

JNS 03126

## A light and electron microscopical study of B-50 (GAP-43) in human intramuscular nerve and neuromuscular junctions during development

L. F. G. M. Hesselmans<sup>1</sup>, F. G. I. Jennekens<sup>1</sup>, C. J. M. van den Oord<sup>1</sup>,  
A. B. Oestreicher<sup>2</sup>, H. Veldman<sup>1</sup> and W. H. Gispen<sup>2</sup>

<sup>1</sup>Laboratory for neuromuscular diseases, University Hospital, Utrecht (The Netherlands), and <sup>2</sup>Division of Molecular Neurobiology, Institute of Molecular Biology, University of Utrecht, Utrecht (The Netherlands)

(Received 23 September, 1988)

(Accepted 31 October, 1988)

---

### SUMMARY

The growth associated protein B-50 (GAP-43) is demonstrated in human intramuscular nerves and neuromuscular junctions by light microscopy and electron microscopy. Nearly all fetal endplates are shown to be immunoreactive for B-50. The percentage of B-50 positive endplates decreases significantly during the peri- and neonatal period, but in children and adults a low percentage of B-50 positive endplates remains present. These data indicate that also in the human peripheral nervous system the expression of B-50 is developmentally regulated.

---

**Key words:** Phosphoprotein B-50; GAP-43; Intramuscular nerve; Neuromuscular junction; Human development

---

### INTRODUCTION

B-50 is an acidic neuron specific phosphoprotein. It belongs to a group of growth associated proteins (GAPs) and is identical to GAP-43, pp46 and F1 (Benowitz and Routtenberg 1987; Nielander et al. 1987; Zwiers et al. 1987). Recent investigations using DNA cloning methods show the sequence of GAP-43 to be identical with that

---

*Correspondence to:* Dr. L. F. G. M. Hesselmans, Laboratory for Neuromuscular Diseases, University Hospital, Catharijnesingel 101, 3511 GV Utrecht, The Netherlands.

of a neuron-specific calmodulin-binding protein, termed P-57 (Cimler et al. 1987). B-50 may be part of a regulatory mechanism for the intracellular calcium concentration, which plays a central role in neurite outgrowth and synaptogenesis (Kater et al. 1988; Mattson et al. 1988). B-50 is a 226 amino acid polypeptide, that is synthesized and axonally transported in greatly elevated amounts relative to other proteins, during periods of neuronal differentiation, axon outgrowth and after axon injury (Skene and Willard 1981a,b; Kalil and Skene 1986; Perrone-Bizzozero et al. 1986; Gorgels et al. 1987; Perrone-Bizzozero and Benowitz 1987; Perry et al. 1987). B-50 is transported along the axons by fast transport (Skene and Willard 1981a). After crushing of peripheral nerves the synthesis of B-50 is raised and high amounts of B-50 appear in the newly formed sprouts. When reinnervation is completed the amount of B-50 in the peripheral nerve returns to the control level (Skene and Willard 1981a; Verhaagen et al. 1986). B-50 is found at high levels in recently reinnervated rat neuromuscular junctions. Within weeks following the completion of muscle reinnervation, B-50 immunoreactivity in the nerve terminals returns to the control level (Verhaagen et al. 1988).

In the rat central nervous system (CNS) B-50 is a protein of the presynaptic membrane (Gispén et al. 1985) and has been implicated in both the development and modulation of neuronal connections (Pfenninger et al. 1983; De Graan et al. 1985, 1986; Van Hooff et al. 1987; Zwiers et al. 1987). In the immature neuron, B-50 is an integral constituent of the growth cone, and although overall levels drop considerably during the transition to mature synapses, significant amounts continue to be associated with the presynaptic terminals of certain neurons throughout life (Jacobson et al. 1986; Oestreicher and Gispén 1986; Skene et al. 1986).

In human neonatal CNS B-50 is uniformly expressed throughout the brain. In the adult human brain B-50 expression varies markedly among different regions. High levels are expressed in the visual cortex and the orbital frontal gyrus. In contrast to the adult rat hippocampus low levels of B-50 are found in the adult human hippocampus (Neve et al. 1987; Shi-Shung et al. 1988).

In view of its presence in growth cones B-50 has been used as a marker for growth of neuronal tissue in animals. It is not known whether the results of animal investigations concerning the presence of B-50 can be freely translated to human peripheral nervous tissue. The aim of the present study is to investigate whether B-50 is also present in human peripheral nerves and neuromuscular junctions.

## MATERIALS AND METHODS

Following abortion of 2 human fetuses of 15 and 20 weeks, biopsies of their lower limb muscles were obtained. Biopsies of intercostal muscle were taken from patients, who underwent surgery for intrathoracic disorders: one 27-week-old human fetus, 8 children and 7 adults, without neuromuscular disease. In one of the adults this disorder involved a malignant process. No neurotoxic medication had been used before the operations.

