Selective Distribution of the 57 kDa Neural Intermediate Filament Protein in the Rat CNS

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In order to determine the CNS distribution of the 57 kDa neural intermediate filament protein (NIFP), a specific antiserum was used in immunofluorescence studies on serial sections taken from each spinal cord level and at 300-μm intervals through the rat brain. The labeling pattern was recorded onto camera lucida tracings of adjacent sections stained with hematoxylin and eosin/luxol fast blue. Three major immunolocalization patterns were revealed. (1) Both large-caliber and fine-caliber axons of optic and all brain stem cranial nerves and their tracts except for the auditory portion of the VIIIth nerve. (2) An extensive array of fine-caliber fibers in the cerebellar white matter and brain stem with region-specific variation in pattern and density. Prominent among the regions with dense arrays of labeled processes were selective cerebellar afferent systems, particularly olivocerebellar fibers, visual afferents arising in the retina, and selective regions of reticular formation. In contrast to the brain stem, the telencephalon contained rare label. (3) Filamentous labeling of neuronal cytokarya in sensory ganglia and a small number of CNS nuclear groups, including all autonomic nuclei and the cholinergic pontine nuclei. On sections of selected CNS regions, distribution patterns of the 57 kDa NIFP were correlated to results obtained by in situ hybridization of a cDNA probe for the 57 kDa NIFP. The data suggest the possible existence of neuronal IF proteins specifically related to selective anatomic and/or neurotransmitter systems.

Intermediate filaments (IF) are prominent and ubiquitous components of eukaryotic cells. These filaments are composed of one or more members of a family of fibrous proteins that have a conserved α-helical core region and variable end domains. Variation of the size and amino acid sequences within the α-helical core domain indicates a division of the IF family into 5 types (Steinert and Roop, 1988). Type IV IF are composed of a triplet of proteins that are restricted to the nervous system and thought to be present in most CNS neuronal cell bodies and axons (Shaw et al., 1981; Trojanowski et al., 1986). Al-

though polyacrylamide gel electrophoresis of neuronal IF preparations from various areas of the nervous system has consistently illustrated the triplet proteins (200, 150, 68 kDa) to be major components, other bands, often in the 55–60 kDa range, have been noted (Liem et al., 1978; Schlaepfer and Freeman, 1978; Czosnek and Soifer, 1980; Brown et al., 1981; Yen and Fields, 1981; Zackroff et al., 1982).

An IF protein of 56–57 kDa has been identified as one of the major IF polypeptides present in both the neuron-like cell line PC12 and in neuroblastoma cell lines (Portier et al., 1982, 1984a, b; Parysek and Goldman, 1987). Immunolocalization studies have shown that the 56 kDa IF protein, peripherin (Escurat et al., 1988), and the 57 kDa neural intermediate filament protein (NIFP; Parysek and Goldman, 1988) are localized exclusively in neuronal elements of rat tissues. In situ hybridization showed that the 57 kDa NIFP mRNA was detectable in areas of the nervous system that labeled with 57 kDa NIFP antiserum (Parysek et al., 1988). In another laboratory, a cDNA clone (clone 73) representing a message that increased markedly during nerve growth factor stimulated PC12 cell differentiation (Leonard et al., 1988) was found to encode an IF protein. In situ hybridization using RNA transcripts of portions of clone 73 indicated that message is present in peripheral nervous system (PNS), sensory ganglia, and a subset of spinal cord and brain stem nuclei (Leonard et al., 1988). A portion of the sequence reported for clone 73 is identical to that of the cDNA for the 57 kDa NIFP (Parysek et al., 1988). The predicted amino acid sequence of the 57 kDa NIFP cDNA indicates that it is a type III IF protein as are vimentin, desmin, and GFAP. Thus, these recent studies (Leonard et al., 1988; Parysek and Goldman, 1988; Parysek et al., 1988) indicate that a type III IF protein is present in mature neurons and, furthermore, that it has a restricted distribution in comparison to the type IV neurofilament triplet (NFT) proteins. In this study, we have examined the distribution of the 57 kDa NIFP in the rat CNS using fluorescence–immunolocalization methods. This work complements studies on the distribution of the mRNA for this protein. An analysis of the complete, unique anatomic distribution of the 57 kDa NIFP in both neuronal cell bodies and axonal processes of the CNS has illuminated systems-specific labeling patterns.

Materials and Methods

Tissue preparation. The brains of albino rats were removed without fixation immediately following sacrifice of the animals and were sliced in the coronal plane into 3-mm blocks utilizing a measured rat brain cradle (Zivic-Miller). Blocks of cervical, thoracic, lumbar, and sacral cord were taken in the transverse plane. All blocks were embedded in 7.0% gelatin, oriented in flat cryomolds (Tissue-Tek), and frozen in dry

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ice-cooled ethanol. Ten serial 4–5 μm sections (forming a serial set) were taken from each brain block every 60 sections until the block was exhausted. One set of serial sections was taken from each spinal cord block. Sections were air-dried and stored in desiccated boxes at −40°C. The first slide of each series was stained with hematoxylin and eosin/luxol fast blue (H&E/LFB) (Luna, 1968). Adjacent slides were immunolabeled and other slides in the same series were processed for *in situ* hybridization.

Immunofluorescence. 57 kDa NIFP antiserum production was initiated by injection of an emulsified mixture of Freund's adjuvant and approximatley 1 mg of 57 kDa NIFP in a polyacrylamide gel slice. After 3 weeks, a booster injection of half of the original amount of antigen without adjuvant was given. Bleed one was drawn 7 d later; serum from only one (199) of the 2 rabbits produced a reaction to the antigen as assayed by immunoblot. Serum from bleed 2 of the antibody-producing rabbit, diluted 1:50 in Tris-saline, pH 8.2, 0.1% BSA added, was used for this study.

Immunolabeling with 57 kDa NIFP antiserum or the monoclonal antibody (15G1) that cross-reacts with the 200 kDa and 150 kDa NFT proteins (Parysek and Goldman, 1987) was carried out as described previously (Parysek and Goldman, 1988). One slide from alternate or, in some cases, every serial set was incubated sequentially with normal goat serum, 57 kDa NIFP antiserum, and fluoresceinated goat anti-rabbit IgG (Kirkegaard and Perry). Sections from the midbrain, pons, and medulla were double-labeled with 57 kDa NIFP antiserum and 15G1 followed by a mixture of fluorescein-labeled goat anti-rabbit IgG and rhodamine-labeled goat anti-mouse IgG. The fluorescein- and rhodamine-labeled secondary antibodies showed no cross-reaction with mouse and rabbit IgG, respectively.

Processing of CNS sections with preimmune serum from rabbit 199 showed no labeling of structures that contained the 57 kDa NIFP. Other control slides in which serum was omitted from the labeling procedure showed identical results.

Immunoblot analyses indicate that the reaction of 15G1 in whole brain or whole spinal cord tissue is restricted to the 150 kDa and 200 kDa neural IF proteins and does not include reaction with any other NIFP or other phosphorylated proteins.

