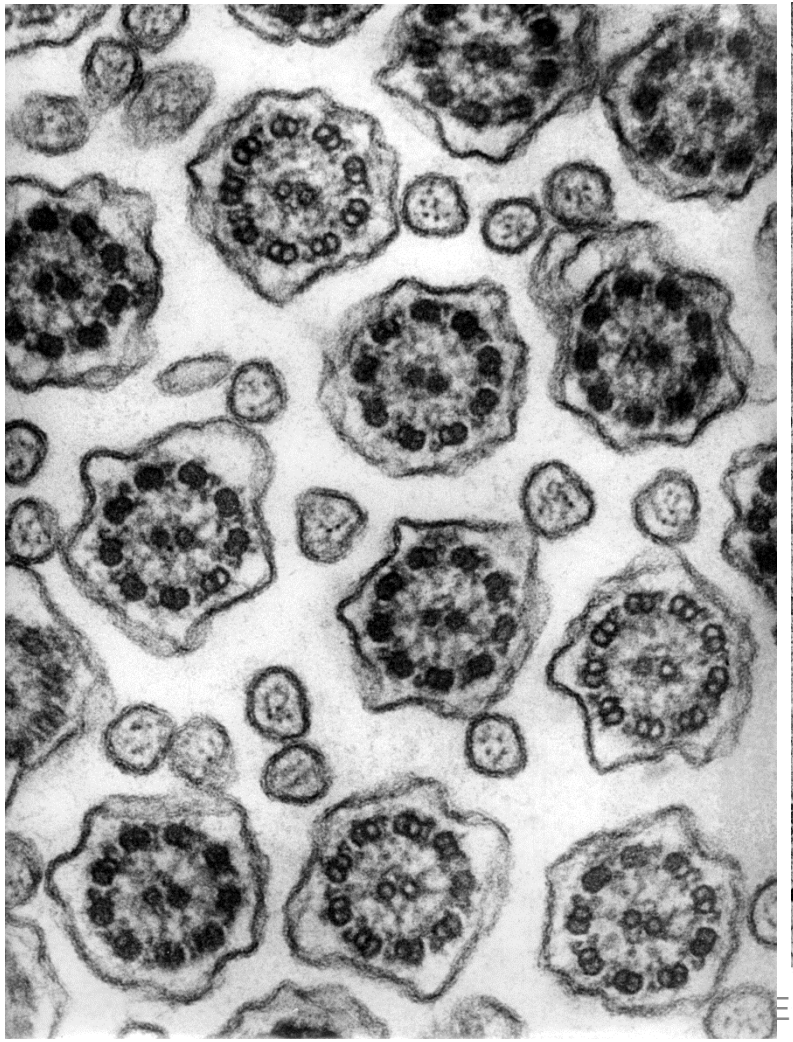
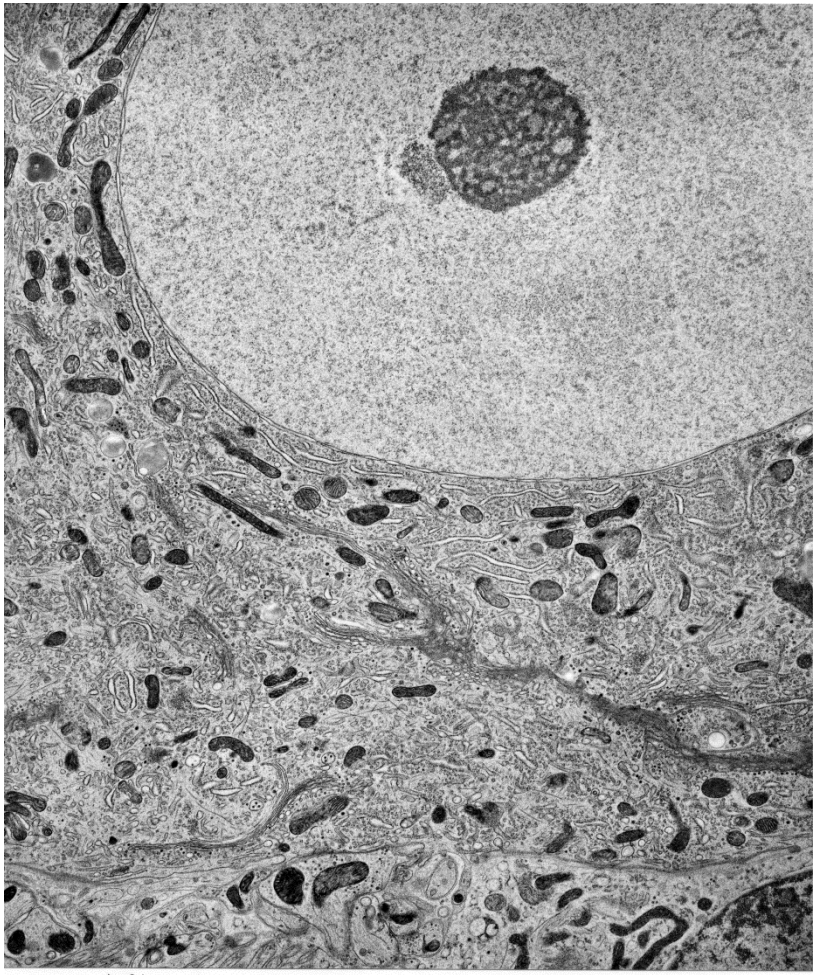
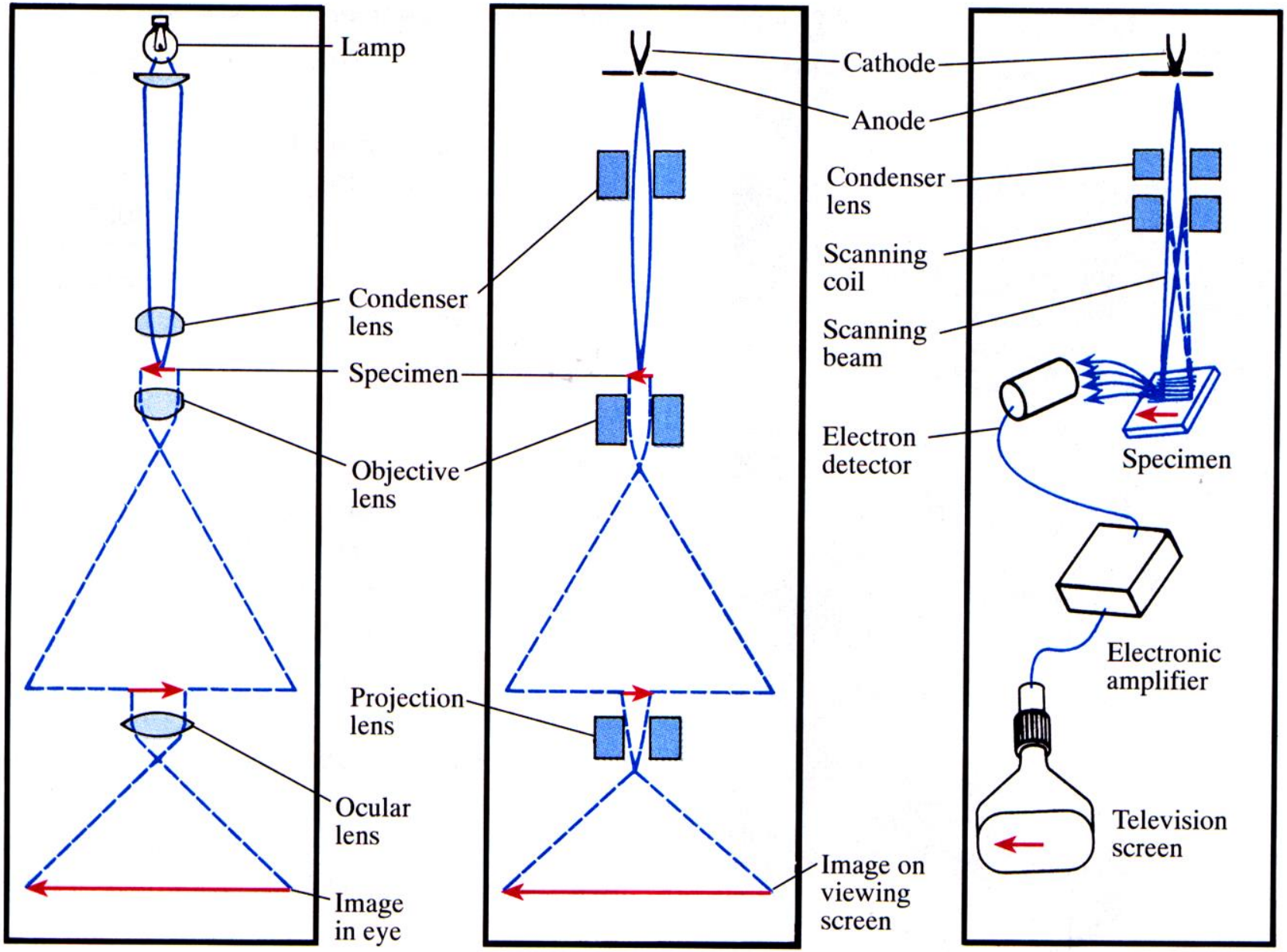
Electron- Microscopic Techniques
How to Prepare a good EM Photo





A thin section of the quick-frozen and substitution electron micrograph shows the cytoplasm of the Purkinje cell of the rat cerebellum. (× 11, 400.)


