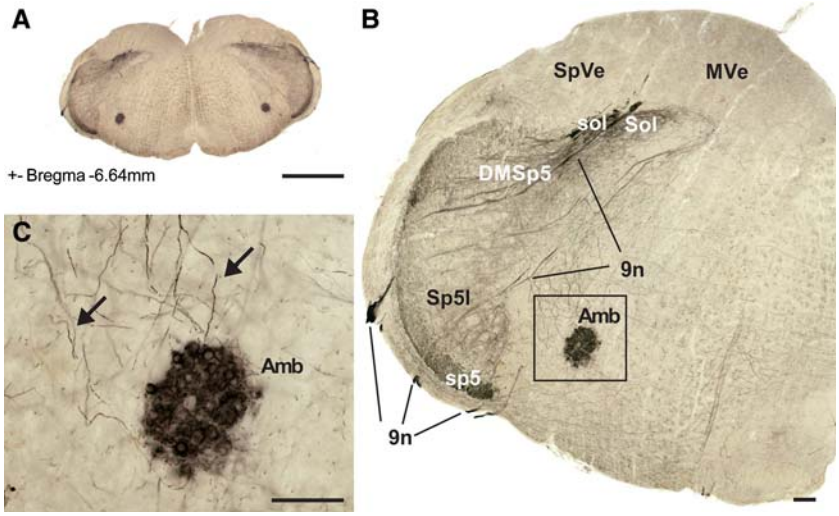

Fig. 5 Peripherin immunoreactivity in a transverse section of the medulla. **a** Low magnification transverse section showing peripherin immunolabelling in the trigeminal structures and in the pars compacta region of the nucleus ambiguus, presented in detail in the following panels. **b** Peripherin immunostaining is observed in nerve fibres in the dorsomedial (*DMSp5*) and interpolar (*Sp5I*) spinal trigeminal nucleus, and the spinal trigeminal tract (*sp5*). Immunoreactivity is also found in the glossopharyngeal nerve fibres (*9n*) which project to the sol and the nucleus of the Sol. Note the intense immunolabelling of neurons nucleus ambiguus pars compacta region (*Amb*, detailed in **c**). No peripherin immunolabelling is observed in the medial or spinal vestibular nucleus (*MVe* and *SpVe*). **c** Nucleus ambiguus neurons show strong peripherin expression in both their cell bodies and neurite projections (*arrows*). *Scale bars* **a** = 1 mm, **b**, **c** = 100 μm