All tissue was fixed for 2.5 h in 2% paraformaldehyde/50 mM phosphate buffer,

pH 7.4 containing 0.1 M lysine and 0.2% sodium periodate (PLP) (McLean and Nakane 1974). The muscles were cryoprotected by immersion in graded sucrose (7.5, 15, 25, and 35%).

To demonstrate B-50 immunoreactivity in intramuscular nerves a double labelling experiment was performed using mouse monoclonal anti-70 and -200 kDa neurofilament (NF) antibodies (Monosan, Uden, The Netherlands) and affinity purified rabbit anti-B-50 antibodies (antiserum 8420). In 7- $\mu$ m thick cryostat sections endogenous peroxidase activity was inhibited using periodic acid and sodium borohydride according to Heyderman and Neville (1977). Sections were incubated overnight with mouse anti-NF antibodies (dilution 1:10) in PBS containing 0.2% Triton, rinsed with PBS, and incubated with horseradish peroxidase (HRP)-conjugated rat anti-mouse IgG (dilution 1:80; Dako, Copenhagen, Denmark) for 60 min. Sections were stained for peroxidase according to the 3,3'-diaminobenzidine (DAB) method of Graham and Karnovsky (1966). This staining method gives little suppression of immunofluorescence. Cross-reaction between rabbit anti-mouse antibodies and swine anti-rabbit antibodies was prevented by washing the sections in an acidic 0.1 M glycine solution, pH 2.5, during 8 h after the peroxidase reaction had been performed. In this way cross-reacting rabbit anti-mouse antibodies were eluted. Afterwards the same sections were incubated with purified rabbit anti-B-50 antibodies (1:1000) for 16 h, rinsed with PBS/Triton and incubated with fluorescein isothiocyanate (FITC)-conjugated swine anti-rabbit IgG (1:100; Dako, Copenhagen, Denmark) for 60 min. The sections were mounted in 90% glycerol in veronal-buffered saline, containing 0.1% paraphenylene diamine.

To demonstrate B-50 immunoreactivity at neuromuscular junctions a double labelling experiment was performed using rat monoclonal antibodies against acetylcholine receptors (AChR) (mcAb35, gift from Dr. M.H. De Baets, Laboratory of Immunology, University of Limburg, The Netherlands; Smit et al. 1988) and purified rabbit anti-B-50 antibodies. In 7- $\mu$ m thick cryostat sections endogenous peroxidase activity was inhibited using periodic acid and sodium borohydride according to Heyderman and Neville (1977). The sections were incubated with anti-acetylcholine receptor antibodies (1:1000) for 8 h, rinsed with PBS, and incubated with HRP-conjugated goat anti-rat antibodies (1:5; Pel-Freeze Laboratories, Rogers, AR, U.S.A.) for 16 h. Sections were stained for peroxidase according to the DAB method of Graham and Karnovsky (1966). After rinsing the sections with acetone and PBS, they were incubated with anti-B-50 antibodies (1:1000) for 16 h, rinsed with PBS and incubated with FITC-conjugated swine anti-rabbit IgG (1:100) for 60 min. The sections were mounted in 90% glycerol in veronal-buffered saline, containing 0.1% paraphenylene diamine.

The cryostat sections were examined with a Leitz Orthoplan microscope. All endplates within the sections were examined and scored for B-50 immunofluorescence. When a AChR immunoreactive endplate was contacted by a B-50 fluorescent axon branch, this endplate was scored as B-50 positive. The number of B-50 positive endplates divided by the number of AChR stained endplates, multiplied by 100, yields the percentage of B-50 positive endplates.

Data were statistically tested by linear regression analysis corrected for sample size.

To demonstrate B-50 at an ultrastructural level, 20- $\mu$ m sections of lower limb muscles from a 20-week-old human fetus were incubated overnight in anti-B-50 antibodies (1:1000 in PBS containing 0.2% BSA and 0.2% Triton), rinsed in PBS containing 0.2% Triton, incubated with swine anti-rabbit IgG (1:100) for 8 h, rinsed with PBS, and incubated in HRP-conjugated rabbit antibodies against peroxidase (1:160) overnight. Sections were stained for peroxidase according to the DAB-Ni-Co method of Adams (1981). The 20- $\mu$ m sections were postfixed in 1% osmium tetroxide for 60 min at room temperature, dehydrated and embedded in Epon as in routine EM procedures. Thin sections of endplate or nerve containing regions were contrasted with lead citrate for 1 min. Sections were photographed in a Zeiss electron microscope.