Lamp
Condenser lens
Specimen
Objective lens
Ocular lens
Image in eye
LIGHT MICROSCOPE
Cathode
Anode
Condenser lens
Projection lens
Image on viewing screen
TRANSMISSION ELECTRON MICROSCOPE
Condenser lens
Scanning coil
Scanning beam
Electron detector
Specimen
Electronic amplifier
Television screen
SCANNING ELECTRON MICROSCOPE


N208a
200KV透過型電顕室
走査型透過型電顕室
火元責任者
廣川 信隆

High Voltage EM: (JEOL JEM-3100FEF) 300 KV and the accessory for the Cooling

TEM: JEOL JEM-2000EX II

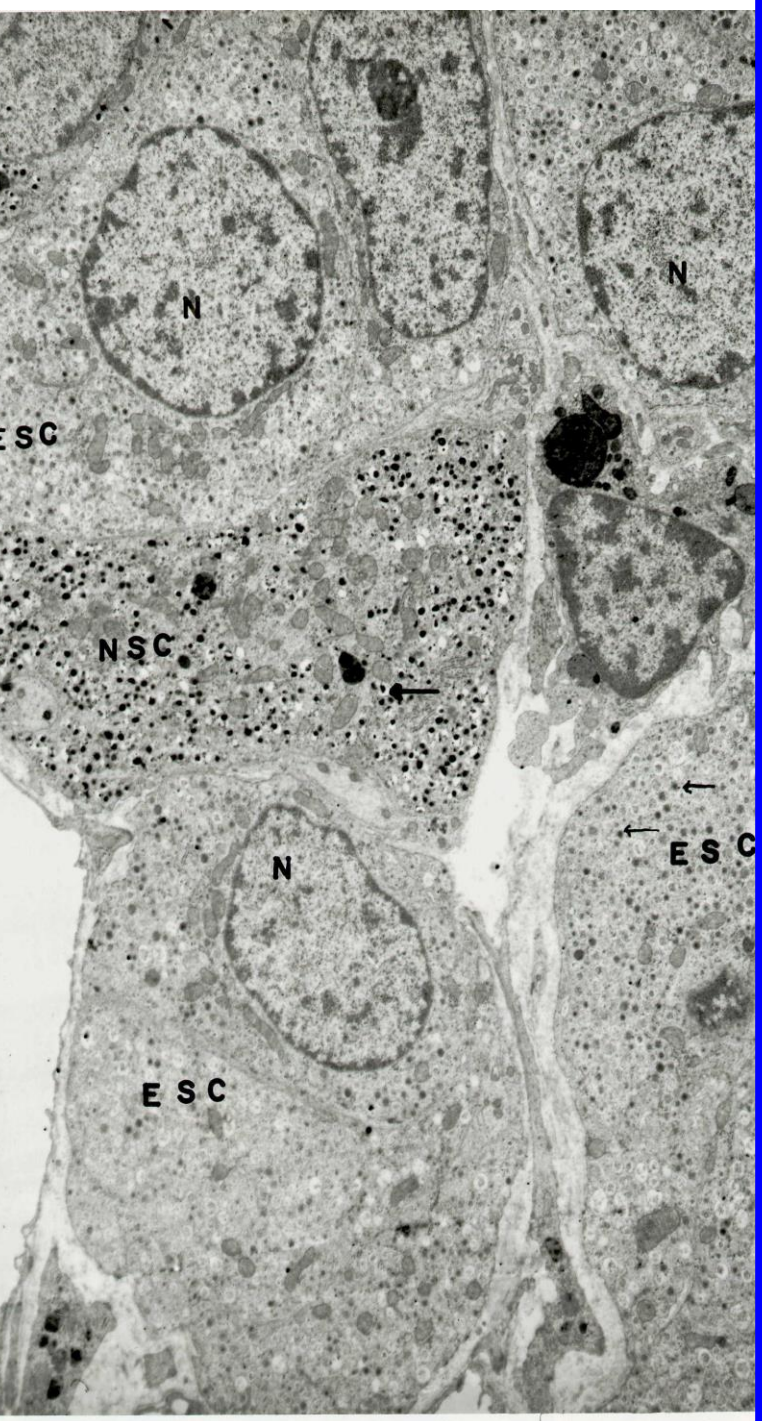
TEM: HITACHI H-7100

TEM: HITACHI H-7500

TEM: HITACHI H-7700

https://www.youtube.com/watch?time_continue=156&v=WbG4F2-jGu0&feature=emb_logo

TEM: HITACHI H-7800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N
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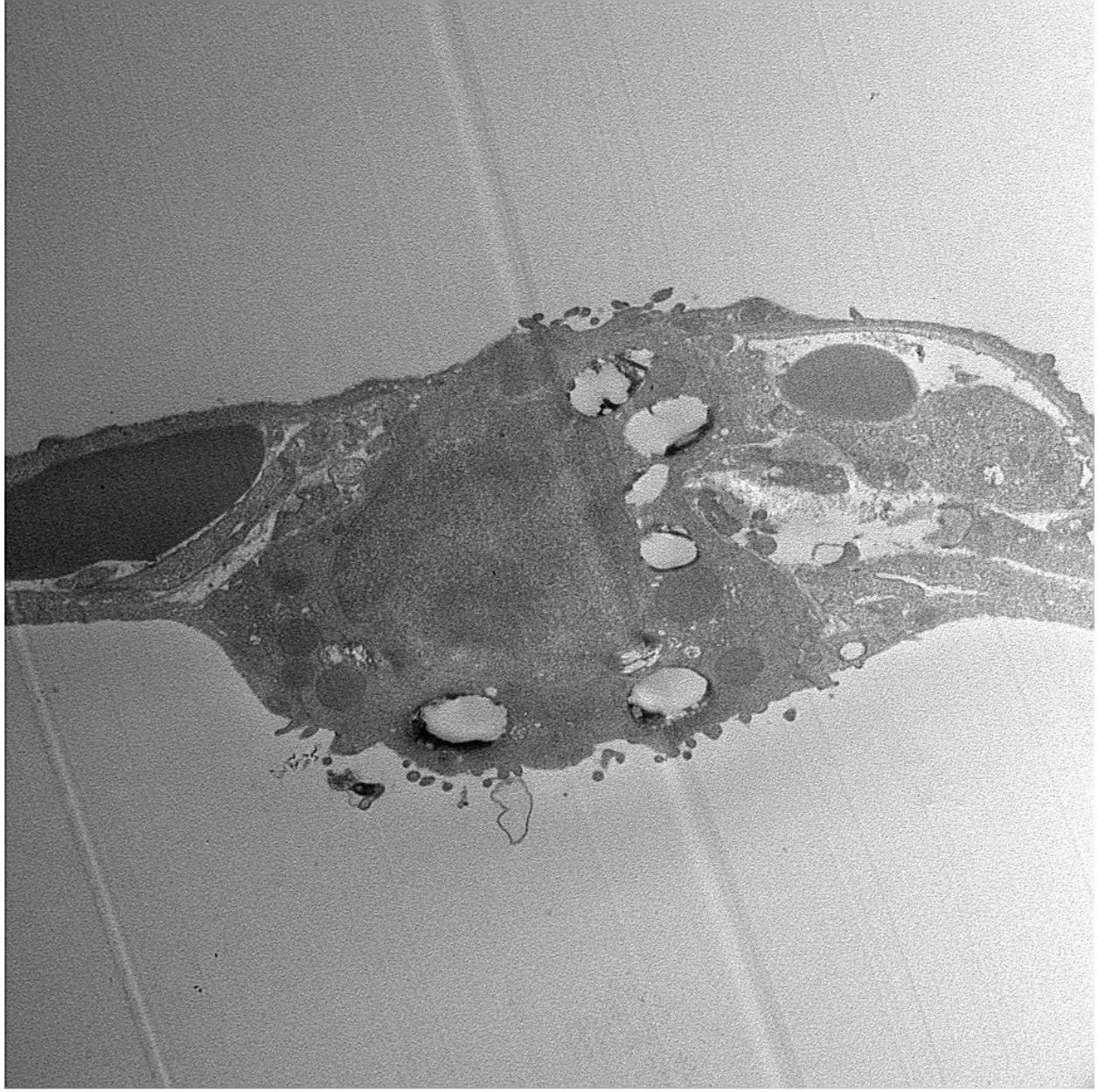

