Virchows Arch A (1987) 411:53–59

Virchows Archiv A

Synaptophysin: a reliable marker for medulloblastomas

K. Schwechheimer [1], B. Wiedenmann [2], and W.W. Franke [3]

[1] Institut für Neuropathologie der Universität, Im Neuenheimer Feld 220/221, 6900 Heidelberg 1, Federal Republic of Germany
[2] Medizinische Universitätsklinik, Abteilung für Innere Medizin IV, Bergheimer Straße 58, 6900 Heidelberg 1, Federal Republic of Germany
[3] Institut für Zell- und Tumorbiologie, Abteilung Membranbiologie und Biochemie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg 1, Federal Republic of Germany

Summary. Synaptophysin is an acidic, integral membrane glycoprotein (M_r 38000) of presynaptic vesicles in various neurons and neuroendocrine cells, and in tumours derived from such cells. By indirect immunofluorescence microscopy of cryostat sections, using the monoclonal antibody SY 38 to synaptophysin, a consistent positive immunoreactivity was observed in all medulloblastomas ($n=6$) and neuroblastomas ($n=3$) as well as a ganglioneuroma and a glioneuronal hamartoma. The presence of synaptophysin in medulloblastomas was confirmed biochemically by immunoblotting experiments. For purpose of comparison, the expression of intermediate-sized filament (IF) proteins was also examined. While neurofilament proteins were consistently expressed in the neuroblastomas (3/3), the ganglioneuroma and the glioneuronal hamartoma, IF distribution in medulloblastomas was variable. A neurofilament-positive type of tumour (1/6) could be distinguished from vimentin-expressing neoplasms (4/6) by immunocytochemistry. These data indicate that synaptophysin is a reliable marker for medulloblastomas as well as other differentiated and undifferentiated neuronal tumours and in this respect is superior to the more heterogeneous expression patterns of IF proteins in these tumours.

Key words: Synaptophysin – Medulloblastomas – Neuronal tumours – Intermediate-sized filament proteins – Immunocytochemistry – Immunoblotting

Synaptophysin is an integral, acidic membrane glycoprotein of presynaptic vesicles (M_r 38000) which can be immunocytochemically identified by the monoclonal antibody SY 38 (Wiedenmann and Franke 1985) and other specific antibodies (Jahn et al. 1985). Recently, this protein has also been identified as a major calcium-binding protein of the synaptic vesicle membrane (Rehm et al. 1986). Synaptophysin has been immunocytochemically demonstrated in diverse types of neurons, in adrenal medulla, in neuroendocrine cells, in pancreatic islet cells and in a variety of other cells as well (Wiedenmann and Franke 1985). It is conserved in neuronal and neuroendocrine tumours, notably in pheochromocytomas, paragangliomas, ganglioneuroblastomas, islet cell tumours of the pancreas, carcinoids of the gastrointestinal tract and the lung as well as in medullary thyroid carcinomas (Wiedenmann et al. 1986).

Neurofilament (NF) proteins, the neuron-specific intermediate-sized filament (IF) components, usually appear in the form of three polypeptides of different molecular weights (NF-L, NF-M, NF-H; Hoffman and Lasek 1975; Lee et al. 1972; for reviews see: Dahl and Bignami 1977; Liem et al. 1978; Osborn et al. 1982; Osborn and Weber 1983; Weber et al. 1983). NF proteins are exquisite markers for differentiated neuronal tumours but are often poorly or variably expressed in undifferentiated neural tumours such as medulloblastomas, neuroblastomas and malignant peripheral neuroectodermal tumours (Osborn et al. 1982; Osborn and Weber 1983; Trojanowski and Lee 1983; Lehto et al. 1983; Roessmann et al. 1983; Carlei et al. 1984; Trojanowski et al. 1984; Trojanowski and Lee 1985; Miettinen et al. 1985; Schmidt et al. 1985; Tremblay et al. 1985; Mukai et al. 1986; Osborn et al. 1986). The aim of the present study was to examine the immunoreactivity of synaptophysin in primitive neuroectodermal tumours, such as medulloblastomas of the brain and neuroblasto-

Offprint requests to: K. Schwechheimer at the above address

Table 1. Synaptophysin and intermediate filament immunoreactivity in different types of neuronal tumours