## RESULTS

Intramuscular nerve fibres were identified by means of immunohistochemical staining with monoclonal mouse anti-NF antibodies. In 15- and 20-week-old fetuses a nearly perfect co-localization of B-50 and NF immunoreactivity was observed (Fig. 1). B-50 immunofluorescence was only present within the intramuscular nerves and nerve terminals. The number of nerve fibres showing immunofluorescence for B-50 and the intensity of immunofluorescence decreased with age. No quantitative measurements of these observations were attempted.

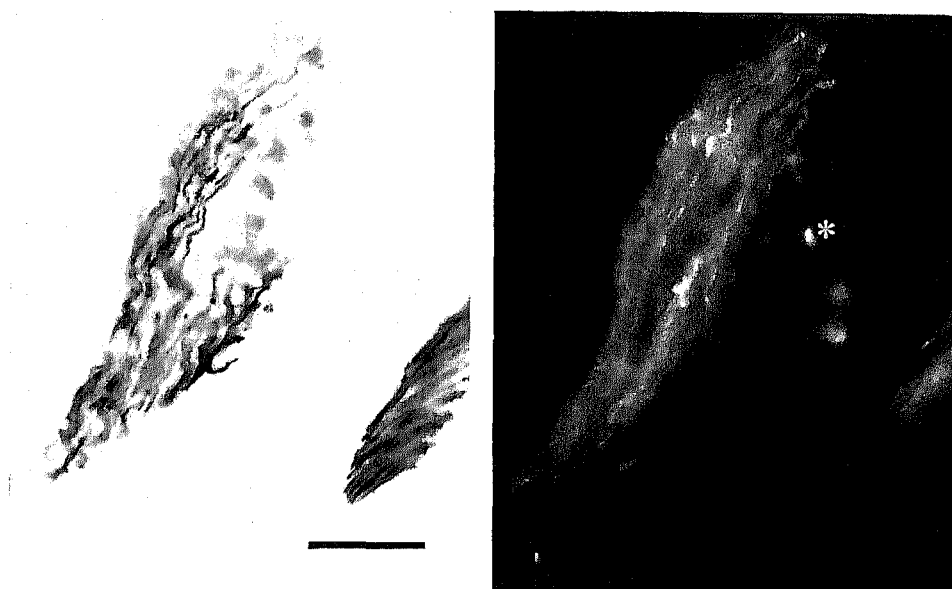


Fig. 1. Double labelling of 20-week-old human fetal intramuscular nerve. Left: immunoperoxidase labelling with anti-NF antibodies. Right: immunofluorescence labelling with anti-B-50 antibodies. A nearly perfect co-localization of NF and B-50 immunoreactivity is observed, indicating B-50 to be present in human intramuscular nerves, autofluorescence is seen in erythrocytes (asterisk). Bar = 50  $\mu$ m.



Fig. 2. Double labelling of 20-week-old human fetal endplates. Left: immunoperoxidase labelling with anti-AChR-antibodies. Right: immunofluorescence labelling with anti-B-50 antibodies. B-50 immunofluorescence is present in small intramuscular nerve fibres reaching the neuromuscular junctions (arrows). Bar = 50  $\mu$ m.

Endplates were identified by immunohistochemical staining with anti-AChR antibodies. Immunofluorescence demonstrating B-50 immunoreactivity, could be clearly visualized in the small nerve terminals reaching the endplates (Fig. 2). The number of endplates examined in each section varied between 1 and 200 endplates. In

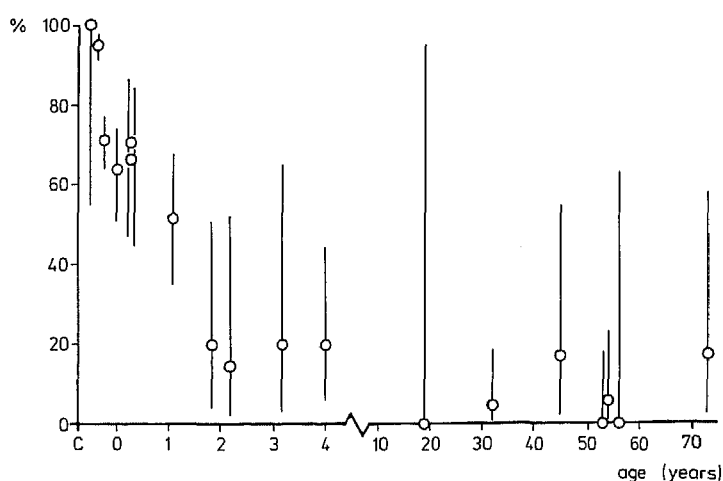


Fig. 3. Percentage of B-50 positive endplates at different ages (with 90% confidence limits). Each datapoint represents 1 subject.

material of fetal muscle usually more endplates were present than at an older age. The percentage of B-50 positive endplates (see Methods) was 90–95% in fetal muscle of 15 and 20 weeks. This percentage decreased with increasing age. At the time of birth the percentage was about 70%. It dropped to 20% at the age of 2 years. The decline in B-50 positive endplates during the fetal and neonatal period was highly significant ( $p < 0.001$ ). In between 2 and 73 years of age no significant further decrease in B-50 immunoreactivity was observed (Fig. 3).