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2 microns
HV=100kV
Direct Mag: 6000x
Tilt:
AMT Camera System

a01.tif
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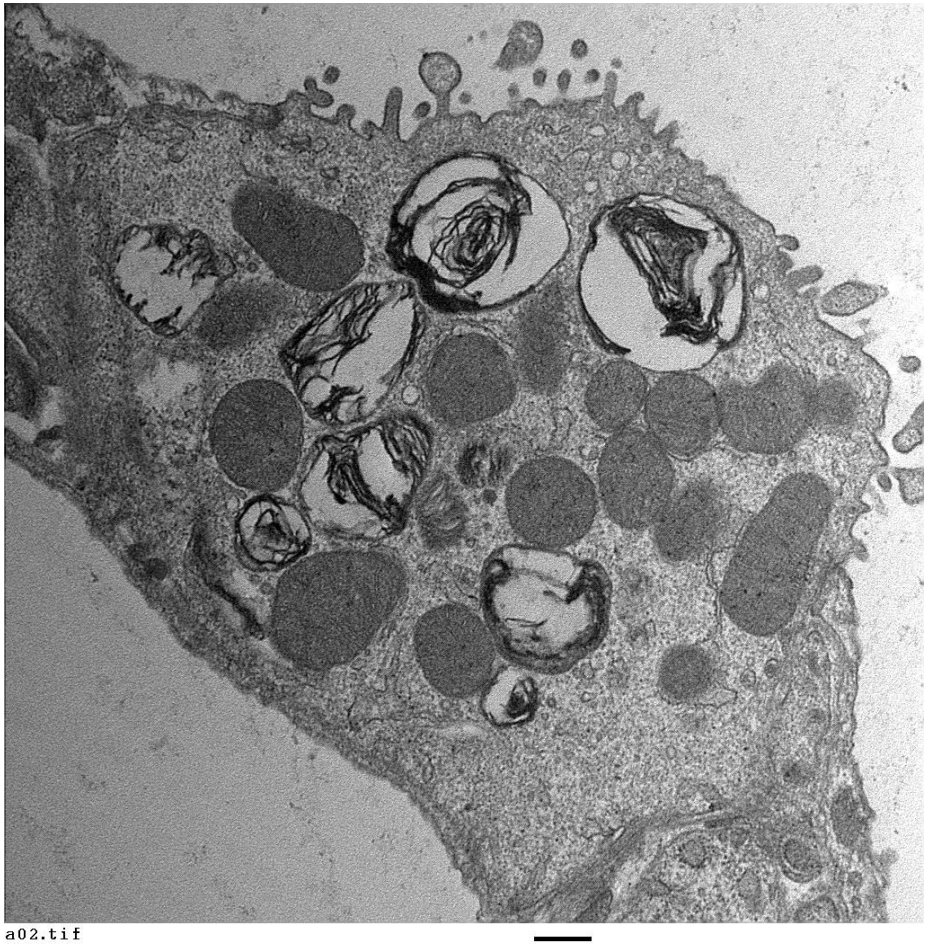

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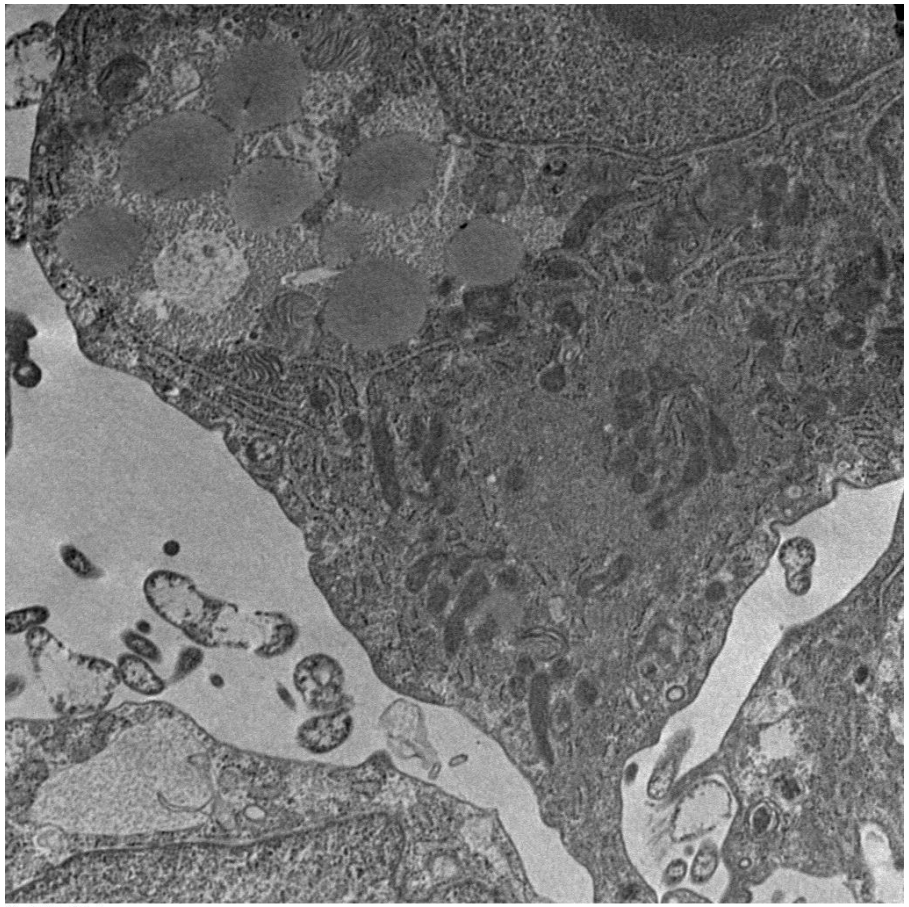

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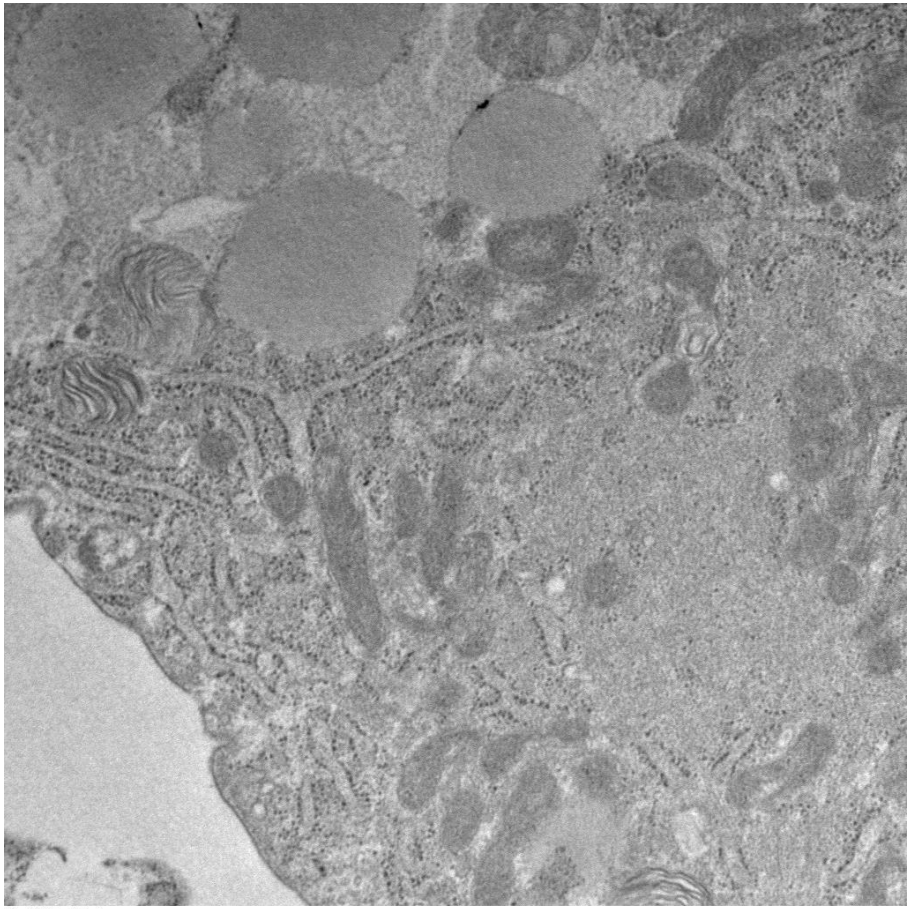