In situ hybridization. In situ hybridization was carried out on brain stem sections at the level of the V, VII, and XII cranial nerve nuclei. The procedure utilized ^{35}S-labeled RNA transcripts of a cDNA that encodes three-quarters of coil 2 of the rod domain and the entire carboxyl terminus of the 57 kDa NIFP. Characterization of the cDNA (designated clone 199E) and *in situ* labeling methods were described previously (Parysek et al., 1988).

Section analysis. The stereotaxic coordinates of each H&E/LFB stained section were correlated to the plates of a stereotaxic rat brain atlas (Paxinos and Watson, 1986), and specific anatomic regions were identifed by comparison to their positions in the atlas. Sections were examined on a Zeiss Axioskop, and the labeling patterns were recorded onto camera lucida tracings of the adjacent H&E/LFB-stained sections. The presence of vessels, leptomeninges, and neurons, identified by phase or dark-field optics, allowed for accurate placement of labeled structures onto the tracings.

Results

Immunolabeled brain and spinal cord sections revealed the presence of 57 kDa NIFP both in the cytokarya of select neuronal groups and in neuronal processes of varying sizes (Fig. 1).

The cytokarya of most neurons in the CNS were 57 kDa NIFP antiserum-negative. In a few specific nuclei, however, clusters of neurons having a bright fibrillar pattern of fluorescence were consistently present in all relevant coronal sections (Table 1). These nuclei include the caudal magnocellular hypothalamic group; the autonomic motor nuclei, i.e., the intermediolateral nucleus of the spinal cord, the dorsal vagus, ambiguous, and Edinger-Westphal nuclei, and a group of lateral pontine neurons possibly representing salivatory neurons; the β-subnucleus of the inferior olive; and the paragenual nucleus. In addition, a subset of cells in sensory ganglia, including the dorsal root ganglion, mesencephalic ganglion, and trigeminal ganglion, contained the 57 kDa NIFP; retinal ganglion cells also were labeled.

No label was identified in olfactory receptor neurons within olfactory epithelium. Fibrillar labeling was also present in numerous neurons scattered throughout the region of the peripeduncular tegmental nucleus (PPTg) and the lateral dorsal tegmental nucleus (LDTg) in the upper pons. In contrast, rare labeled neurons were present in layers III and V of the cerebral cortex without apparent regional predilection (Fig. 2).

Immunoreactive neurons were identified rarely among the motor neurons of the V and VII cranial nerve nuclei (less than 1/section) and somewhat more frequently among motor neurons in the oculomotor nucleus and the ventral horn of the spinal cord (1–3/section). The axons of the motor outflow were labeled, and the motor nuclei often contained short segments of these axons apparently arising from unlabeled cytokarya. In order to determine whether mRNA for the 57 kDa NIFP was present in these unlabeled cytokarya, *in situ* hybridization was performed. ^{35}S-labeled antisense RNA transcripts of the cDNA labeled the cytokarya of the V, VII, and XII cranial nerve nuclei (Fig. 3), the dorsal vagus, and the nucleus ambiguous, as well as several inferior olive (IO) subnuclei. In the sections examined, *in situ* hybridization revealed no label in cytokarya in the cerebellum or in other nuclear regions of the brain stem.

Immunolabeling also revealed a unique restricted distribution of the 57 kDA NIFP in CNS axons (Table 2). Except for the auditory and olfactory nerves, all spinal nerve roots and cranial nerves as well as their intraparenchymal tracts were labeled. The intensity of axonal labeling was uniform for all axons of motor nerves (Fig. 4A) as well as sensory components of VII, IX, and X. In contrast, somatosensory nerves and tracts (the dorsal roots and columns and the 5th cranial nerve and tracts) and the optic nerve and tract contained at least 2 populations of axons of small and large caliber with different labeling characteristics (Fig. 4B). In these nerves and tracts, small-caliber axons labeled intensely. The label of the adjacent large-caliber axons, however, was not as intense as that of the small-caliber axons or large-caliber axons of other cranial nerves. Labeled processes of primary sensory neurons could be detected in otherwise unlabeled regions of the respective second-order nuclei [e.g., optic tract in the lateral geniculate nucleus (LGN)]. Consistent with the lack of labeling in the auditory and olfactory nerves, neither the cochlear nucleus (auditory) nor the olfactory bulb contained any labeled processes.

In addition to spinal roots and cranial nerves and their respective tracts, only a small number of well-defined white matter tracts were labeled with the 57 kDa NIFP antiserum. Most of the long tracts, peduncles, and commissures, identifiable with the NFT antibody 15G1, were unlabeled with 57 kDa NIFP antiserum. In contrast, prominent 57 kDa NIFP labeling was present in certain pathways having a high proportion of very thin axons. Such pathways included the collateral visual pathway to the superior colliculus (Fig. 4C), the olivocerebellar bundles, the inferior cerebellar peduncle (ICP), and the uncinate (Fig. 5). In the cerebellum, the heaviest array of 57 kDa NIFP-containing fibers was present adjacent to the deep cerebellar nuclei and in the vermal white matter. There was a posterior to anterior decrease in the density of labeled fibers in cerebellar white matter. In particular, labeling was very sparse in the subcortical white matter of the anterolateral lobules (Fig. 5). The ventral spinocerebellar tract, medial longitudinal fasciculus, and rubrospinal tract were the only tracts besides cranial nerve components to contain labeled large-caliber axons.

In addition to their presence in well-defined white matter

Table 1. 57 kDa NIFP in gray matter

CNS region	Cytokarya	Labeled processes[a]	
		Thin	Thick
Motor nuclei			
VHSpC, VII, V, III	Few	—	E
XII, VI, IV	—	—	E
Autonomic motor			
IML, DVg, Amb, EW, ? Salivatory	Many	+ +	—
Sensory nuclei			
DRG, GnV, MesV, RetGn	Many	—	E
DH1, LG (afferents to deep layers)	—	+ +	—
DH2-3, Sol, MVst, SpVst	—	+	—
SpV, PrV, Cu, SVst, LVst	—	—	X
Clk, Gr, ECu, thalamus	—	—	—
VC, SO, TzB, LL, IC, MG, SC (gray)	—	—	—
Precerebellar/cerebellar nuclei			
IOb, PGe	Many	+	—
GCL (vermis)	—	+	—
IO (all other), Pn, DCN, GCL (lat)	—	—	—
RN (midline decussation)	—	—	X
Brain stem reticular formation			
Cholinergic			
LDTg, SPTg, PPTg	Many	+ +	—
Catecholaminergic			
RVL/C1, C3, A1, A4, A5, A7, LC	—	—	—
Medulla and pons			
MdV, LPGi, PnC, MnR	—	+ +	—
LRt, ROb, RMg, RPn, VLTg, PnO, PMnRt	—	+	—
RPa, DMTg	—	—	X
PrH, MdD, DTg, Gi, DPGi, PCRt, PB, KF	—	—	—
Diencephalon/midbrain			
TMC, CMC, PCMC	Many	+ +	—
CTgT, DpMe, VTA/SuM, LM	—	+ +	—
region medial to MG, PP, PIL	—	+ +	—
CnF, PAG, RtTh, SNC, LH	—	+	—
PTA	—	+	X
MiTg, SNR, thalamus (except LG, RtTh)	—	—	—
IPN, hypothalamus (all other regions)	—	—	—
Telencephalon			
Cerebral cortex	Few	—	—
ZI, B, lat preoptic area	—	+	—
SA, CPu, GP, VP, Amy, Hp	—	—	—