The ultrastructural investigations were performed on lower limb muscles taken from a 20-week-old human fetus. Intramuscular nerves contained mostly small non-myelinated axons. Frequently one Schwann cell enveloped 2 or 3 axons. Usually only 1 or 2 of these axons showed B-50 immunoreactivity. In cases when a Schwann cell contained only 1 axon, this axon sometimes showed B-50 immunoreactivity. As expected, only a few myelinated axons were seen. B-50 immunoreactivity was not seen within Schwann cells or other cells (Figs. 4 and 5).

Neuromuscular junctions usually showed 2 or more nerve terminals, containing synaptic vesicles and mitochondria. Due to long incubation times no optimum membrane structure was attained. Intense B-50 immunoreactivity could be demonstrated within the nerve terminals. The peroxidase reaction product was concentrated near the

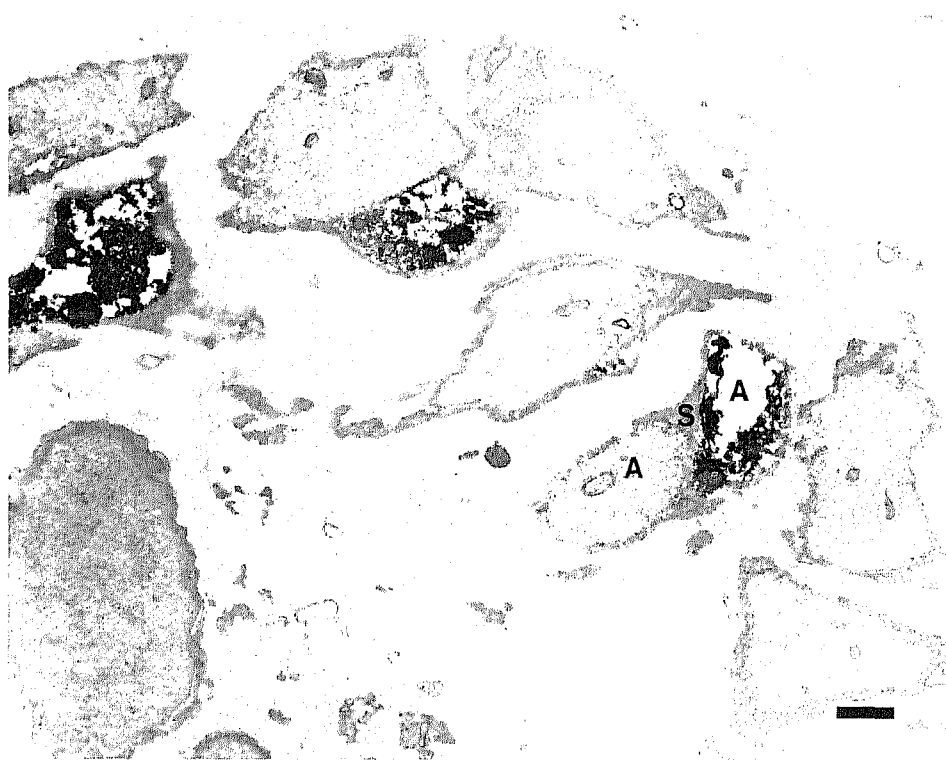


Fig. 4. Transverse section of 20-week-old human fetal intramuscular nerve. B-50 immunoreactivity product is shown within some non-myelinated axons. A = axon, S = Schwann cell. Bar = 1  $\mu$ m.