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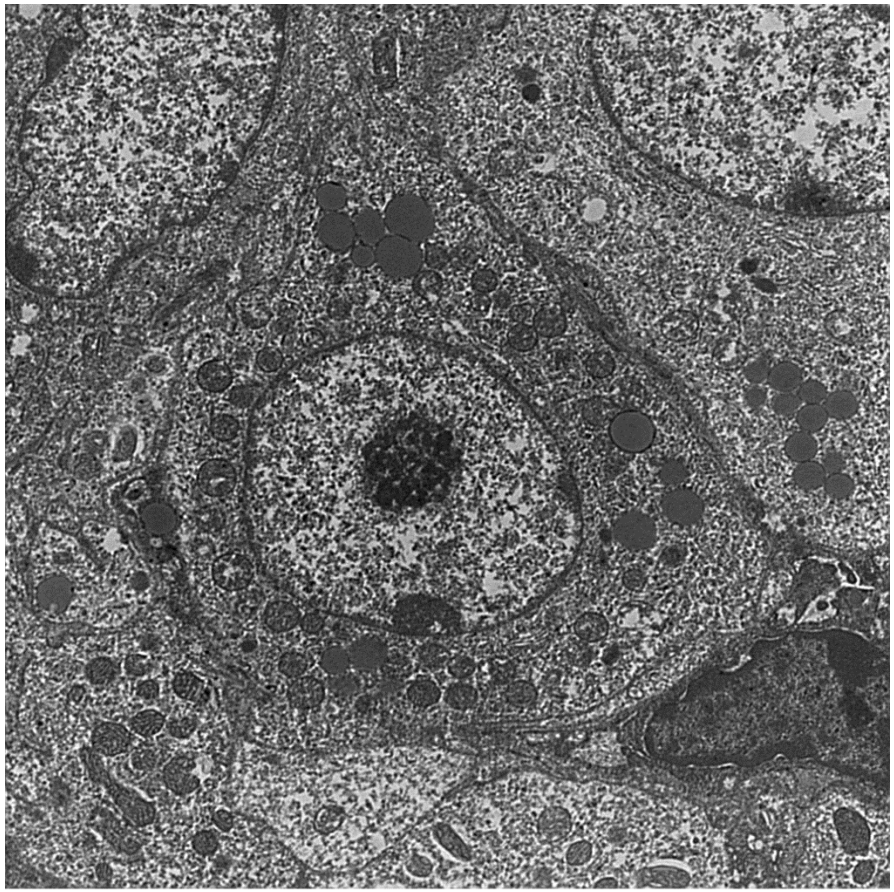

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2 microns
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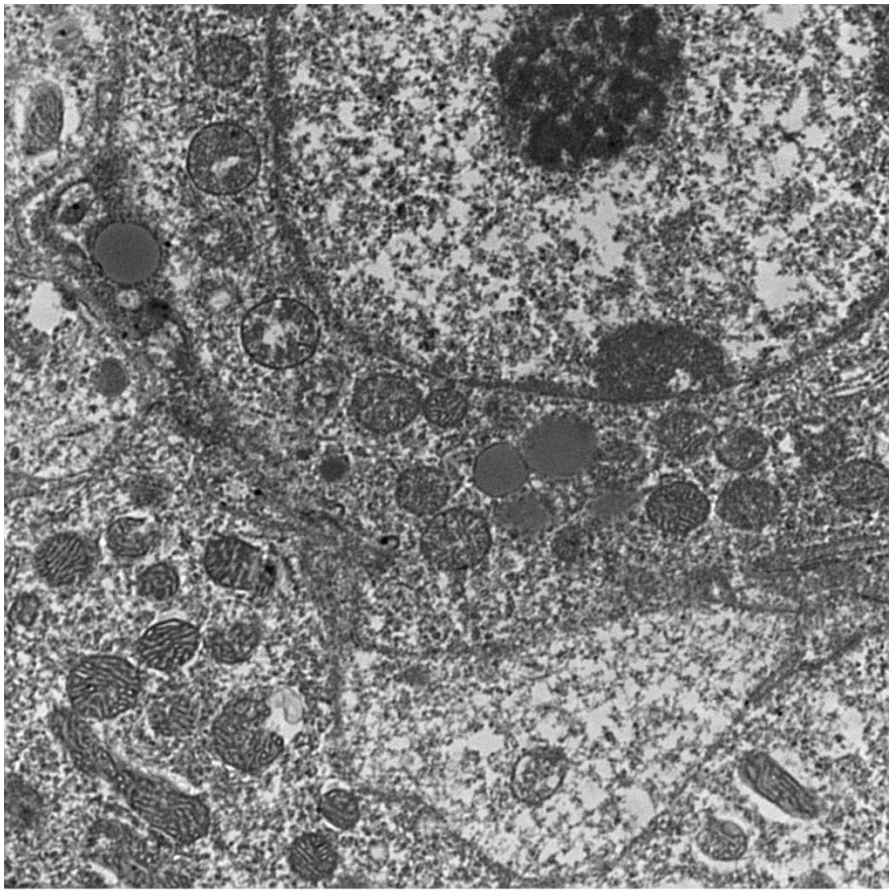