[a] + = sparse array of labeled processes; + + = dense array of labeled processes; E = labeled axons exiting nucleus of origin; X = thick axons crossing nuclear region. Anatomic abbreviations are defined in the Key to Figure 1.

tracts, thin fibers were abundant in brain stem, particularly in well-delimited portions of the reticular formation (RF), and in those nuclei having neuronal cytoplasmic staining (Table 1). Cerebral cortex, dorsal hypothalamus, anterior and lateral cerebellum, and the parvocellular RF of caudal pons and medulla contained only occasional thin fibers. Regions of the brain notable for the absence of immunodetectable 57 kDa NIFP included the olfactory bulb, basal ganglia, hippocampus, amygdala, habenula, all nuclei of the auditory pathway including the inferior colliculus, and most of thalamus except for reticular thalamus and axons entering the LGN.

Discussion

The results of this study indicate that the 57 kDa NIFP has a prominent CNS presence largely confined to the brain stem and spinal cord. In contrast to the widespread distribution of the NFT proteins, the distribution of the 57 kDA NIFP is restricted to specific neuronal groups and axonal pathways. 57 kDa NIFP antiserum-labeling of CNS axons falls into 3 main categories. These include 1) cranial nerves and their tracts, 2) a specific subset of vestibulocerebellar pathways, and 3) a subset of brain stem RF. The immunofluorescence and in situ hybridization data indicate that the neuronal groups containing 57 kDa NIFP fall into 5 functional categories: 1) Primary sensory ganglia, 2) skeletal and autonomic motor nuclei, 3) brain stem cholinergic nuclei, 4) nuclei thought to modulate vestibular information, the caudal medial accessory nucleus (CMAO) and the paragenual nucleus (efferents to the vestibular labyrinth), and 5) the caudal magnocellular nuclear groups of the hypothalamus. Leonard et al. (1988) additionally identified mRNA for this

KEY

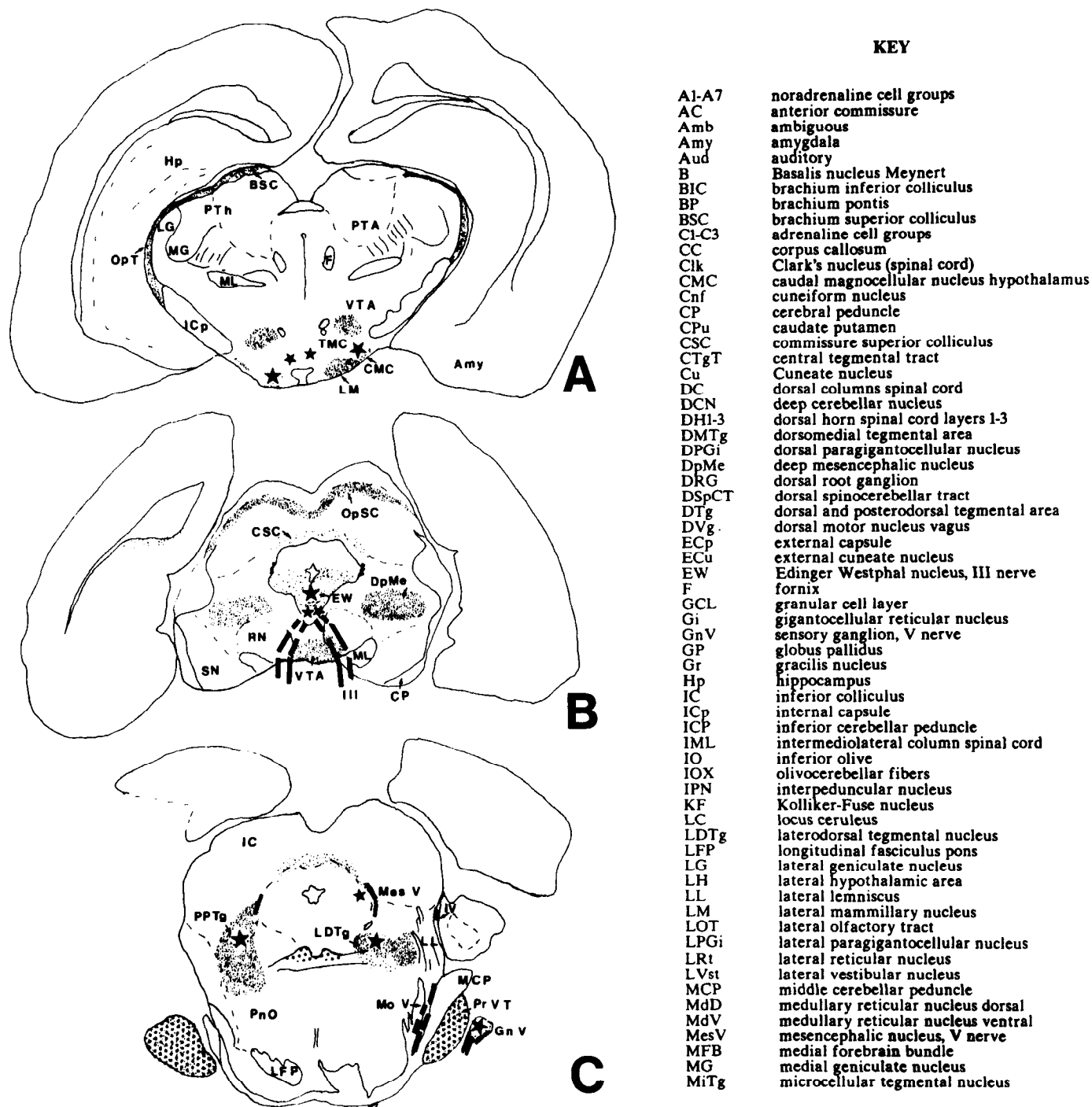|	
A1-A7	noradrenaline cell groups
AC	anterior commissure
Amb	ambiguous
Amy	amygdala
Aud	auditory
B	Basalis nucleus Meynert
BIC	brachium inferior colliculus
BP	brachium pontis
BSC	brachium superior colliculus
C1-C3	adrenaline cell groups
CC	corpus callosum
Clk	Clark's nucleus (spinal cord)
CMC	caudal magnocellular nucleus hypothalamus
Cnf	cuneiform nucleus
CP	cerebral peduncle
CPu	caudate putamen
CSC	commissure superior colliculus
CTgT	central tegmental tract
Cu	Cuneate nucleus
DC	dorsal columns spinal cord
DCN	deep cerebellar nucleus
DH1-3	dorsal horn spinal cord layers 1-3
DMTg	dorsomedial tegmental area
DPGi	dorsal paragigantocellular nucleus
DpMe	deep mesencephalic nucleus
DRG	dorsal root ganglion
DSpCT	dorsal spinocerebellar tract
DTg	dorsal and posterodorsal tegmental area
DVg	dorsal motor nucleus vagus
ECp	external capsule
ECu	external cuneate nucleus
EW	Edinger Westphal nucleus, III nerve
F	fornix
GCL	granular cell layer
Gi	gigantocellular reticular nucleus
GnV	sensory ganglion, V nerve
GP	globus pallidus
Gr	gracilis nucleus
Hp	hippocampus
IC	inferior colliculus
ICp	internal capsule
ICP	inferior cerebellar peduncle
IML	intermediolateral column spinal cord
IO	inferior olive
IOX	olivocerebellar fibers
IPN	interpeduncular nucleus
KF	Kolliker-Fuse nucleus
LC	locus ceruleus
LDTg	laterodorsal tegmental nucleus
LFP	longitudinal fasciculus pons
LG	lateral geniculate nucleus
LH	lateral hypothalamic area
LL	lateral lemniscus
LM	lateral mammillary nucleus
LOT	lateral olfactory tract
LPGi	lateral paragigantocellular nucleus
LRt	lateral reticular nucleus
LVst	lateral vestibular nucleus
MCP	middle cerebellar peduncle
MdD	medullary reticular nucleus dorsal
MdV	medullary reticular nucleus ventral
MesV	mesencephalic nucleus, V nerve
MFB	medial forebrain bundle
MG	medial geniculate nucleus
MiTg	microcellular tegmental nucleus