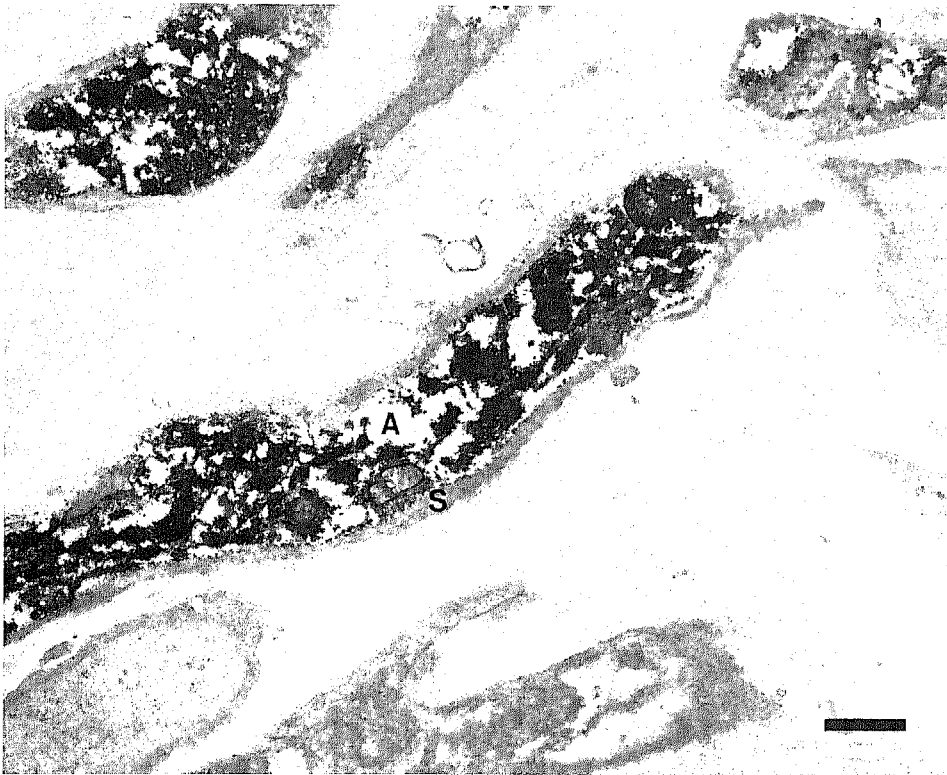


Fig. 5. Longitudinal section of 20-week-old human fetal intramuscular nerve. B-50 immunoreactivity product is clearly visible within the axon. No immunoreactivity is seen within the Schwann cell. A = axon, S = Schwann cell. Bar = 1  $\mu$ m.

presynaptic membrane and sometimes on the synaptic vesicle membrane and mitochondrial membrane. At a few neuromuscular junctions small amounts of peroxidase reaction product were observed at the postsynaptic membrane. B-50 immunoreactivity was not seen outside the junctional regions (Fig. 6).

## DISCUSSION

This paper shows that intense B-50 immunoreactivity is present in fetal human intramuscular nerve and neuromuscular junctions. Its presence at neuromuscular junctions decreases during the fetal and neonatal period. At the time of birth the majority of endplates still show B-50 immunoreactivity. This indicates that B-50 is not only present while the first neuromuscular contacts are being made, but also some months afterwards. Data on B-50 immunoreactivity in the developing peripheral nervous system of laboratory animals are not available. In rat CNS, B-50 immunoreactivity remains present in adult animals particularly in regions where continuously new plastic changes may occur, like in the hippocampal region (Oestreicher and Gispén 1986; Benowitz