35-9.tif
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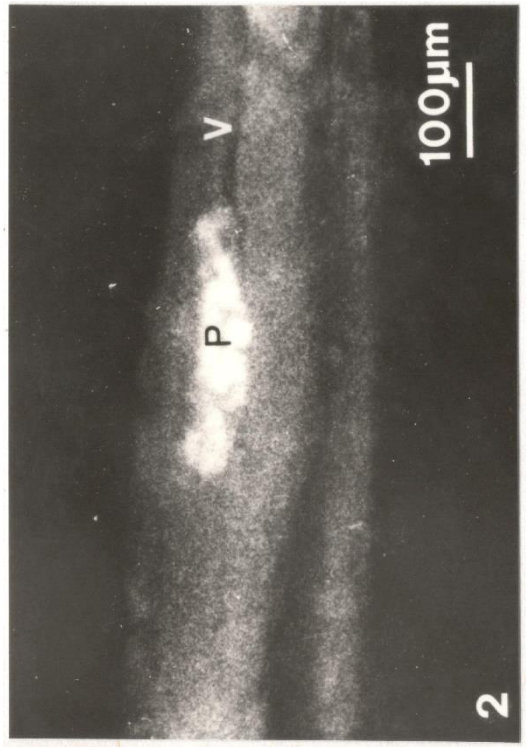
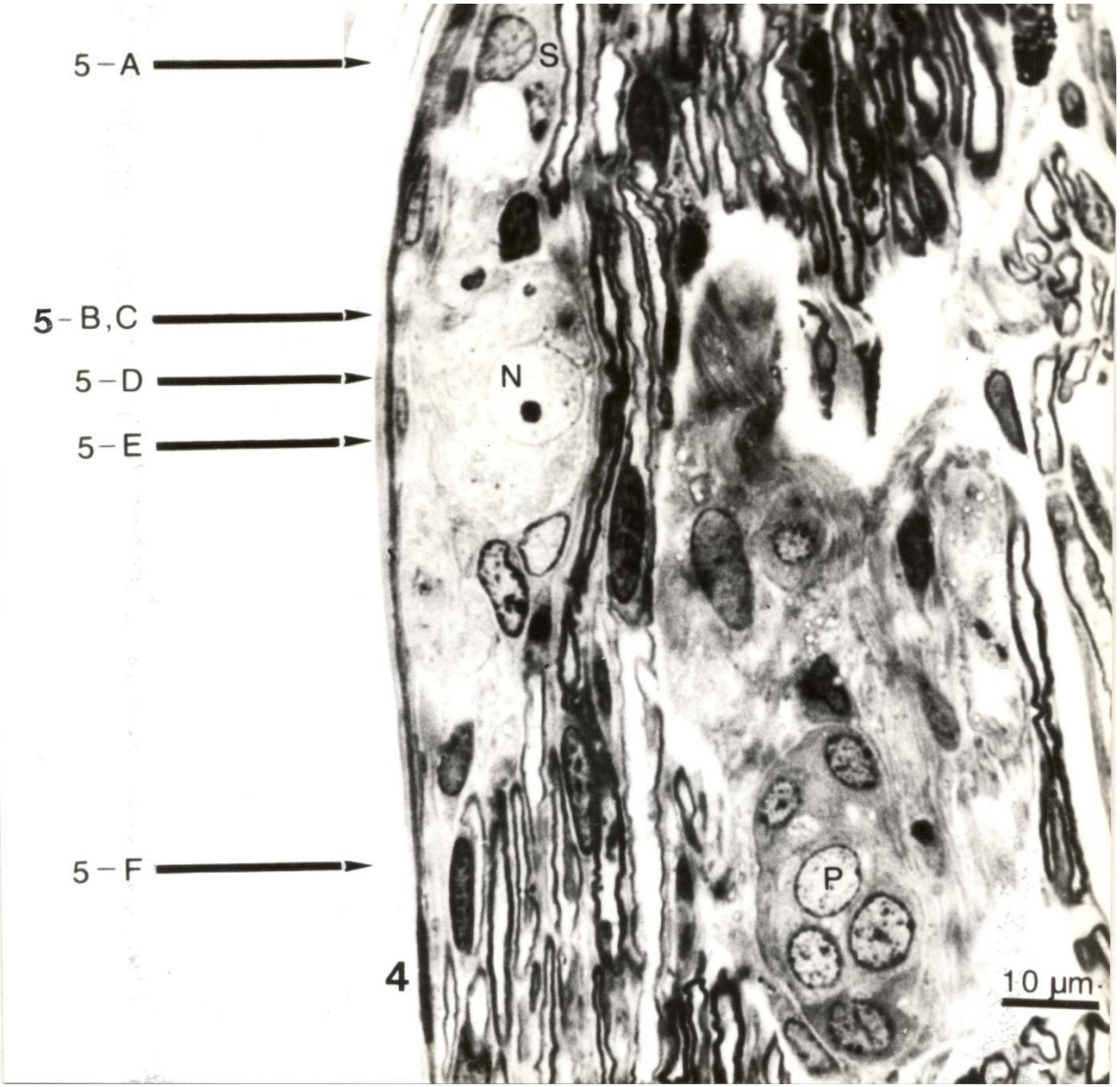
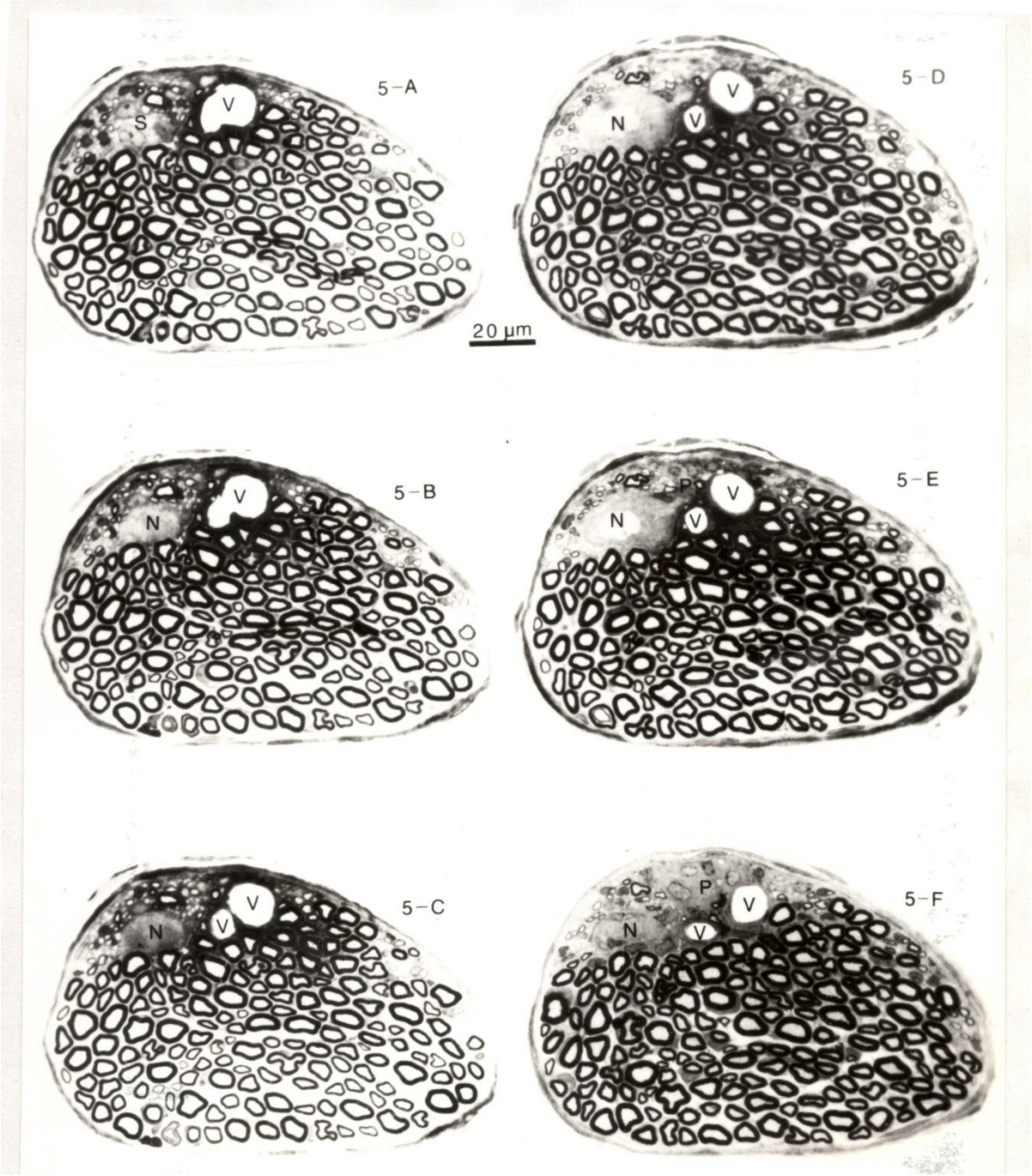
500 nm
HV=100kV
Direct Mag: 10000x

Paraganglia in Recurrent laryngeal nerve (Chien, 1989)





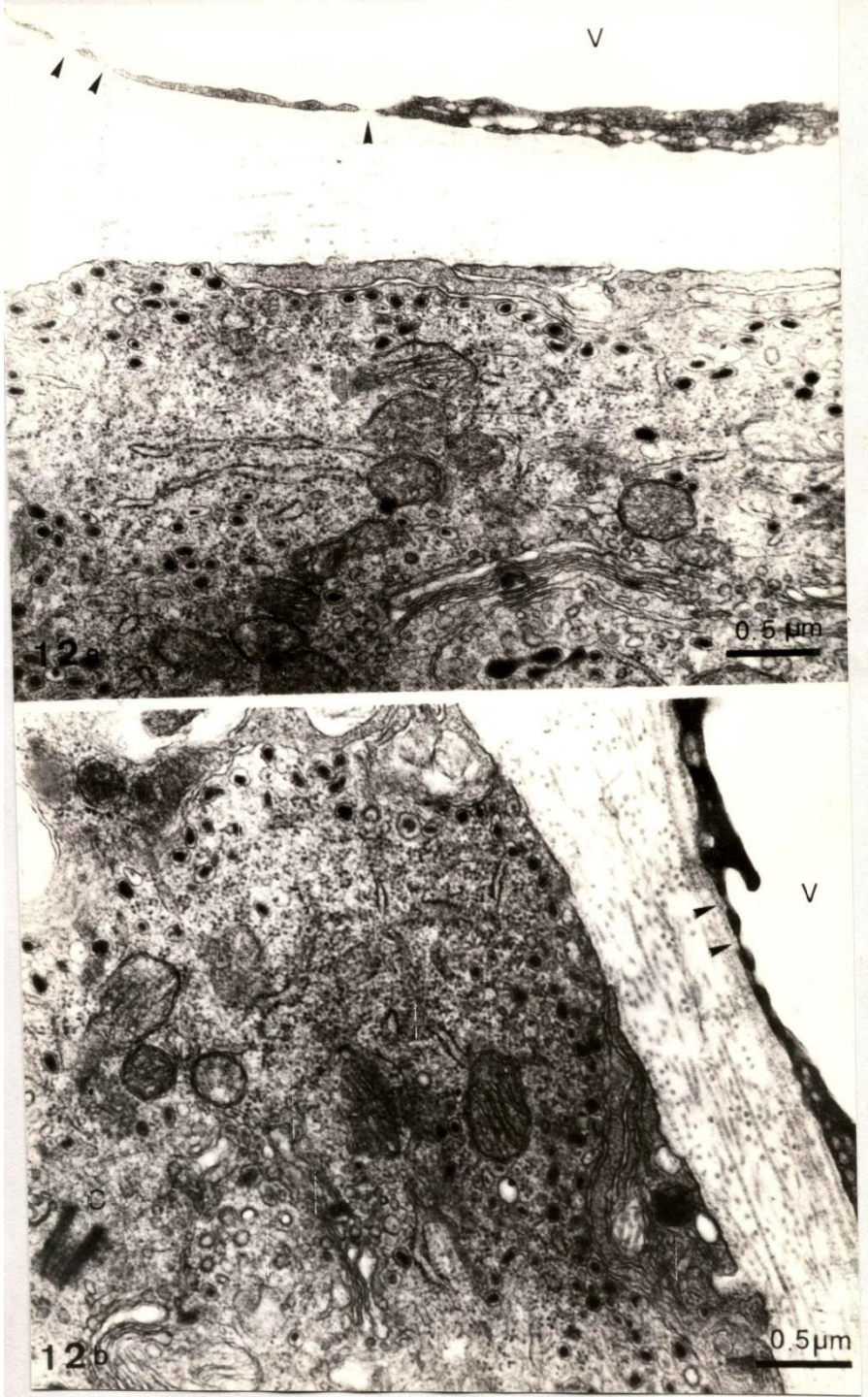
Find the ganglia neuron (N) and **Paraganglia (P)** from the Semi-thin sections of nerves