Figure 1. Overview of the 57 kDa NIFP in the CNS. Labeled areas indicate the distribution of the 57 kDa NIFP by immunofluorescence in the telencephalon–diencephalon (A), midbrain (B), rostral pons (C), caudal pons (D), rostral medulla (E), mid-medulla (F) and spinal cord (G). Sections rostral to A contain label only within optic tract, medial forebrain bundle, and cingulum. Fine continuous lines indicate anatomic and gray–white matter boundaries. Fine dashed lines indicate gray matter divisions, e.g., the granule cell–molecular layer division in the cerebellum. All sections are coronal and slightly asymmetric; the left side is approximately 1 mm rostral to the right. Anatomic abbreviations are defined in the Key.

protein in neurons of vestibular and deep cerebellar nuclei.

Among the CNS nuclei described above, there is a subset in which the 57 kDa NIFP antiserum identifies antigen in the axonal process but not within the cytokaryon of most of the neurons. These include the somatic motor cranial nerve nuclei, the red nucleus, the medial deep cerebellar nucleus, and the ventral horn nuclei. The varied ability for polyclonal and mono-

clonal antibodies to recognize the NFT proteins in the cytokaryon versus axonal process (Shaw et al., 1981; Goldstein et al., 1983; Sternberger and Sternberger, 1983) or among neurons of the same type (Trojanowski et al., 1985) has been well described and has usually been attributed to post-translational modifications of the IF causing structural changes that either reveal or block epitopes (Sternberger and Sternberger, 1983;
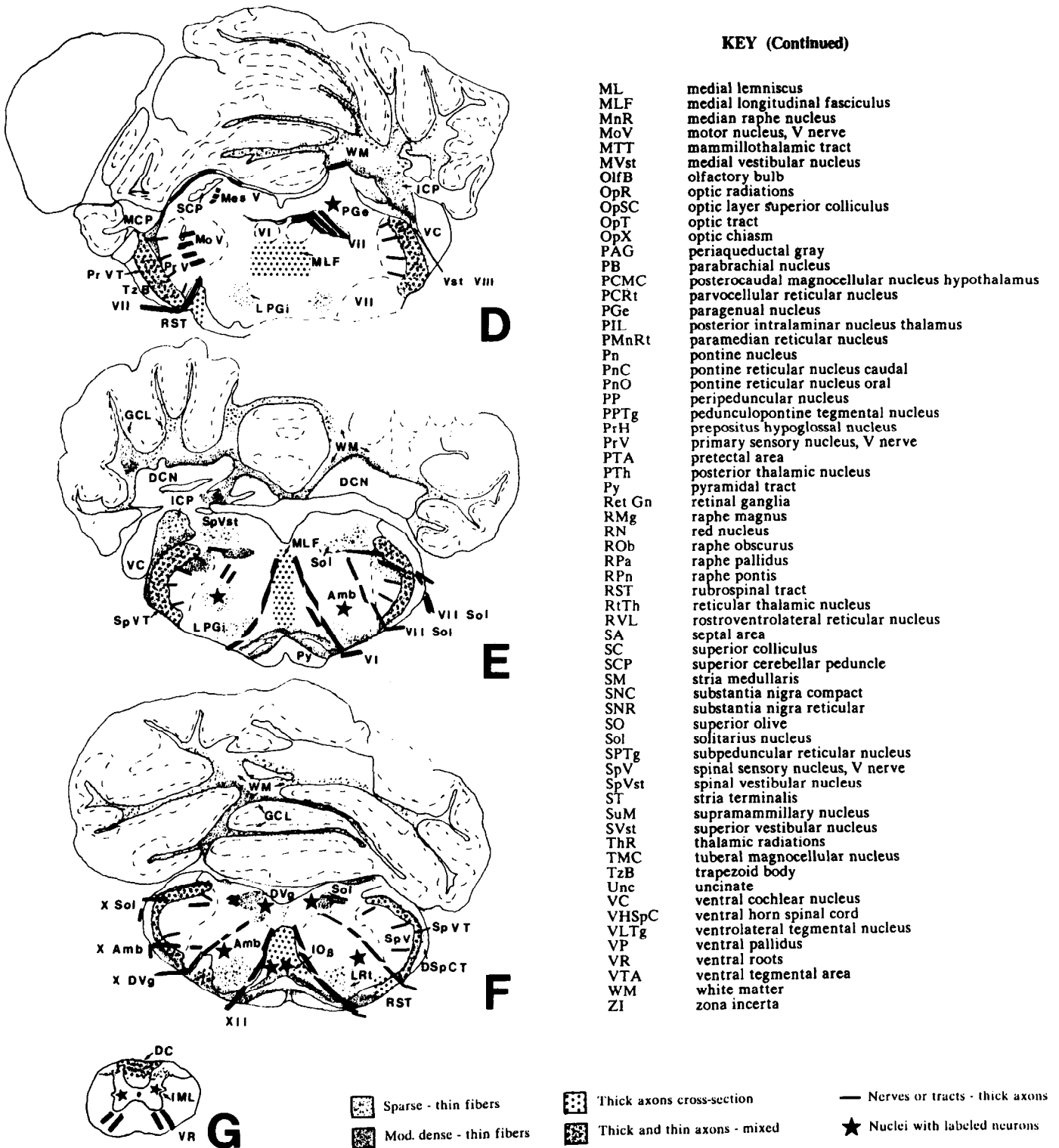
KEY (Continued)