Tumour type	Sex	Age (yrs)	Site	SY 38	NF 68	NF 160	NF 200	Vim	GFP
Medulloblastoma	M	7	cerebellum	⊕	−	−	−	+	−
$n=6$	M	9	IVth ventricle	⊕	−	−	−	−	−
	M	26	cerebellum	+	+	+	+	−	+[a]
	M	9	IVth ventricle	+	−	−	−	+	+[a]
	M	1	cerebellum	⊕	−	−	−	+	+[a]
	M	11	cerebellum	⊕	−	−	−	+	−
Neuroblastoma	M	2	intraspinal	+	+	+	+	+[b]	−
$n=3$	M	1	adrenal medulla[h]	+	+	+	−	+[b]	−
	M	1	liver metastasis[h]	+	+	+	+	+[b]	−
Ganglioneuroma $n=1$	M	26	abdomen	+[c]	+[d]	+[d]	+[d]	+[e]	+[f]
Glio-neuronal hamartoma $n=1$	M	1	cerebral hemisphere	+[c]	+[d]	+[d]	+[d]	+[g]	+[g]

[a] enclosed normal and reactive astrocytes within the tumour
[b] single or clusters of neuroblastoma cells
[c] punctate pattern delineating neurons and synapses
[d] neurofilament immunoreactivity within neurons and axons
[e] satellite and Schwann cells
[f] many, but not all Schwann cells and satellite cells in ganglioneuroma
[g] vimentin- and GFP-co-expression in the glial compartment of the tumour
[h] identical case: primary tumour and metastasis

⊕ denotes focal synaptophysin immunoreactivity in 4/6 medulloblastomas
Abbreviations: yrs = years; SY = synaptophysin; NF-L, NF-M, NF-H = neurofilament polypeptides; Vim = vimentin; GFP = glial fibrillary protein

mas, in comparison with IF proteins, particularly neurofilament proteins, in order to evaluate its potential as a differentiation marker for this group of tumours.

Material and methods

Biopsy specimens of tumours were obtained immediately after surgical removal from the Department of Neurosurgery of the Surgical Center, University of Heidelberg. The series examined in this study was obtained from males (ages range from 1 to 26 years; see Table 1) and comprised 6 medulloblastomas, 3 neuroblastomas (including one case in which the primary tumour as well as a liver metastasis was available), 1 ganglioneuroma and 1 glioneuronal hamartoma. Biopsies were immediately frozen in isopentane precooled with liquid nitrogen to about −130° C and were usually stored at −80° C until use.

Immunofluorescence microscopy was performed on 4–6 µm cryostat sections (Frigocut 2 800 E, Fa. Jung, Nußloch, FRG), fixed with acetone at −20° C for 10 min as previously described (Franke et al. 1978, 1979). Secondary antibodies raised in goats were coupled with either tetramethyl-rhodamine-isothiocyanate (TRITC) or Texas Red sulfonyl chloride (TR), respectively (Dianova, Hamburg, FRG). Fluorescence was visualized with a Zeiss fluorescence photomicroscope (photomicroscope III, Carl Zeiss, Oberkochen, FRG). The following primary monoclonal antibodies were used: (a) murine monoclonal antibody (IgG) against synaptophysin (SY 38; Boehringer, Mannheim, FRG) 5 µg/ml; (b) murine monoclonal antibodies (IgG) against neurofilament proteins (anti-NF-L, anti-NF-M, anti-NF-H; Boehringer, Mannheim, FRG) 4–20 µg/ml; (c) murine monoclonal antibodies (IgG) against vimentin (Boehringer, Mannheim, FRG) 4 µg/ml; (d) murine monoclonal antibody (IgG) against glial fibrillary protein (GFP; Boehringer, Mannheim, FRG) 2–4 µg/ml or anti-GFP 12.24 (PROGEN, Heidelberg, FRG). The specifity of these antibodies was documented by immunocytochemistry and immunoblotting. Controls, performed by omitting the first antibody, were always negative thus indicating the absence of non-specific binding of the secondary antibodies.

For electrophoretic and immunoblotting studies tissue samples were homogenized in 2–3 volumes of ice-cold buffer (140 mM NaCl, 2–6 mM KCl, 6.4 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 0.1 mM dithioerythritol, 0.005% phenylmethylsulfonyl fluoride, pH 7.4) by 10 strokes with a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 5000 × g for 10 min, and the pellet was discarded. An aliquot of this "low-speed supernatant" was used at different protein concentrations for gel electrophoresis. Another aliquot was ultracentrifuged at 130000 × g for 120 min, and the resulting sediment was designated as the "high speed pellet". SDS-polyacrylamide gel electrophoresis and immunoblotting were performed as previously described (Wiedenmann and Franke 1985).