(Chien, 1989)

Blood vessel (V)
Fenestrated capillary
Next to the paraganglia cells

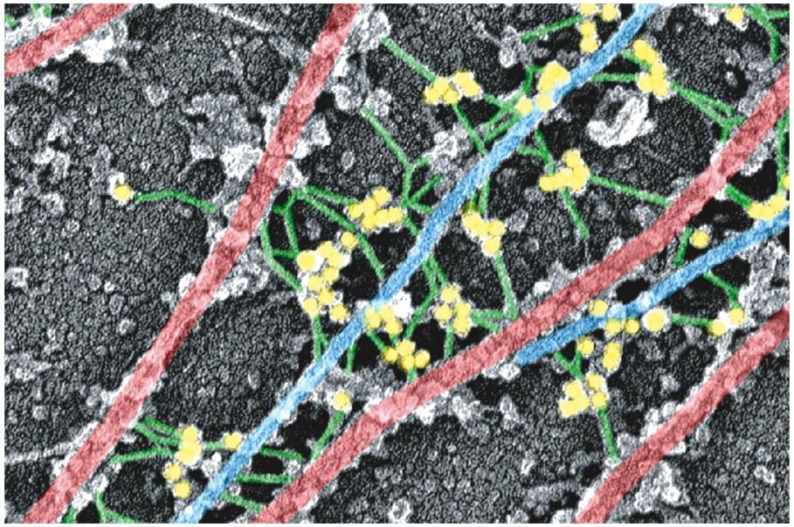
(Chien, 1989)