ML	medial lemniscus
MLF	medial longitudinal fasciculus
MnR	median raphe nucleus
MoV	motor nucleus, V nerve
MTT	mammillothalamic tract
MVst	medial vestibular nucleus
OlfB	olfactory bulb
OpR	optic radiations
OpSC	optic layer superior colliculus
OpT	optic tract
OpX	optic chiasm
PAG	periaqueductal gray
PB	parabrachial nucleus
PCMC	posterocaudal magnocellular nucleus hypothalamus
PCRt	parvocellular reticular nucleus
PGe	paragenual nucleus
PIL	posterior intralaminar nucleus thalamus
PMnRt	paramedian reticular nucleus
Pn	pontine nucleus
PnC	pontine reticular nucleus caudal
PnO	pontine reticular nucleus oral
PP	peripeduncular nucleus
PPTg	pedunculopontine tegmental nucleus
PrH	prepositus hypoglossal nucleus
PrV	primary sensory nucleus, V nerve
PTA	pretectal area
PTh	posterior thalamic nucleus
Py	pyramidal tract
Ret Gn	retinal ganglia
RMg	raphe magnus
RN	red nucleus
ROb	raphe obscurus
RPa	raphe pallidus
RPn	raphe pontis
RST	rubrospinal tract
RtTh	reticular thalamic nucleus
RVL	rostroventrolateral reticular nucleus
SA	septal area
SC	superior colliculus
SCP	superior cerebellar peduncle
SM	stria medullaris
SNC	substantia nigra compact
SNR	substantia nigra reticular
SO	superior olive
Sol	solitarius nucleus
SPTg	subpeduncular reticular nucleus
SpV	spinal sensory nucleus, V nerve
SpVst	spinal vestibular nucleus
ST	stria terminalis
SuM	supramammillary nucleus
SVst	superior vestibular nucleus
ThR	thalamic radiations
TMC	tuberal magnocellular nucleus
TzB	trapezoid body
Unc	uncinate
VC	ventral cochlear nucleus
VHSpC	ventral horn spinal cord
VLTg	ventrolateral tegmental nucleus
VP	ventral pallidus
VR	ventral roots
VTA	ventral tegmental area
WM	white matter
ZI	zona incerta

Sparse - thin fibers

Mod. dense - thin fibers

Thick axons cross-section

Thick and thin axons - mixed

— Nerves or tracts - thick axons

★ Nuclei with labeled neurons

Figure 1. Continued.

Carden et al., 1985). Although we do not know the factor(s) responsible for masked 57 kDa NIFP epitopes in the cell bodies of some neurons, the correlative data obtained by *in situ* hybridization have allowed an accurate examination of 57 kDa NIFP distribution (Leonard et al., 1988; Parysek et al., 1988; present study). The data suggest that the 57 kDa NIFP indeed has a restricted distribution and that the findings are not simply because of restricted antigen recognition by the particular antiserum used in this study.

The recently reported immunolocalization study for peripherin indicates that it, too, has a highly restricted CNS distribution (Escurat et al., 1988). Like the 57 kDa NIFP, peripherin is present in sensory ganglia including retinal ganglion cells and in spinal motor neurons. In contrast to the 57 kDa NIFP,
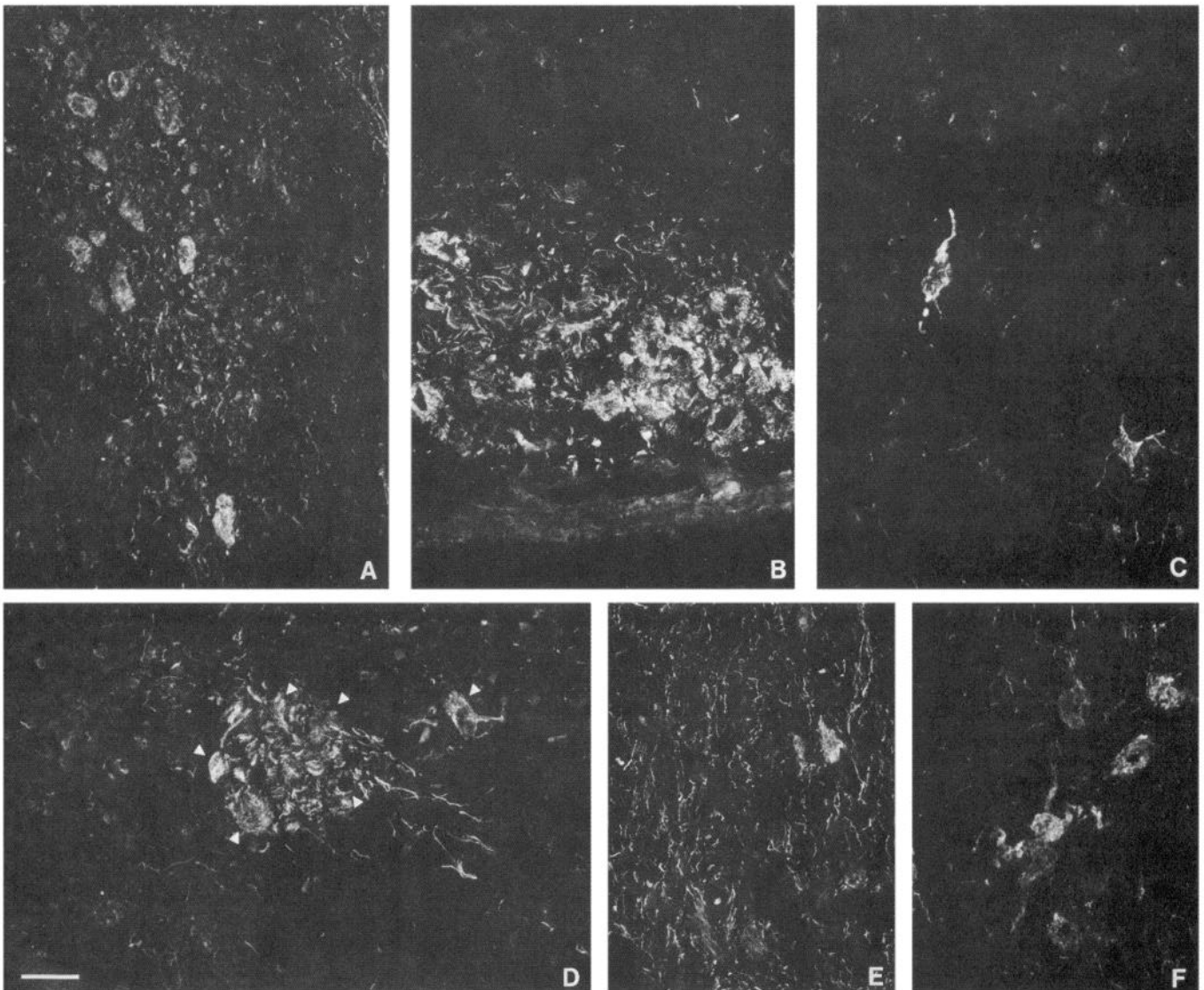
Figure 2. Distribution of the 57 kDa NIFP in gray matter. The 57 kDa NIFP antiserum labels cytokarya and thin processes of a select group of CNS nuclei including the dorsal motor nucleus of X (*A*), the caudal magnocellular nucleus of the hypothalamus (*B*), and the intermediolateral cell column of the thoracic spinal cord (*filled triangles indicate cell bodies*) (*D*). Rare labeled cytokarya are present within very sparsely labeled neuropil in the cerebral cortex (*C*). In contrast, fibrillary label is present in the cytokarya of numerous neurons that are widely scattered among a moderately dense array of labeled processes (*E*) in a region that extends diagonally within the rostral pons from the rostral end of the PPTg (*F*) just caudal to the substantia nigra to its more dorsal caudal end and then extends beneath the SCP to include the area of the LDTg in the periaqueductal gray matter (see Fig. 1*B*). Scale bar, 25 μm.