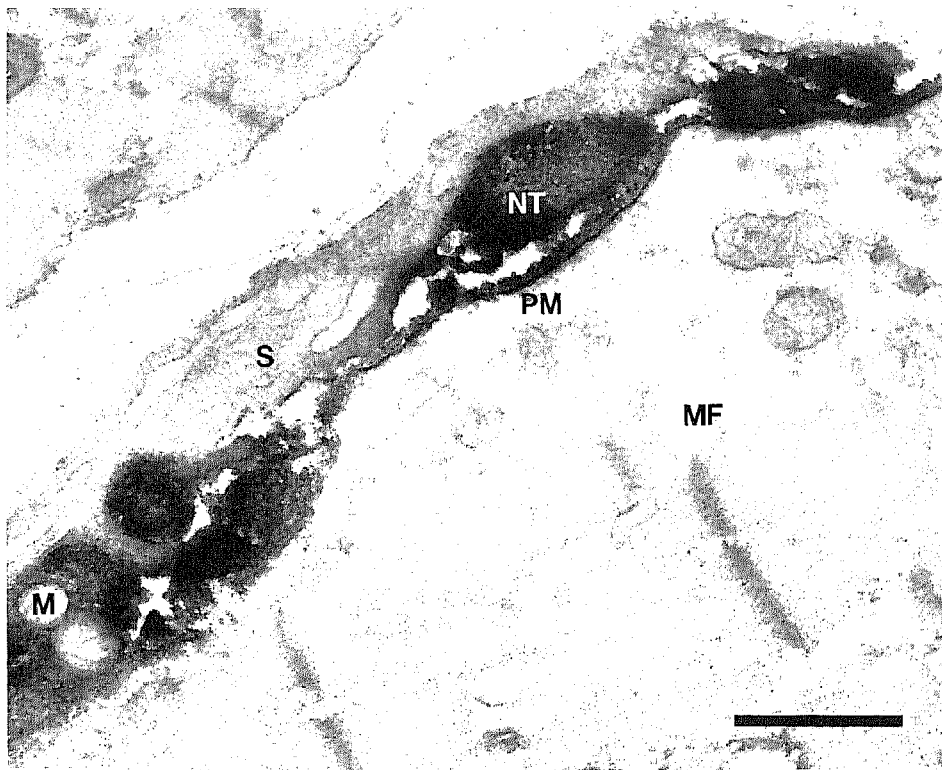


Fig. 6. 20-Week-old human fetal neuromuscular junction. B-50 immunoreactivity product is shown within the nerve terminals. Two mitochondria within a nerve terminal are seen at the left. M = mitochondrion, MF = muscle fibre, NT = nerve terminal, PM = postsynaptic membrane, S = Schwann cell.  
Bar = 1  $\mu$ m.

et al. 1988). In the human CNS a high level of mRNA for B-50 remains expressed in the orbital frontal region (Shi-Shung et al. 1988). B-50 (F1) has been suggested to play a role in the plasticity of CNS synapses (Benowitz and Routtenberg 1987). A similar role for B-50 in neuromuscular junctions is well possible (Verhaagen et al. 1988) and would explain why some endplates in adults are positive for B-50, as these endplates might be undergoing a remodelling process. Evidence indicating on-going remodelling in neuromuscular junctions throughout life has been obtained in studies of animals (Cardes 1983).

At the ultrastructural level B-50 immunoreactivity is seen in non-myelinated axons of the developing human peripheral nervous system. Only a few myelinated axons are seen, none of these showing B-50 immunoreactivity. A preponderance of B-50 in non-myelinated axons is to be expected since myelination follows axon outgrowth. Moreover, growth of peripheral nerve fibres takes place mostly in the non-myelinated distal part of the nerve fibre. On the other hand, it is possible that in pre-embedding procedures myelin is difficult for antibodies to penetrate and therefore the absence of B-50 immunoreactivity in myelinated fibres may be a result of the method we used.



Recent investigations demonstrating B-50 in developing rat pyramidal tract, with use of a pre-embedding method with anti-B-50 antibodies, however, do demonstrate B-50 immunoreactivity within myelinated axons. This indicates that B-50-antibodies are able to pass the myelin membranes (Gorgels et al. 1988).

Frequently 2 or more axons are found surrounded by 1 Schwann cell. This is a normal finding during the fetal differentiation of peripheral nerves (Fidzianska 1971; Webster and Favilla 1984). When 1 Schwann cell envelops 2 or 3 axons, usually one is showing B-50 immunoreactivity while the others do not (Fig. 4). This cannot be explained by a difference in penetrating capacity of antibodies into the axons, and therefore reflects a real difference in B-50 immunoreactivity. On cross-section the axons are similar in appearance. It is possible that the axons not containing any B-50 have already formed a completely developed neuromuscular junction that needs no further readjustments, while the axons containing B-50 immunoreactivity are still forming and remodelling their endplates. Since in a 20-week-old fetus polyneuronal innervation is probable (Fidzianska 1980), it is also possible that the axons devoid of B-50 immunoreactivity are retracting their terminals instead of growing to the endplate. Another possibility is that one of the axons may arise from a motor neuron, while the other is a dendrite from a sensory neuron. B-50 can be expected to be present in developing sensory axons since it is known to be present in the olfactory system of neonatal and adult rats (Verhaagen et al. 1989) and in neurites from fetal rat dorsal root ganglion cells in culture (Schmidt et al. 1988). However, if peripheral motor and sensory neurons develop during a different fetal period, the expression of B-50 in motor and sensory neurons may not coincide.

At an ultrastructural level B-50 is seen in the nerve terminals of fetal neuromuscular junctions. The peroxidase reaction product demonstrating the presence of B-50 is concentrated on the presynaptic membrane, synaptic vesicle membranes and other membranes. A minor degree of peroxidase reaction product is sometimes seen at the postsynaptic membrane, which may be the result of inadequate localisation due to the method used for visualization. Because B-50 is a membrane associated protein a preference of B-50 for the synaptic membranes can be expected.

Our results indicate B-50 to be present in human peripheral nerves and neuromuscular junctions. The intense immunoreactivity for B-50 during the fetal period decreases during the peri- and neonatal period. These findings point to a developmentally regulated expression of B-50 in human peripheral nerves similar to that seen in laboratory animals. Therefore B-50 may be a marker for growth of peripheral nerve fibres and as such be of value in histological studies of regeneration processes.

#### ACKNOWLEDGEMENTS

This investigation was supported by the Princes Beatrix Fonds. Dr. J.F. Hitchcock and Dr. J.R. Lahpor took the biopsies. S.A. van Eeuwijk performed the statistical analysis.