La^{+3} 鑭離子灌流探討 Blood-Nerve Barrier

La^{+3} 鑭離子灌流探討 Blood-Nerve Barrier

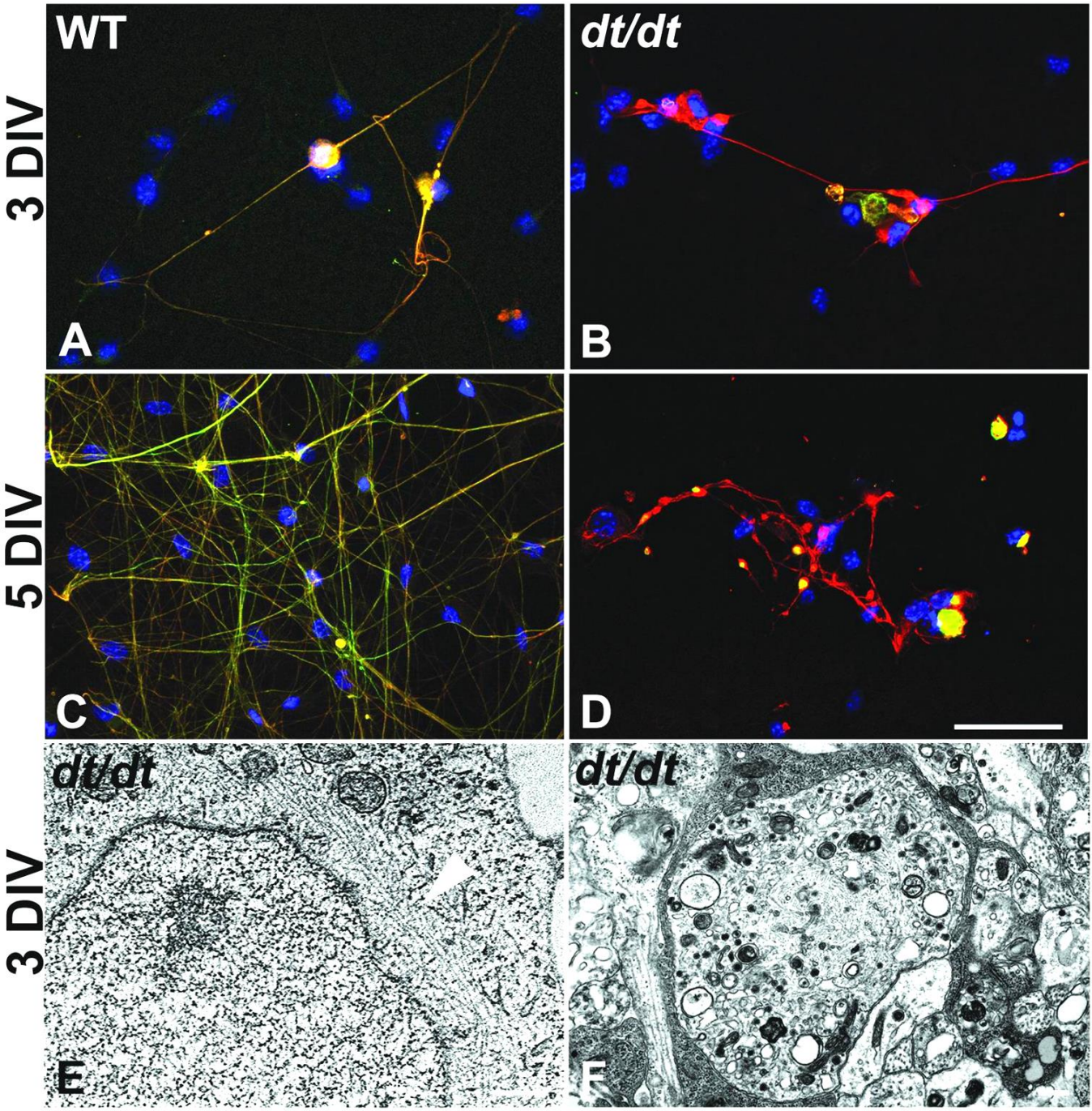
Nature Mutant for Neuronal Degeneration

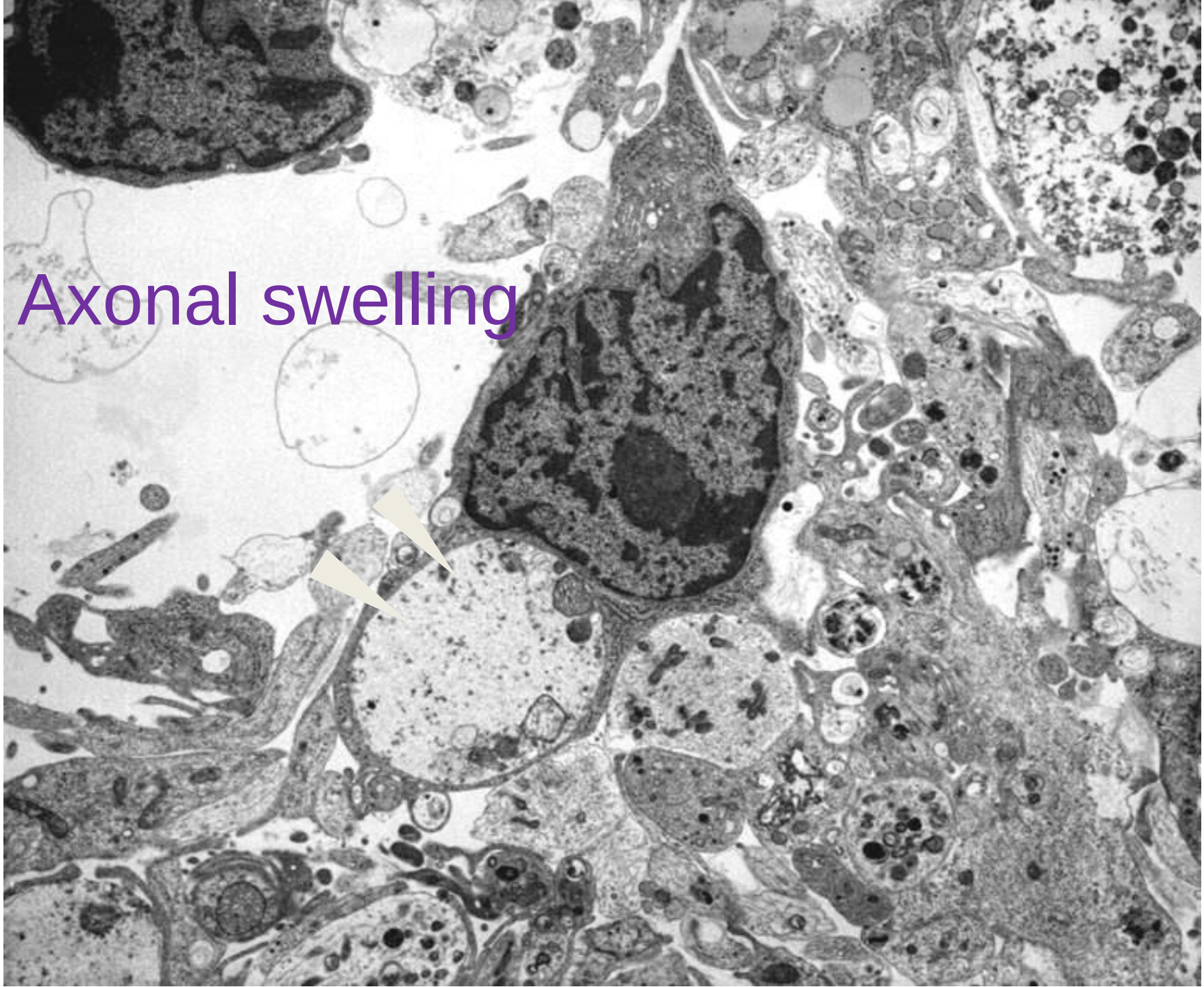


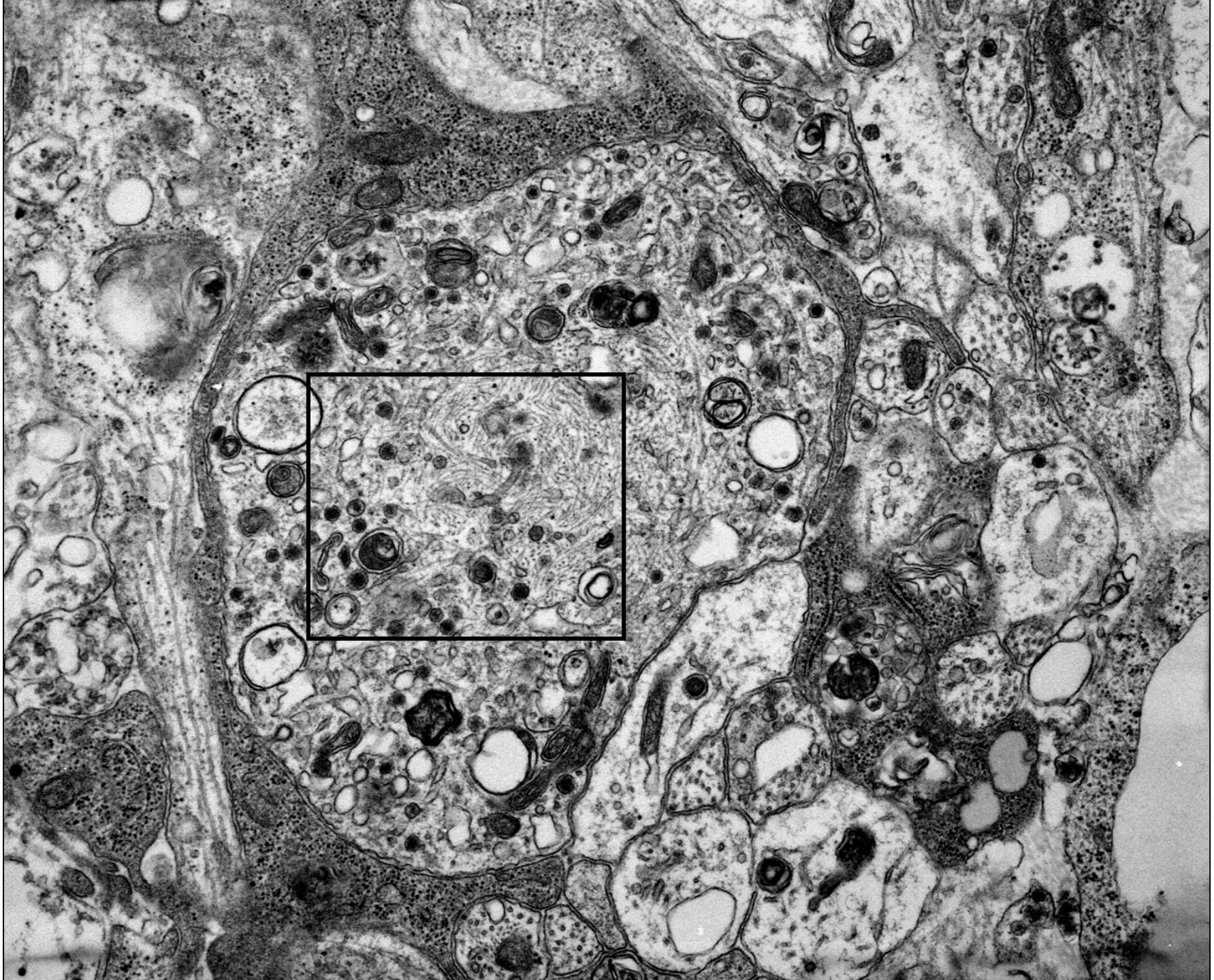
- *Dystonia musculorum (dt)* mouse is a recessive hereditary sensory neuropathy of the mutant mouse, which is defective in *BPAG1* gene.
- Mice affected with *dt* are seemingly normal at birth, but by 10–12 days they begin twitching, writhing, and exhibiting uncoordinated movements.
- **BPAG1** cross-links the intermediate filaments and other cytoskeletons.

Cultured DRG neurons from E15.5 embryos

a-interenxin proteins are accumulated in the cell bodies as well as in the processes of *dt/dt* neurons.