Results

As demonstrated by immunofluorescence microscopy synaptophysin immunoreactivity was found in all medulloblastomas tested (6/6). The typically granular cytoplasmic pattern was either strong and uniform (Fig. 1a) or more sparse and focal (4/6; Fig. 1c). These results indicate tumour-to-tumour differences in the frequency of synaptophysin-posi-

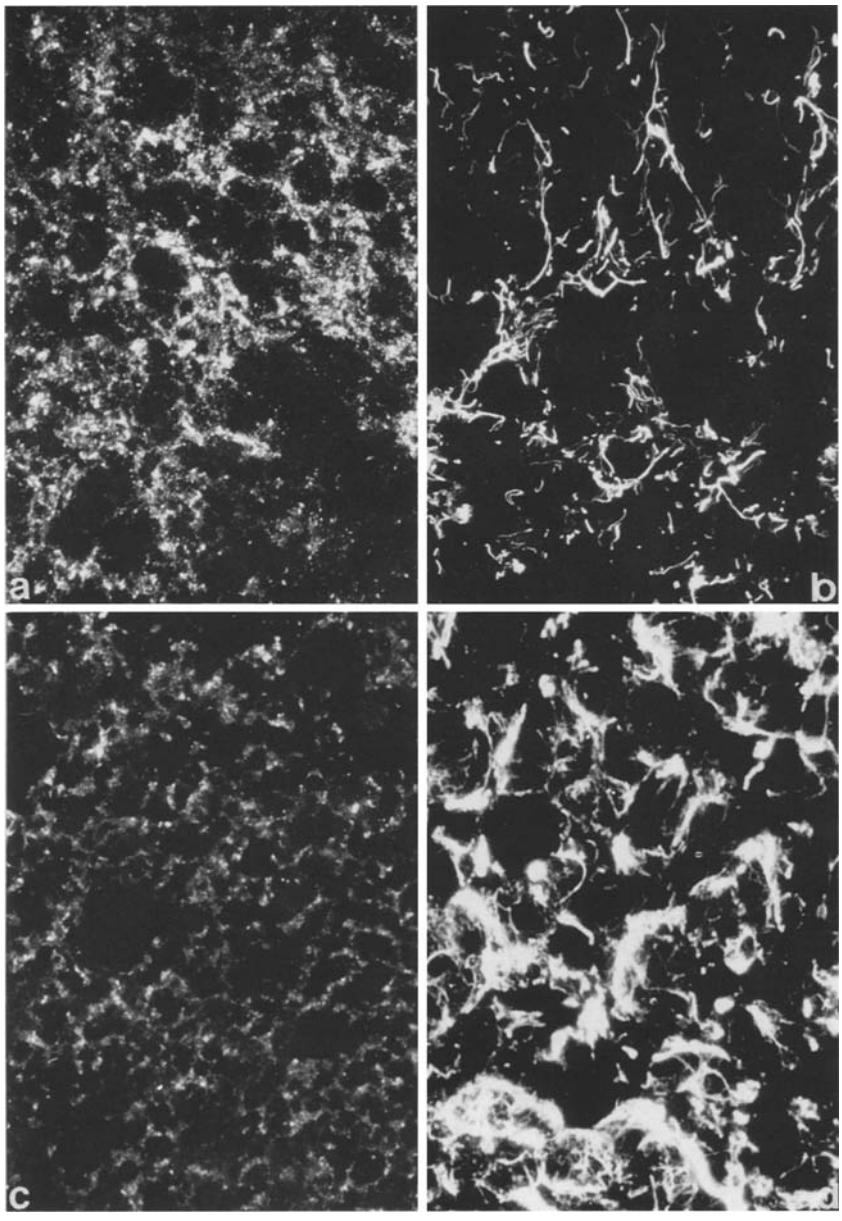

Fig. 1 a–d. Immunofluorescence microscopy of different types of *medulloblastomas*. **a, b.** Neurofilament-positive medulloblastoma: **(a)** Synaptophysin immunoreactivity showing punctate patterns; **(b)** NF-L immunoreactivity. **c, d.** Vimentin-positive medulloblastoma: **(c)** Synaptophysin immunoreactivity with small fluorescent spots; **(d)** immunoreactivity for vimentin. × 500

tive structures, i.e. neurosecretory vesicles, as well as cell-to-cell heterogeneity in the amounts of synaptophysin expressed. The full complement of the neurofilament polypeptide triplet was only found in one case (1/6). In this tumour, the reaction was rather weak and restricted to a small cytoplasmic rim of the medulloblastoma cells, whereas it was relatively strong in the slender cellular processes (Fig. 1b). In four medulloblastomas, vimentin was the only IF protein detected (Fig. 1d). The absence of immunoreactive neurofilaments in five medulloblastomas was in marked contrast to the heterogeneous, but consistent synaptophysin immunoreactivity (Fig. 1c). One medulloblastoma was negative for all IF proteins, but, again, synaptophysin could be demonstrated in this case. The expression of GFP co-localized with vimentin in three cases was mostly associated with reactive astrocytes entrapped in the tumour.