## REFERENCES

- Adams, J.C. (1981) Heavy metal intensification of DAB-based HRP reaction products. *J. Histochem. Cytochem.*, 29: 775–780.
- Benowitz, L.I. and A. Routtenberg (1987) A membrane phosphoprotein associated with neural development, axonal regeneration, phospholipid metabolism, and synaptic plasticity. *Trends Neurosci.*, 10: 527–532.
- Benowitz, L.I., P.J. Apostolides, N. Perrone-Bizzozero, S.P. Finklestein and H. Zwiers (1988) Anatomical distribution of the growth-associated protein GAP-43/B-50 in the adult rat brain. *J. Neurosci.*, 8: 339–352.
- Cardasis, C.A. (1983) Ultrastructural evidence of continued reorganization at the aging (11–26 months) rat soleus neuromuscular junction. *Anat. Rec.*, 207: 399–415.
- Cimler, B.M., D.H. Giebelhaus, B.T. Wakim, D.R. Storm and R.T. Moon (1987) Characterization of murine cDNAs encoding P-57, a neural-specific calmodulin-binding protein. *J. Biol. Chem.*, 262: 12158–12163.
- De Graan, P.N.E., C.O.M. Van Hooff, B.C. Tilly, A.B. Oestreicher, P. Schotman and W.H. Gispen (1985) Phosphoprotein B-50 in nerve growth cones from fetal rat brain. *Neurosci. Lett.*, 61: 235–241.
- De Graan, P.N.E., A.B. Oestreicher, L.H. Schrama and W.H. Gispen (1986) Phosphoprotein B-50: localization and function. *Prog. Brain Res.*, 69: 37–50.
- Fidzianska, A. (1971) Electron microscopic study of the development of human foetal muscle, motor end-plate and nerve. *Acta Neuropathol.*, 17: 234–247.
- Fidzianska, A. (1980) Human ontogenesis. II. Development of the human neuromuscular junction. *J. Neuropath. Exp. Neurol.*, 39: 606–615.
- Gispen, W.H., J.L.M. Leunissen, A.B. Oestreicher, A.J. Verkleij and H. Zwiers (1985) Presynaptic localization of B-50 phosphoprotein: the (ACTH)-sensitive protein kinase substrate involved in rat brain polyphosphoinositide metabolism. *Brain Res.*, 328: 381–385.
- Gorgels, T.G.M.F., A.B. Oestreicher, E.J.M. De Kort and W.H. Gispen (1987) Immunocytochemical distribution of the protein kinase C substrate B-50 (GAP43) in developing rat pyramidal tract. *Neurosci. Lett.*, 83: 59–64.
- Gorgels, T.G.M.F., A.B. Oestreicher, M.M.A. Helsen, E.J.M. De Kort and W.H. Gispen (1988) Ultrastructural localization of B-50/GAP43 in the developing cervical pyramidal tract in the rat (Abstract). *E.N.A. Zurich*, in press.
- Graham, R.C. and M.J. Karnovsky (1966) The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney; ultrastructural cytochemistry by a new technique. *J. Histochem.*, 41: 291–302.
- Heyderman, E. and A.M. Nevill (1977) Shorter immunoperoxidase technique for the demonstration of carcinoembryonic antigen and other cell products. *J. Clin. Pathol.*, 30: 138–140.
- Jacobson, R.D., I. Virág and J.H.P. Skene (1986) A protein associated with axon growth, GAP-43, is widely distributed and developmentally regulated in rat CNS. *J. Neurosci.*, 6: 1843–1855.
- Kalil, K. and J.H.P. Skene (1986) Elevated synthesis of an axonally transported protein correlates with axon outgrowth in normal and injured pyramidal tracts. *J. Neurosci.*, 6: 2563–2570.
- Kater, S.B., M.P. Mattson, C. Cohan and J. Conner (1988) Calcium regulation of the neuronal growth cone. *Trends Neurosci.*, 11: 315–321.
- Mattson, M.P., A. Taylor-Hunter and S.B. Kater (1988) Neurite outgrowth in individual neurons of a neuronal population is differentially regulated by calcium and cyclic AMP. *J. Neurosci.*, 8: 1704–1711.
- McLean, I.W. and P.K. Nakane (1974) Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. *J. Histochem. Cytochem.*, 22: 1077–1083.
- Neve, R.L., N.I. Perrone-Bizzozero, S. Finklestein, H. Zwiers, E. Bird, D.M. Kurnit and L.I. Benowitz (1987) The neuronal growth-associated protein GAP-43 (B-50, F1): neuronal specificity, developmental regulation and regional distribution of the human and rat mRNAs. *Mol. Brain Res.*, 2: 177–183.
- Nieler, H.B., L.H. Schrama, A.J. van Rozen, M. Kasperaitis, A.B. Oestreicher, P.N.E. De Graan, W.H. Gispen and P. Schotman (1987) Primary structure of the neuron-specific phosphoprotein B-50 is identical to growth-associated protein GAP-43. *Neurosci. Res.*, 1: 163–172.
- Oestreicher, A.B. and W.H. Gispen (1986) Comparison of the immunocytochemical distribution on the phosphoprotein B-50 in the cerebellum and hippocampus of immature and adult rat brain. *Brain Res.*, 375: 267–279.
- Perrone-Bizzozero, N.I. and L.I. Benowitz (1987) Expression of a 48-kilodalton growth-associated protein in the goldfish retina. *J. Neurochem.*, 48: 644–652.