peripherin was not recognized in the brain stem but was present in olfactory nerves and receptor neurons where other neuronal IF proteins previously were shown to be absent (Schwob et al., 1986). Thus, either peripherin and the 57 kDa NIFP are similar but not identical NIFP or the antisera utilized for the respective immunolocalization studies have different specificities for the same protein.

On the basis of the data available prior to this study, hypotheses have been formed that correlate the unique distribution of 57 kDa NIFP with neuronal function. The suggestion that the 57 kDa NIFP has some function specific to first-order axons (Parysek and Goldman, 1988) is supported by the finding that none of the second-order somatosensory neurons or their axons contains the 57 kDa NIFP. Another hypothesis advanced was that the 57 kDa NIFP provides a structural stabilizing function

for very long axons which cross from CNS to PNS or vice versa (Escurat et al., 1988; Leonard et al., 1988). The absence of the 57 kDa NIFP from certain CNS long tracts such as the corticospinal tract and from the auditory nerve and pathway indicates that the presence of the protein is not essential to stabilize all long axons or all nerves crossing from PNS to CNS nor is it present in all first-order sensory axons. The prominent presence of the 57 kDa NIFP in 2nd-order visual and proprioceptive-cerebellar axons as well as certain RF pathways arising within the CNS indicates that this NIFP is not confined to long-tract axons or to first-order axons. Thus, the additional data of this study corroborate prior observations but also provide findings that do not support past hypotheses.

The functional and anatomic characteristics of the small subset of nuclear groups containing fibrillary labeling suggest 2 dif-
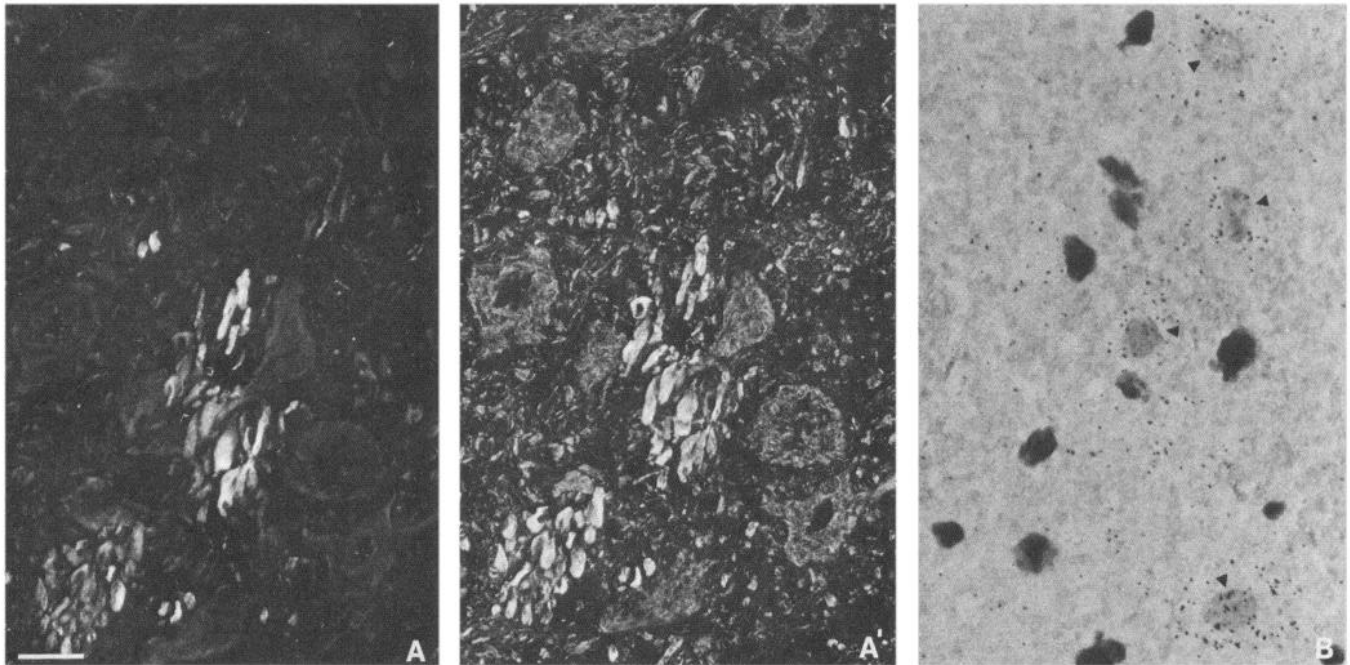
Figure 3. Distribution of immunodetectable 57 kDa NIFP and 57 kDa NIFP mRNA in motor nuclei. Double-label immunofluorescence of a section of the VII nucleus shows labeled large fibers and weak or no labeling of motor neurons with 57 kDa NIFP antiserum (*A*). The large axons (presumptive axons of VII) and motor neurons are labeled with 15G1 (*A'*). *In situ* hybridization of a 57 kDa NIFP-specific cDNA to a similar but nonadjacent section reveals the presence of the 57 kDa NIFP mRNA in these cells. *Filled triangles* identify nuclei of labeled neurons in *B*. Scale bar, 25 μm.