Synaptophysin immunoreactivity in neuroblastomas (3/3) was consistent and usually strong (Fig. 2c). In contrast to medulloblastomas, all neuroblastomas examined were NF-positive (Fig. 2a). Additionally, single or clusters of tumour cells were vimentin-positive (Fig. 2b). GFP was always negative (Table 1).

In the *ganglioneuroma* studied sparse, small synaptophysin-immunofluorescent spots were seen

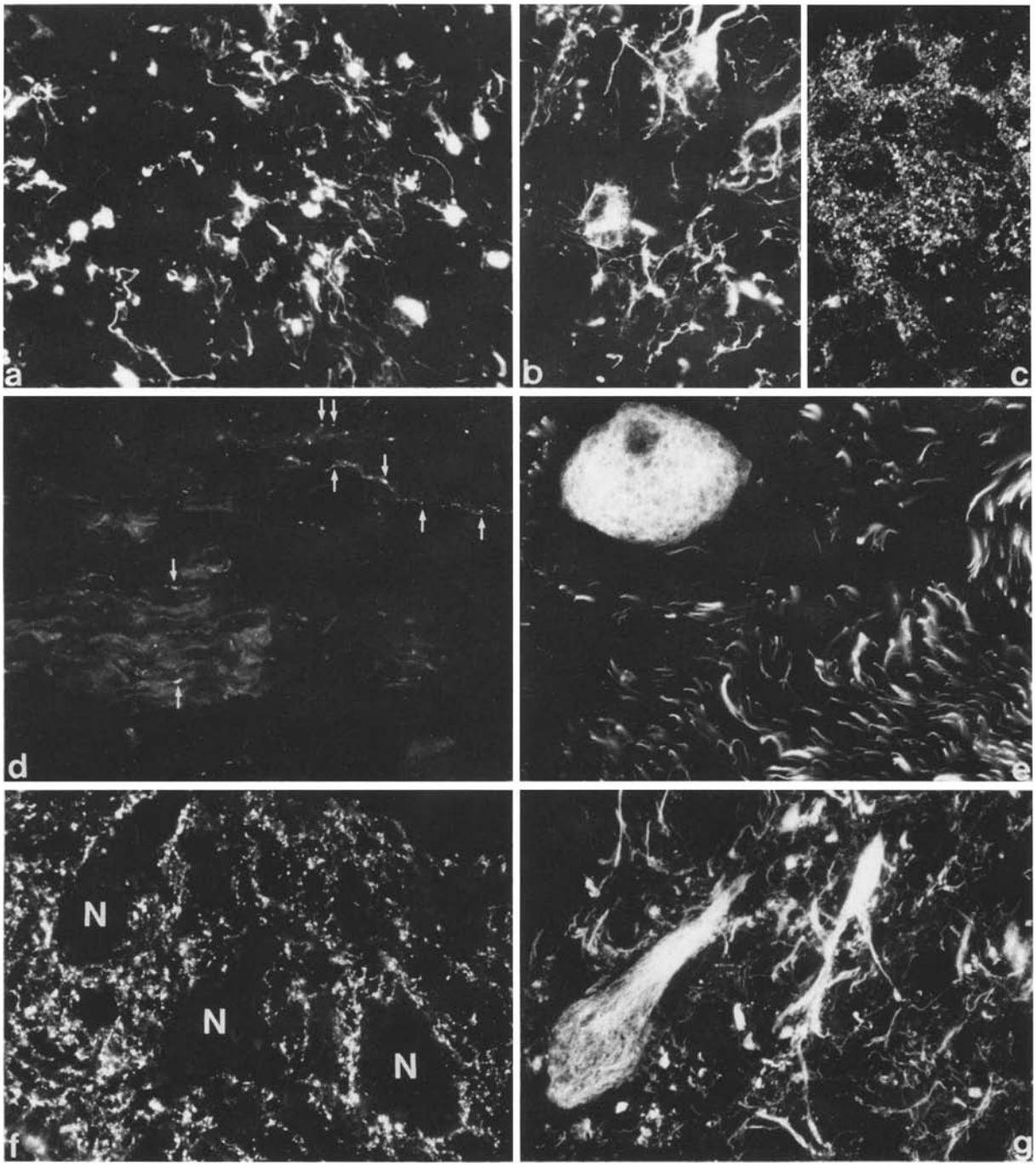

Fig. 2a–g. Immunofluorescence microscopy of other neuronal tumours. **a–c**. *Neuroblastoma*, liver metastasis: **(a)** Neurofilament (NF-L) immunoreactivity; **(b)** distribution of vimentin-positive cells; **(c)** punctate pattern of synaptophysin immunoreactivity. **d, e.** *Ganglioneuroma:* **(d)** Sparse fluorescent dots denote some synaptophysin immunoreactivity in the neuroma compartment of the tumour (*arrows*); **(e)** neurofilament (NF-L) immunoreactivity in the cytoplasm of a ganglion cell (*upper left quadrant*) and strong reaction in the axons. **f, g.** *Glio-neuronal hamartoma:* **(f)** Strong synaptophysin immunoreactivity around the ganglion cell bodies (N, neuron); **(g)** neurofilament (NF-L) immunoreactivity within the ganglion cell bodies and surrounding axonal and dendritic processes. × 500

around large ganglion-cells (data not shown; cf. Gould et al. 1986) and also in scattered patterns in the neuroma portion of the tumour (Fig. 2d). Neurofilament proteins were detected in cell bodies of neurons and their axons in the neuroma compartment (Fig. 2e). In contrast, Schwann cells were negative for synaptophysin and neurofilament proteins, but were positive for vimentin as were the

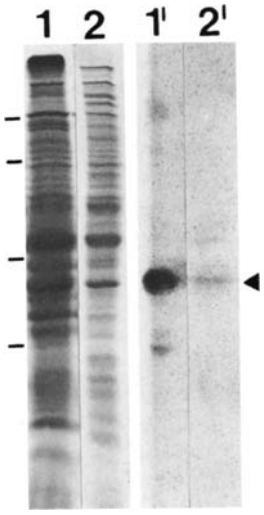

Fig. 3. Identification of synaptophysin in a medulloblastoma by one-dimensional gel electrophoresis and immunoblot analysis. Lanes 1 and 1′: human brain "low speed supernatant"; lanes 2 and 2′: "high speed pellet" of human medulloblastoma. Polypeptides separated by SDS-PAGE were either stained with Coomassie blue (lanes 1 and 2) or transferred to nitrocellulose paper sheets and reacted with antibody SY 38 against synaptophysin, followed by autoradiographic visualization using ^{125}I-labeled secondary antibodies (lanes 1′ and 2′). The major immunoreactive band migrates with an apparent molecular weight of about M_r 38000. Positions of molecular weight markers, run in parallel, are denoted on the left margin as horizontal bars (from top to bottom: M_r 180000, 96000, 50000, and 33000, respectively; for details, see Wiedenmann and Franke 1985). The arrowhead on the right margin side indicates the position of synaptophysin