- Perrone-Bizzozero, N.I., S.P. Finklestein and L.I. Benowitz (1986) Synthesis of a growth-associated protein by embryonic rat cerebrocortical neurons in vitro. *J. Neurosci.*, 6: 3721-3730.
- Perry, G.W., D.W. Burmeister and B. Grafstein (1987) Fast axonally transported proteins in regenerating goldfish optic axons. *J. Neurosci.*, 7: 792-806.
- Pfenninger, K.H., L. Ellis, H.P. Johnson, L. Friedman and S. Somlo (1983) Nerve growth cones isolated from fetal rat brain: subcellular fractionation and characterization. *Cell*, 35: 573-584.
- Schmidt, M., P. Edwards, A.B. Oestreicher and W.H. Gispen (1989) Colchicine effect on growth-associated protein GAP-43 (B-50) localization in rat dorsal root ganglion in culture. *Neurosci. Lett.*, in press.
- Shi-Chung, Ng., S.M. De La Monte, G.L. Conboy, L.R. Karns, M.C. Fishman (1988) Cloning of human GAP-43: Growth association and ischemic resurgence. *Neuron*, 1: 133-139.
- Skene, J.H.P. and M. Willard (1981a) Changes in axonally transported proteins during axon regeneration in toad retinal ganglion cells. *J. Cell Biol.*, 89: 86-95.
- Skene, J.H.P. and M. Willard (1981b) Axonally transported proteins associated with axon growth in rabbit central and peripheral nervous systems. *J. Cell Biol.*, 89: 96-103.
- Skene, J.H.P., R.D. Jacobson, G.J. Snipes, C.B. McGuire, J.J. Norden and J.A. Freeman (1986) A protein induced during nerve growth (GAP-43) is a major component of growth-cone membranes. *Science*, 233: 783-786.
- Smit, L.M.E., H. Veldman and F.G.I. Jennekens (1988) Immunohistochemical localization of acetylcholinereceptors in human endplates using a monoclonal antibody. *J. Histochem. Cytochem.*, 110: 1061-1079.
- Van Hooff, C.O.M., P.N.E. De Graan, A.B. Oestreicher and W.H. Gispen (1988) B-50 phosphorylation and polyphosphoinositide metabolism in nerve growth cone membranes. *J. Neurosci.*, 8: 1789-1795.
- Verhaagen, J., C.O.M. Van Hooff, P.M. Edwards, P.N.E. De Graan, A.B. Oestreicher, P. Schotman, F.G.I. Jennekens and W.H. Gispen (1986) The kinase substrate protein B-50 and axonal regeneration. *Brain Res. Bull.*, 17: 737-741.
- Verhaagen, J., A.B. Oestreicher, P.M. Edwards, H. Veldman, F.G.I. Jennekens and W.H. Gispen (1988) Light- and electron-microscopical study of phosphoprotein B-50 following denervation and reinnervation of the rat solcus muscle. *J. Neurosci.*, 8: 1759-1766.
- Verhaagen, J., A.B. Oestreicher, W.H. Gispen and F.L. Margolis (1989) The expression of the growth associated protein B-50/GAP-43 in the olfactory system of neonatal and adult rats. *J. Neurosci.*, in press.
- Webster, H. DeF. and J. Favilla (1984) Development of peripheral nerve fibres. In: Dyck, P.J., P.K. Thomas, E.H. Lambert and R. Bunge (Eds.), *Peripheral Neuropathy*, W.B. Saunders Co., Philadelphia, PA, pp. 329-360.
- Zwiers, H., A.B. Oestreicher, M.A. Bisby, P.N.E. De Graan and W.H. Gispen (1987) Protein kinase C substrate B-50 in adult and developing rat brain is identical to axonally transported GAP-43 in regenerating peripheral rat nerve. In: Bisby, M.A. and R.S. Smith (Eds.), *Axonal Transport*, A.R. Liss Inc., New York, NY, pp. 421-433.