Table 2. 57 kDa NIFP in white matter

CNS region	Axon caliber[a]		
	Thin	Moderate	Thick
Motor roots and nerves			
III, IV, V, VI, VII, ventral roots	—	—	++
IX, X (exit ventrally)	++	+	—
Sensory nerves and tracts			
II, OpX, OpT	+	++	—
BSC, CSC, OpSC	++	—	—
MesVT	+	—	++
DC, SpVT, PrVT	+	++	+
Vestibular VIII	+	—	—
SolT, IX, X (exit laterally)	++	+	—
Aud VIII, TzB, LL, BIC, ML, ThR	—	—	—
OpR, OlfB, LOT	—	—	—
Motor/cerebellar tracts and peduncles			
VSpCT, MLF, RST	—	++	—
IOX, Unc, ICP (lacy pattern laterally)	++	—	—
DSpCT, MCP, SCP, BP, Py, LFP, CP	—	—	—
Cerebellar white matter			
Vermis, amiculum DCN	+/++	—	—
Central, lateral folia	+/−	—	—
Telencephalon/diencephalon			
Cingulum, ST, MFB	+	—	—
ECp, CC, ICp, AC, F, MTT, SM	—	—	—

[a] + = sparse array of labeled axons; ++ = dense array of labeled axons. Anatomic abbreviations are defined in the Key to Figure 1.
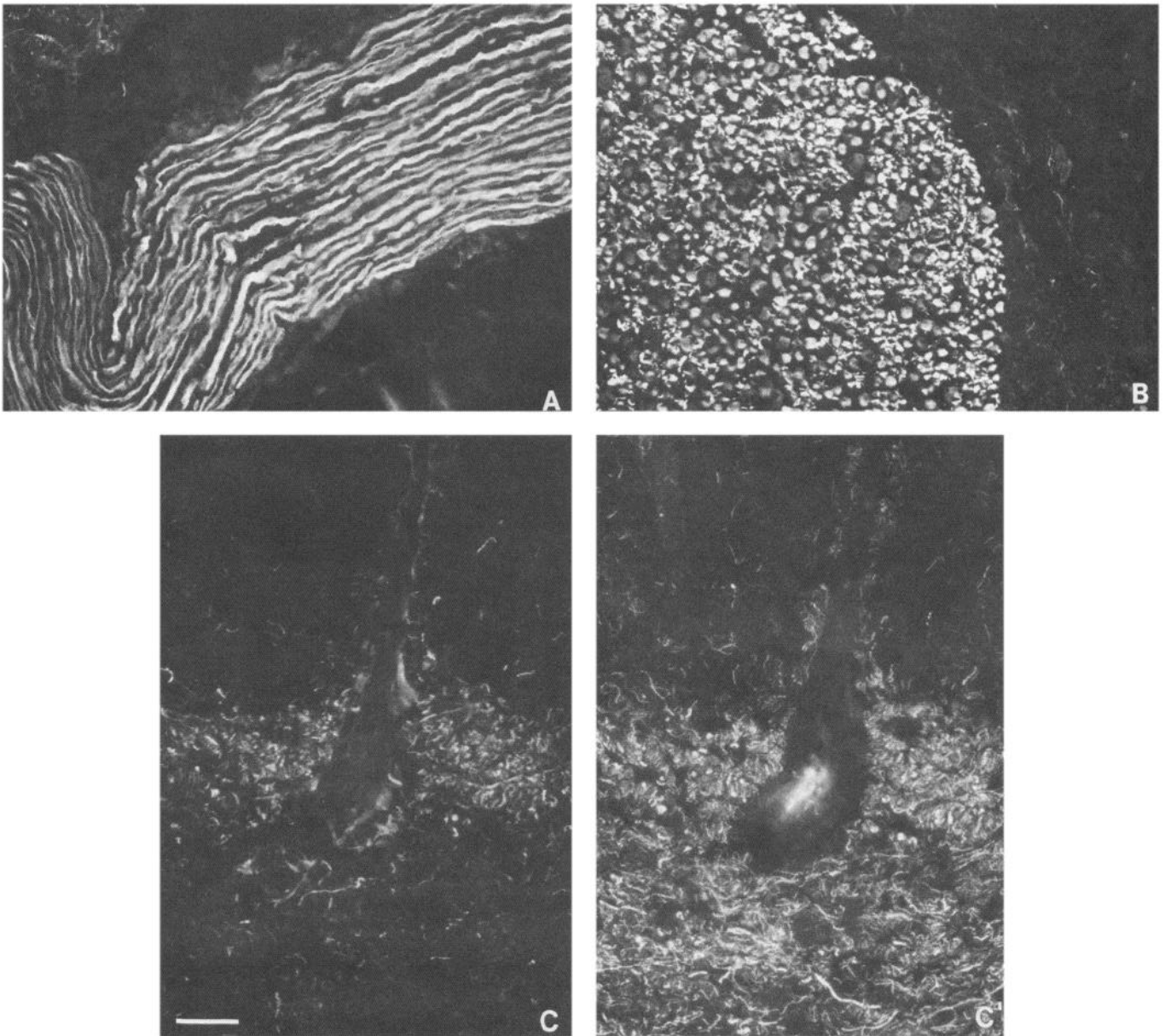
Figure 4. Labeling patterns of cranial nerves and tracts with 57 kDa NIFP antiserum. The XII cranial nerve in the leptomeningeal space (*A*), spinal tract of V (*B*), and superior colliculus (*C*) are labeled with 57 kDa NIFP antiserum; *C* is double-labeled with 15G1 (*C'*). The labeling patterns shown in *A* are typical of large axons in motor nerves and roots; those shown in *B* are consistently seen in sensory tracts, nerves, and roots; and those shown in *C* typify areas of dense labeling of thin fibers. Comparison of *C* and *C'* reveals the restricted labeling of a single layer, the optic layer, with 57 kDa NIFP antiserum. Scale bar, 25 μm.

ferent and probably independent organizing principles for CNS utilization of the 57 kDa NIFP. First, there appears to be a striking correlation between the distribution of neurons containing 57 kDa NIFP and those containing the enzyme CAT in the brain stem and spinal cord. The largest group of 57 kDa NIFP antiserum-positive neurons includes the autonomic motor neurons, preganglionic sympathetic neurons, and numerous neurons within the region of the PPTg and LDTg. These nuclei as well as the brain stem and spinal cord motor nuclei have in common many densely packed CAT-containing neurons (Sofroniew et al., 1985). The correlation between the presence of 57 kDa NIFP and CAT is not complete since the major cholinergic regions in the telencephalon contain extremely sparse processes

labeled by the 57 kDa NIFP antiserum; however, the degree of correlation is intriguing.