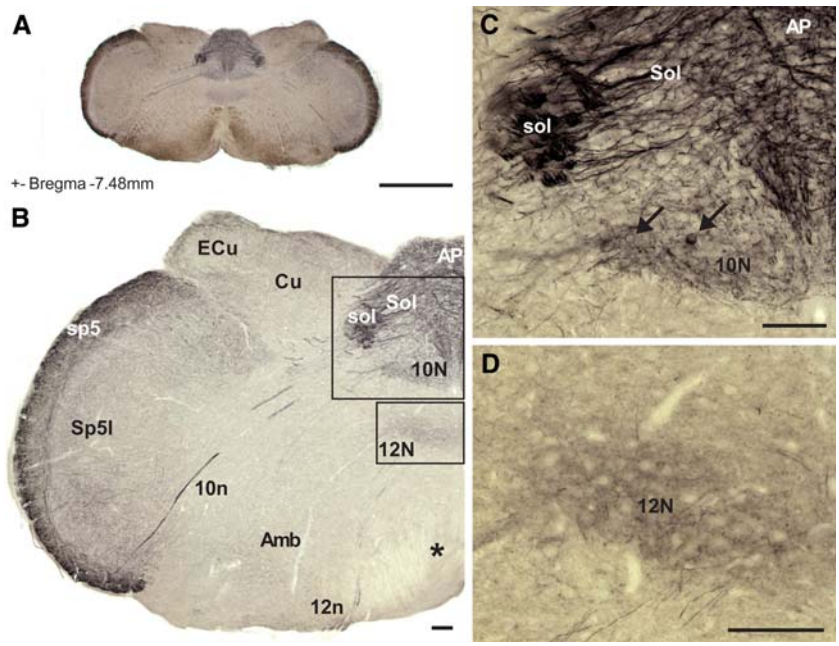

Fig. 6 Peripherin expression in a transverse section of the medulla close to obex. **a** Low power photomicrograph of the mouse medulla shows localisation of peripherin immunoreactivity that is presented at higher magnification in the following panels. **b** Peripherin expression is observed in the area postrema (*AP*), the nucleus of the Sol, sol and the dorsal motor nucleus of the vagus nerve (*10N*), detail of these structures is shown in **c**. Peripherin immunostaining is also seen in the hypoglossal nucleus (*12N*, detailed in **d**), the sp5, the interpolar portion of the spinal trigeminal nucleus (*Sp5I*), and in the nerve fibres that give rise to the vagus nerve (*10n*) and the hypoglossal nerve (*12n*). Note there is no peripherin immunoreactivity in this more caudal region of the nucleus ambiguus (*Amb*) or in the ventromedial region of the medulla (marked with *asterisk*), which contains sub-nuclei of the inferior olive. **c** Peripherin expression in the *AP*, nucleus of the Sol and the sol is restricted to nerve fibres, while in 10N, cell bodies show relatively weaker peripherin expression (*arrows*). **d** Neuronal somata in 12N show weak peripherin immunostaining. *Scale bars* **a** = 1 mm, **b**–**d** = 100 μm

et al., 1990a) did not detect peripherin expressing nerve fibres in the mouse cerebellum. In adult rat, these peripherin positive fibres likely arise from the inferior olive and contribute to the climbing fibres within the molecular layer (Brody et al. 1989; Errante et al. 1998). However, we did not observe immuno-staining in the inferior olive region. This may be resolved in the future by in situ hybridization. Similarly, we failed to detect peripherin immunolabelling in the external cuneate nucleus or in the vestibular nuclei that provide sensory input into the cerebellum via the inferior cerebellar peduncle. Input from fibres arising in Clarke's nucleus in the cervical spinal cord region may also contribute to these peripherin positive fibres in the peduncle. Peripherin expression in nerve fibres within the lateral deep cerebellar nucleus appears to arise from the neurons within this nucleus, even though peripherin labelled cell somata were not observed. This lack of cell body labelling in the presence of axon labelling was also observed by Brody et al. (1989) in the adult rat.

It is unclear why peripherin so clearly demarcates discrete sensory, motor and autonomic neuronal populations. For example, the peripherin immunolabelling of the primary afferent cell bodies of the mesenchephalic trigeminal nucleus (Me5), which convey proprioceptor input from intrafusal muscle spindle afferents of the masticatory muscles (Yoshida and Oka 1998), is prominent. Similarly nucleus ambiguus pars compacta, the site of the esophageal motoneurons which exit the hindbrain via the vagus (Bieger and Hopkins 1987), is an example of the discrete subnuclear representation of motor pathways. The more caudal and ventral aspects of nucleus ambiguus—associated with pharyngeal and laryngeal motor activity, and the vagal parasympathetic outflow to the heart and respiratory premotor control (McAllen and Spyer 1976; Bieger and Hopkins 1987), showed no discrete peripherin immunolabelling in the present study. Peripherin expression was noted in the nucleus ambiguus of adult rat (Brody et al. 1989), however the data were insufficient to distinguish between the different regions.

Peripherin expression has been implicated in neurite outgrowth (Escurat et al. 1990; Troy et al. 1990a; Undamatla and Szaro 2001; Smith et al. 2006) and repair (Oblinger et al. 1989; Troy et al. 1990b; Chadan et al. 1994). A recent study in neuronally differentiated PC12 cells demonstrated that knockdown of peripherin expression using siRNA led to a marked inhibition of the neurite growth and maintenance (Helfand et al. 2003). Up-regulation of peripherin expression is also a pronounced feature of neural repair, which can include neurons not normally exhibiting peripherin expression (Beaulieu et al. 2002), but overexpression of the protein in a transgenic mouse model led to the development of amylolateral sclerosis (ALS)-like neuronal pathology (Beaulieu et al. 1999, 2000). Conversely, peripherin knockout mice exhibited only minor discernable differences in CNS structure, specifically a reduction in small diameter, unmyelinated sensory fibres in the spinal cord that are thought to be the non-peptidergic nociceptive afferents (Lariviere et al. 2002). The fact that the majority of the DRG neurons were retained in the peripherin knockout mouse, even though 67% express peripherin (Troy et al. 1990a), suggests that neurite development proceeded normally for most of the neurons. The limited effect of peripherin knockout may be due to redundancy between peripherin and the type IV neuronal intermediate filaments, namely α-internexin and NF-L, NF-M and NF-H. Up-regulation of α-internexin protein was observed in the motor axons of the peripherin knockout mice (Lariviere et al. 2002). Based on such studies, one may postulate that peripherin contributes to the demarcation and maintenance of the neuronal sub-populations which have been delineated here and it highlights the need for an improved understanding of the significance of this type III intermediate filament protein to the neuronal circuitry of the brain.

The description of peripherin expression in the hindbrain provided here, contributes key normative data in the mouse CNS model. Our study identifies peripherin as a marker for afferent and motor neuronal populations which were largely discrete from the dorsal sensory and ventral motor columns.

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