satellite cells. It is worth emphasizing that vimentin and GFP were co-expressed in satellite cells and in many, although not all Schwann cells (Table 1).

Included in this study was a case of a rare tumour, probably originating from a developmental disorder, which was mainly composed of typical and atypical neuronal and glial cells. Hence, it was designated "glio-neuronal hamartoma". Synaptophysin immunoreactivity was very strong in this lesion and occurred around the numerous axons and mature or atypical ganglion cells (Fig. 2f). Immunoreactivity in the latter case was probably related to the distribution of dendrites. Brilliant neurofilament immunoreactivity was noted, apparently due to the high content of axons dendrites and to the bundles of neurofilaments in the large cell bodies of the ganglion cells (Fig. 2g). The various immunohistological results are summarized in Table 1.

The presence of synaptophysin in medulloblastomas was also demonstrated by the immunoblot technique. Figure 3 presents a case in which the immunoreactive polypeptide of M_r 38000, as typical for synaptophysin was found (Wiedenmann and Franke 1985; Wiedenmann et al. 1986).

Discussion

Synaptophysin, a new marker for neuronal and neuroendocrine cells as well as for tumours derived from such cells (Wiedenmann and Franke 1985; Gould et al. 1986; Wiedenmann et al. 1986; cf. Jahn et al. 1985) was consistently found in all medulloblastomas examined in this study. These results confirm and extend previous data (Gould et al. 1986; Wiedenmann et al. 1986) and document the usefulness of immunocytochemistry for this marker in the diagnosis of medulloblastomas. Therefore, this vesicle membrane protein can be regarded as a reliable marker for this group of tumours. The consistent demonstration of synaptophysin also provides a convincing argument to classify medulloblastomas as neuronal tumours. Moreover, the presence of synaptophysin supports the concept of the inclusion of medulloblastomas amongst the primitive neuroectodermal tumours (PNET; Hart and Earle 1973; Rorke 1983; for a recent critical review, see Rubinstein 1985).