A potential relationship between the 57 kDa NIFP and cholinergic transmitter function is supported by the physiologic characteristics of PC12 cells. PC12 cells have been shown to contain CAT and to synthesize, store, and release acetylcholine (Schubert et al., 1977) in addition to norepinephrine and dopamine (Greene and Rein, 1977). Induction of neuronal differentiation in PC12 cells by NGF results in decreased catecholamine content, decreased numbers of catecholaminergic-type large granules, and an increase in the cholinergic-type agranular vesicles (Tischler and Greene, 1978). Furthermore, while the correspondence between 57 kDa NIFP-positive regions and
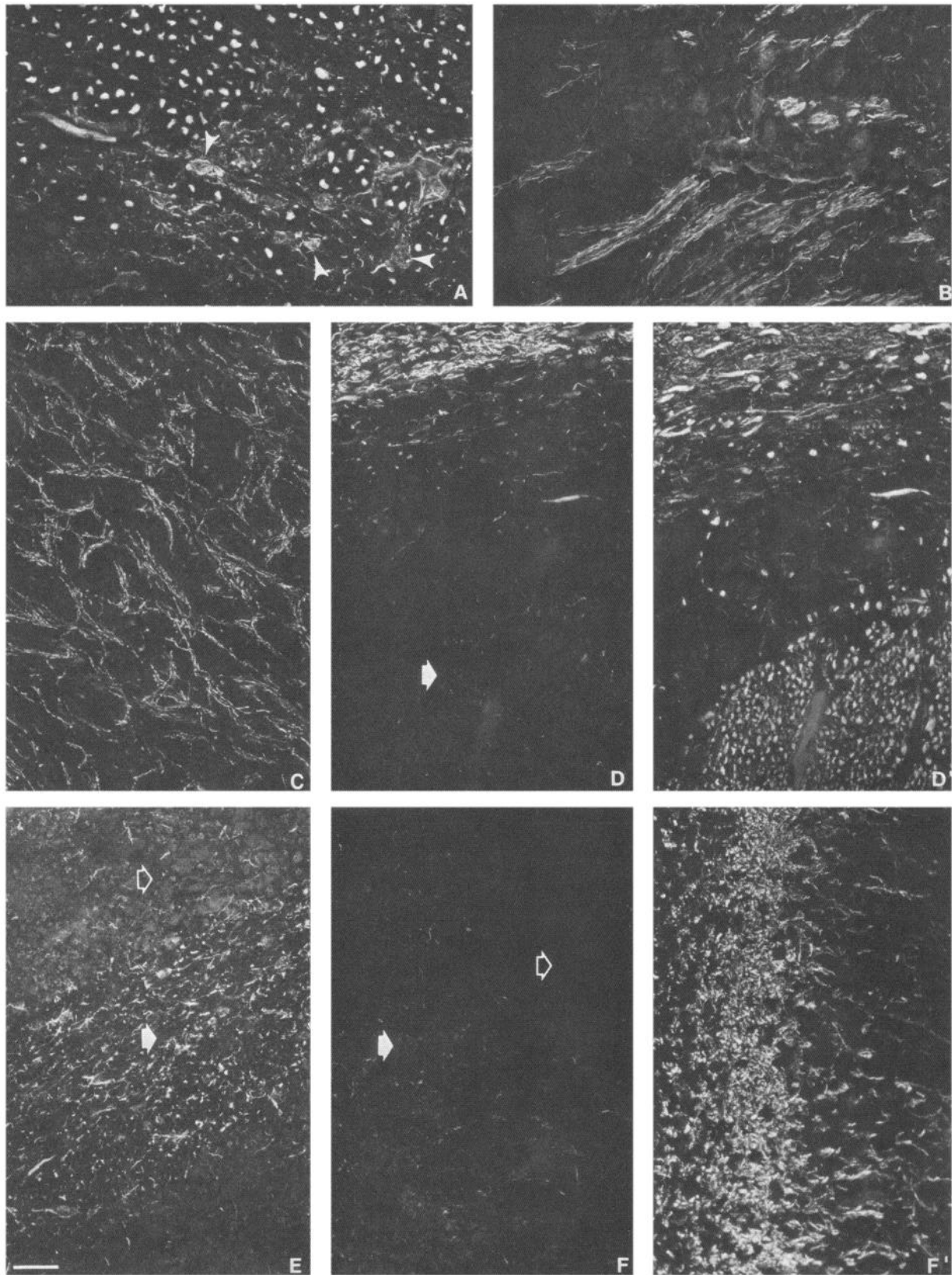
Figure 5. 57 kDa NIFP distribution in olivovestibulocerebellar pathways. 57 kDa NIFP antiserum labels the cytokarya (*arrowheads*) in the β-subnucleus of the inferior olive, seen among labeled thick axons of the MLF (*A*). Labeled tracts of the olivovestibulocerebellar system include olivocerebellar fibers (*B*), a subpopulation of axons in the ICP (*C*), and the uncinate tract (*D*). The SCP, carrying cerebellar efferents to the thalamus, is unlabeled (*D*). A moderately dense array of cerebellar subcortical fibers is labeled in the posterior vermis (*E*) in contrast to the very sparse labeling present in the anterolateral cerebellum (*F*). *Closed arrows* indicate left edge of the SCP in *D* and the white matter in *E* and *F*. *Open arrows* indicate the granule cell layer in *E* and *F*. Double-labeling of *D* and *F* with 15G1 reveals, respectively, the SCP (*D′*) and the subcortical white matter in the anterolateral cerebellum (*F′*). Scale bar, 25 μm.

CAT-positive regions is high, there is a notable lack of correspondence between the distribution of 57 kDa NIFP and neuron systems defined by dopamine or norepinephrine (Lindvall and Bjorklund, 1983), Substance P and enkephalin (Cuello et al., 1985), neuropeptide Y (Chronwall et al., 1985), or serotonin (Tork, 1985).

The second possible organizing principle of 57 kDa NIFP distribution reflects observations that a subset of prominently labeled brain stem and cerebellar regions form an interconnected olivovestibulocerebellar circuit (Table 2). Numerous neuronal cytokarya in the subnucleus β of the CMAO were labeled by the 57 kDa NIFP antiserum. *In situ* hybridization indicated that 57 kDa NIFP message is present in several subnuclei of the CMAO in addition to the IOβ. Neurons of the CMAO provide the afferent climbing fibers to the vestibular zone (medial zone A) of the cerebellar vermis (Brodal, 1980). The vermal medial zone A receives vestibular afferents and projects back to the deep medial cerebellar nuclei and to brain stem vestibular nuclei (these nuclei were labeled by clone 73; Leonard et al., 1988) (Ito, 1980; Walberg, 1980). The present study shows that, in addition to the more posterior regions of the cerebellar white matter, the portions of the vestibulocerebellar system labeled by 57 kDa NIFP antiserum included the uncinate tract and the medial ICP. The uncinate tract carries efferents from the medial cerebellar nucleus to the vestibular nuclei (Carpenter and Batton, 1982). Anatomic studies have indicated that the thin axons of the medial ICP are most likely olivocerebellar fibers originating in the IO (Szentagothai-Schimert, 1942; Voogd and Bigare, 1980). In contrast, the superior and middle cerebellar peduncles carrying telencephalic cerebellar afferent and efferent fibers were unlabeled by the 57 kDa NIFP antiserum. Thus, for both the CNS cholinergic and motor control systems, antiserum to the 57 kDa NIFP apparently labels only the brain stem and spinal cord components.

The molecular function of NIF (and IF in general) remains elusive. The structure of these filaments and their demonstrated molecular linkages with other filaments and cytoplasmic organelles historically has been interpreted in the context of a cytoskeletal function (for review, see Lazarides, 1982; Traub, 1985). This view is reflected in the hypothesis that neurofilaments are a major determinant of axonal caliber (Hoffman et al., 1987). This demonstration of a select CNS NIFP distribution potentially related to behavioral–functional or to neurotransmitter-related anatomic organization suggests that IFs may have other roles in addition to their structural role in neurons. The 57 kDa NIFP may be only one of many possible systems-specific NIFs. The identification of such systems would not only contribute to the understanding of CNS organization on a molecular level but also to the understanding of the function of IFs.

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