In contrast, examination of IF protein distribution in various medulloblastomas yielded variable results. In 4 of the 6 tumours examined, vimentin was the exclusive IF protein detected. The failure to demonstrate vimentin in two cases might be due to the presence of very low conentrations of this protein. Neurofilament proteins, on the other hand, were demonstrated only in one medulloblastoma. On the basis of differences in the immunoreactivity of IF proteins, we propose that at least two distinct subtypes of medulloblastomas exist, i.e. exclusively vimentin-positive ones and those that contain neurofilaments. Of course, the occurrence of tumours – or tumour cells – that contain both vimentin and neurofilament proteins cannot be excluded at present. Heterogeneity in IF protein distribution in medulloblastomas is also indicated in the literature (Trojanowski and Lee 1983; Roessmann et al. 1983; Tremblay et al. 1985). In classical medulloblastomas, single cells or small groups of tumour cells sometimes show GFP immunoreactivity. However, cells expressing considerable amounts of GFP within the tumour appear to be entrapped or reactive astrocytes (see also Herpers and Budka 1985).

The consistent finding of synaptophysin in *neuroblastomas* (see also Gould et al. 1986; Wiedenmann et al. 1986) emphasizes the value of this marker in the diagnosis of this tumour, notably as a criterion allowing a differentiation from other

malignant, small, round-cell tumours which present severe diagnostic problems (Enzinger and Weiss 1983). This is all the more important as reports on neurofilament immunoreactivity in neuroblastomas are controversial (Osborn et al. 1982; Osborn and Weber 1983; Carlei et al. 1984; Trojanowski et al. 1984; Mukai et al. 1986; Osborn et al. 1986). Whether neurofilament polypeptides are immunomorphologically demonstratable or not apparently depends in part on tissue preservation and fixation. Recently, Osborn et al. (1986) reported that when cryostat sections were used, neurofilament IF could be identified in all neuroblastomas tested ($n=10$). Our results are basically in agreement with this study, except for on case which was negative for the NF-H polypeptide and our observation of clusters of vimentin-positive cells within neuroblastomas, i.e. cells that seemed to coexpress both types of IFs. According to our results and those of Osborn et al. (1986) neuroblastomas cannot be subdivided on the basis of their IF protein patterns. In agreement with Trojanowski et al. (1984) and Osborn et al. (1986), we were unable to detect glial filaments in the tumour cells. Again, the possibility of some GFP-positive cells in rare cases of neuroblastoma cannot be excluded on the basis of our limited number of cases.

Our demonstration of a complex IF pattern in a glio-neuronal hamartoma is not unexpected. Neurofilament protein expression has been demonstrated in neuronal cell bodies, dendrites and axons. In the glial compartment of the tumour, colocalization of GFP and vimentin has been seen. This cell type mixture, which is reflected by the heterogeneity of IF expression, is in agreement with the established concept of origins of hamartomas.

The finding that the ganglion cells and axons of ganglioneuroma are positive for neurofilaments is consistent with data of other authors (Osborn et al. 1982; Trojanowski and Lee 1983; Mukai et al. 1986). In contrast, the Schwann and satellite cells consistently show vimentin IF as well as, in a part of both cell populations, strong GFP immunoreactivity. This finding is in agreement with several reports of GFP expression in non-glial cells and tumours outside the central nervous system, especially neurinomas and schwannomas (Memoli et al. 1984; Achtstätter et al. 1986; Gould et al. 1986) and in myeopithelial cells of normal and neoplastic salivary glands (Nakazato et al. 1982, 1985; Achtstätter et al. 1986). As in medulloblastomas and neuroblastomas, synaptophysin immunoreactivity is consistently found in the glio-neuronal hamartoma and the ganglioneuroma, again emphasizing the value of synaptophysin as a broad neuronal marker.

In conclusion, our data prove that synaptophysin is a marker for differentiated and undifferentiated neuronal tumours, including primitive neuroectodermal tumours such as medulloblastomas and neuroblastomas. The consistent expression of synaptophysin in at least a considerable part of the medulloblastoma cell population is of great importance in brain tumour diagnosis. This is especially true as the IF protein in tumours of this group do not present a consistent pattern or can even be totally missed by immunocytochemistry. Therefore, synaptophysin is recommended as the best available broad-range marker for medulloblastomas. Finally, synaptophysin in antibody SY 38 is a valuable adjunct to the panel of neural markers relevant to the immunocytochemical characterization and differential diagnosis of human intracranial neoplasm (Schwechheimer 1986).

Acknowledgments. We thank S. Kunze, Hedi Steiner, and J. Peixoto (Neurosurgical Department, University of Heidelberg), and C. Herfarth (Surgical Center, University of Heidelberg) for providing the biopsy specimens. We are indebted to M. Kaiser, A. Sommer, and C. Wahl for expert technical assistance and to I. Alffermann for excellent typing of the manuscript. We are grateful to F. Longo (Institut für Zell- und Tumorbiologie, Abteilung Membranbiologie und Biochemie, Deutsches Krebsforschungszentrum Heidelberg) for critically reading the manuscript. This study has been supported by a grant of the Deutsche Forschungsgemeinschaft Bonn-Bad Godesberg, FRG, and a special grant of the Tumorzentrum Heidelberg/Mannheim ("Neuro-Onkologie") to K.S.

References

Achtstätter T, Moll R, Anderson A, Kuhn C, Pilz S, Schwechheimer K, Franke WW (1986) Expression of glial filament protein (GFP) in nerve sheaths and non-neural cells re-examined using monoclonal antibodies, with special emphasis on the co-expression of GFP and cytokeratins in epithelial cells of human salivary gland and pleomorphic adenomas. Differentiation 31:206–227

Carlei F, Polak JM, Ceccamea A, Marangos PJ, Dahl D, Cocchia D, Michetti F, Lezoche E, Speranza V (1984) Neuronal and glial markers in tumours of neuroblastic origin. Virchows Arch (Pathol Anat) 404:313–324

Dahl D, Bignami A (1977) Preparation of antisera to neurofilament protein from chicken brain and human sciatic nerve. J Comp Neurol 176:645–657

Enzinger FM, Weiss SW (1983) Soft tissue tumors. The C.V. Mosby Company, St. Louis-Toronto-London

Franke WW, Schmid E, Osborn M, Weber K (1978) Different intermediate-sized filaments distinguished by immunofluorescence microscopy. Proc Natl Acad Sci USA 75:5034–5038

Franke WW, Schmid E, Kartenbeck J, Mayer D, Hacker HJ, Bannasch M, Osborn M, Weber K, Denk H, Wanson JC, Drochmans P (1979) Characterization of the intermediate-sized filaments in liver cells by immunofluorescence and electronmicroscopy. Biol Cell 34:99–110

Gould VE, Moll R, Moll J, Lee J, Schwechheimer K, Franke WW (1986) The intermediate filament complement of the spectrum of nerve sheath neoplasms. Lab Invest: 55:463–474

Gould VE, Wiedenmann B, Lee J, Schechheimer K, Dockhorn-Dworniczak B, Radosevich JA, Moll R, Franke WW (1986) Synaptophysin expression in neuroendocrine neoplasms as determined by immunocytochemistry. Am J Pathol (in press)

Hart MN, Earle KM (1973) Primitive neuroectodermal tumors of the brain in children. Cancer 32:890–897

Herpers MJHM, Budka H (1985) Primitive neuroectodermal tumors including the medulloblastoma: glial differentiation signaled by immunoreactivity for GFAP is restricted to the pure desmoplastic medulloblastoma ("arachnoidal sarcoma of the cerebellum"). Clin Neuropathol 4:12–18

Hoffman PN, Lasek RG (1975) The slow component of axonal transport: identification of majors structural polypeptides of the axon and their generality among mammalian neurons. J Cell Biol 66:351–366

Jahn B, Schiebler W, Ouimet C, Greengard P (1985) A 38000 dalton membrane protein (p 38) present in synaptic vesicles. Proc Natl Acad Sci USA 82:41-37-4141

Lee VM-Y, Wu HL, Schlaepfer WW (1972) Monoclonal antibodies recognize individual neurofilament triplet proteins. Proc Natl Acad Sci USA 79:6089–6092

Lehto VP, Virtanen J, Miettinen M, Dahl D, Kahri A (1983) Neurofilaments in adrenal and extraadrenal pheochromocytoma. Demonstration using immunofluorescence microscopy. Arch Pathol Lab Med 107:492–494

Liem RKH, Yen S-H, Salomon GD, Shelanski ML (1978) Intermediate filaments in nervous tissues. J Cell Biol 79:637–645

Memoli VA, Brown EF, Gould VE (1984) Glial fibrillary acidic protein (GFP) immunoreactivity in peripheral nerve sheath tumours. Ultrastruct Pathol 7:269–275

Miettinen M, Lehto VP, Virtanen J (1985) Immunofluorescence microscopic evaluation of the intermediate filament expression of the adrenal cortex and medulla and their tumors. Am J Pathol 118:360–366

Mukai M, Rotikata C, Iri H, Morikawa Y, Shimizu K, Shimoda T, Nukina N, Ihara Y, Kageyama K (1986) Expression of neurofilament triplet proteins in human neural tumors. An immunohistochemical study of paraganglioma, ganglioneuroma, ganglioneuroblastoma, and neuroblastoma. Am J Pathol 122:28–35

Nakazato Y, Ishizeki J, Takahashi K, Yamaguchi H, Kamel T, Mori T (1982) Localization of S-100 protein and glial fibrillary acidic protein-related antigen in pleomorphic adenoma of the salivary glands. Lab Invest 46:621–626

Nakazato Y, Ishida Y, Takahashi K, Suzuki K (1985) Immunohistochemical distribution of S-100 protein and glial fibrillary acidic protein in normal and neoplastic salivary glands. Virchows Arch (Pathol Anat) 405:299–310

Osborn M, Weber K (1983) Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology. Lab Invest 48:372–394

Osborn M, Altmannsberger M, Shaw G, Schauer A, Weber K (1982) Various sympathetic derived human tumors differ in neurofilament expression. Use in diagnosis of neuroblastoma, ganglioneuroblastoma and pheochromocytoma. Virchows Arch (Cell Pathol) 40:141–156

Osborn M, Dirk T, Käser H, Weber K, Altmannsberger M (1986) Immunohistochemical localization of neurofilaments and neuron-specific enolase in 29 cases of neuroblastoma. Am J Pathol 122:433–442

Rehm H, Wiedenmann B, Betz H (1986) Molecular characterization of synaptophysin, a major calcium-binding protein of the synaptic vesicle membrane. EMBO J 5:535–541

Roessmann K, Velasco ME, Gambetti P, Autilio-Gambetti L (1983) Neuronal and astrocytic differentiation in human neuroepithelial neoplasms. An immunohistochemical study. J Neuropathol Exp Neurol 42:113–121

Rorke LB (1983) The cerebellar medulloblastoma and its relationship to primitive neuroectodermal tumors. J Neuropathol Exp Neurol 41:1–15

Rubinstein LJ (1985) Embryonal central neuroepithelial tumors and their differentiating potential. A cytogenetic view of a complex neurooncological problem. J Neurosurg 62:795–805

Schmidt D, Harms D, Burdach S (1985) Malignant peripheral neuroectodermal tumours of childhood and adolescence. Virchows Arch (Pathol Anat) 406:351–365

Schwechheimer K (1986) Nervale Tumormarker. Verh D Ges Pathol: 70:82–103

Tremblay GF, Lee VM-Y, Trojanowski JQ (1985) Expression of vimentin, glial filament, and neurofilament in primitive childhood brain tumors. A comparative immunoblot and immunoperoxidase study. Acta Neuropathol (Berl) 68:239–244

Trojanowski JQ, Lee VM-Y (1983) Anti-neurofilament monoclonal antibodies: reagent for the evaluation of human neoplasms. Acta Neuropathol (Berl) 59:155–158

Trojanowski JQ, Lee VM-Y (1985) Expression of neurofilament antigens by normal and neoplastic human adrenal chromaffin cells. N Engl J Med 313:101–104

Trojanowski JQ, Lee VM-Y, Schlaepfer WW (1984) An immunohistochemical study of human central and peripheral nervous system tumors, using monoclonal antibodies against neurofilaments and glial filaments. Hum Pathol 15:248–257

Weber K, Shaw G, Osborn M, Debus E, Geisler N (1983) Neurofilaments, a subclass of intermediate filametns: structure and expression. Cold Spring Harbor Symp Quant Biol, Vol XLVIII, 717–729

Wiedenmann B, Franke WW (1985) Identification and localization of synaptophysin, an integral membrane glycoprotein of M_r 38000 characteristic of presynaptic vesicles. Cell 41:1017–1028

Wiedenmann B, Franke WW, Kuhn C, Moll R, Gould VE (1986) Synapatophysin: a marker protein for neuroendocrine cells and neoplasms. Proc Natl Acad Sci USA 83:3500–3504

Accepted December 23, 1986