Axonal swelling



DIC Image with TUNEL staining + EM

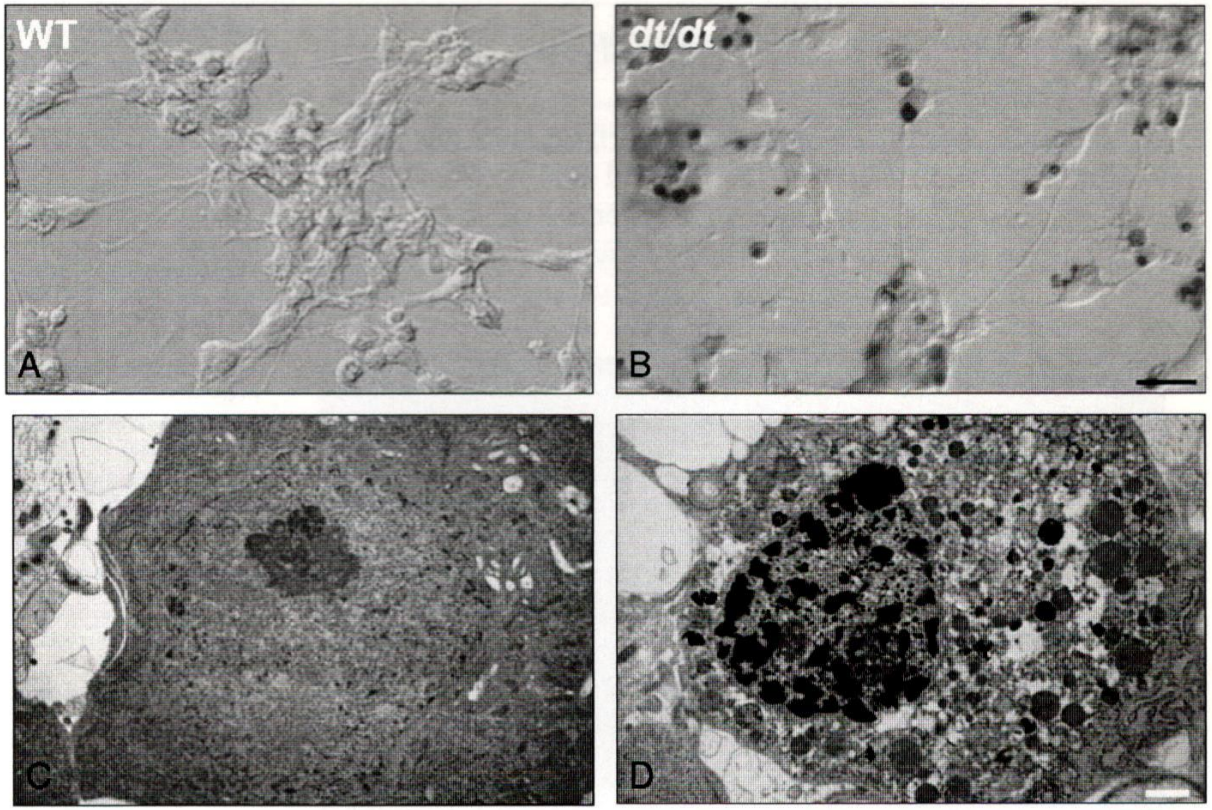
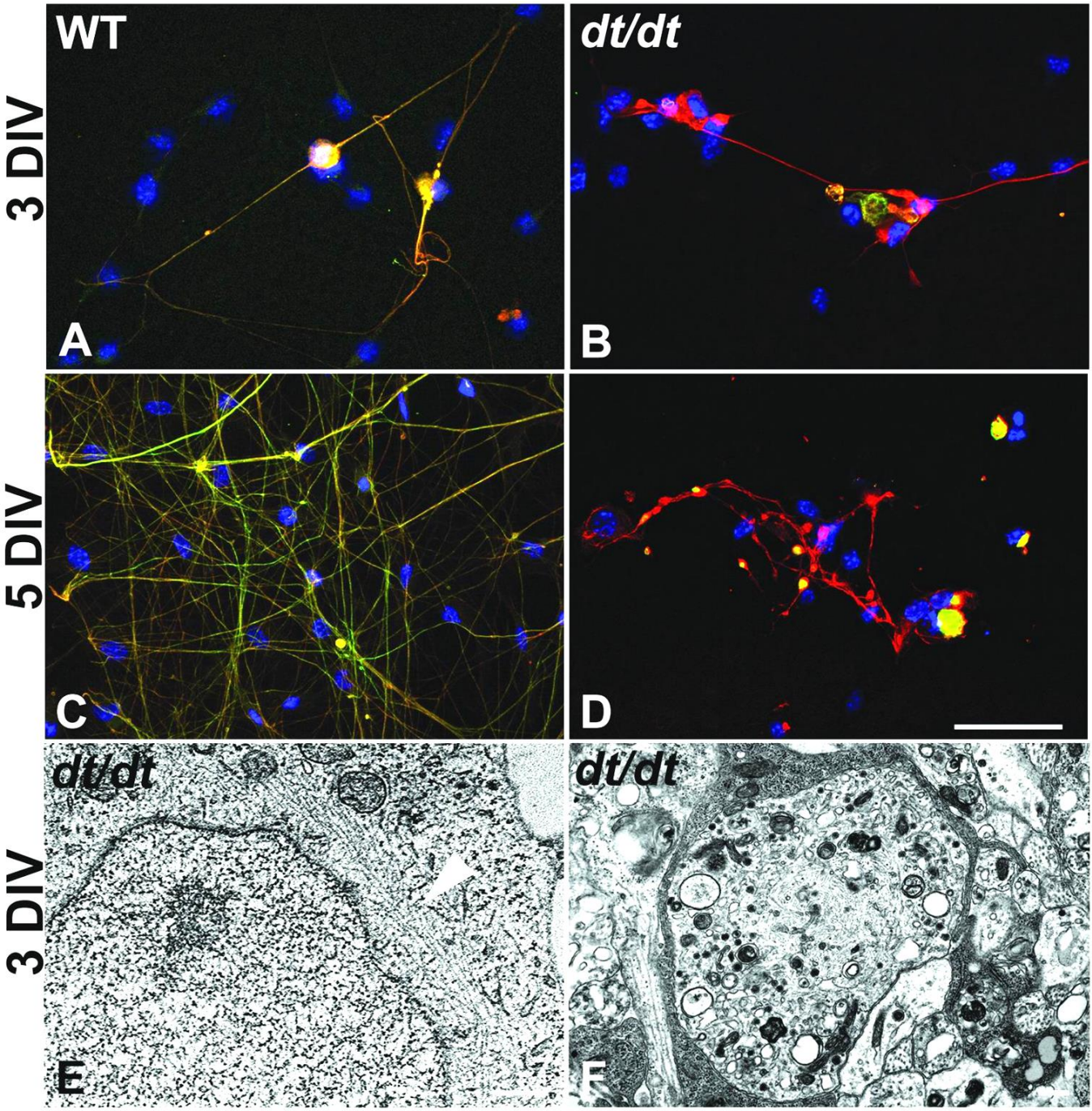


FIGURE 7. TUNEL assays and ultrastructural observations on the cell death of cultured DRG neurons at 5DIV from the wild-type and *dt* mutant mice. No TUNEL-positive cells could be found from the cultured DRG cells of wild-type embryos **(A)**, but some TUNEL-positive cells could be detected from those of *dt/dt* embryos **(B)**. At the ultrastructural level, the cell nucleus of DRG neuron from wild-type embryos exhibited typical nuclear morphology with a nucleolus **(C)**, whereas the nucleus of DRG neuron from *dt/dt* embryos exhibited apoptotic characteristic of chromatin condensation with intact nuclear envelope **(D)**. Scale bars = **(A, B)** 40 μm; **(C, D)** 1 μm.

Cultured DRG neurons from E15.5 embryos

a-interenxin proteins are accumulated in the cell bodies as well as in the processes of *dt/dt* neurons.

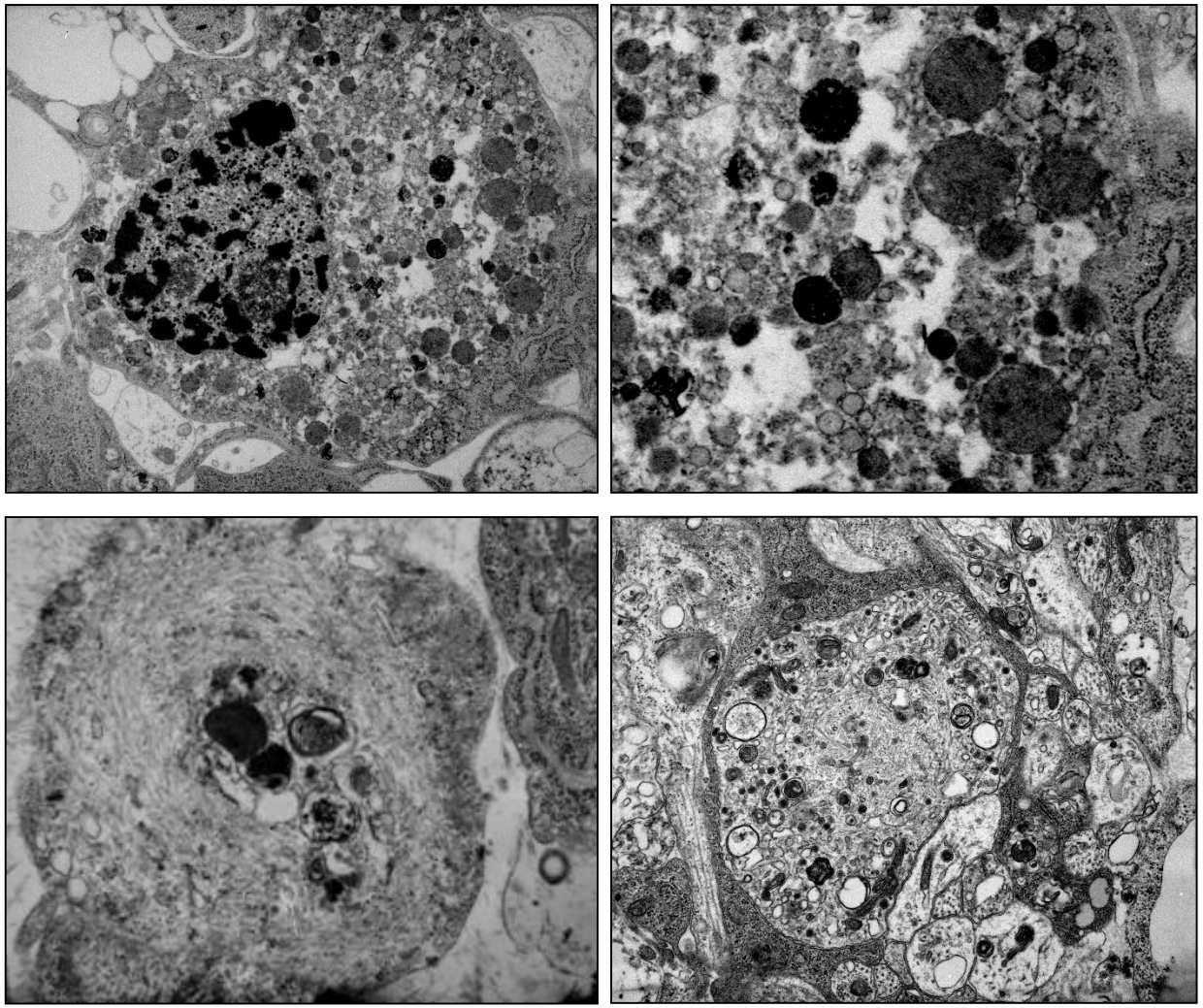


Primary culture of DRG neurons

DRG neurons of *dt/dt* mice observation by Electron microscope

- Chromatin condensation
- IFs accumulation
- Axonal swelling

➢ Cell apoptosis



Pre-embedding immunoEM

Tseng et al.,
J. Comp. Neurol. 2008

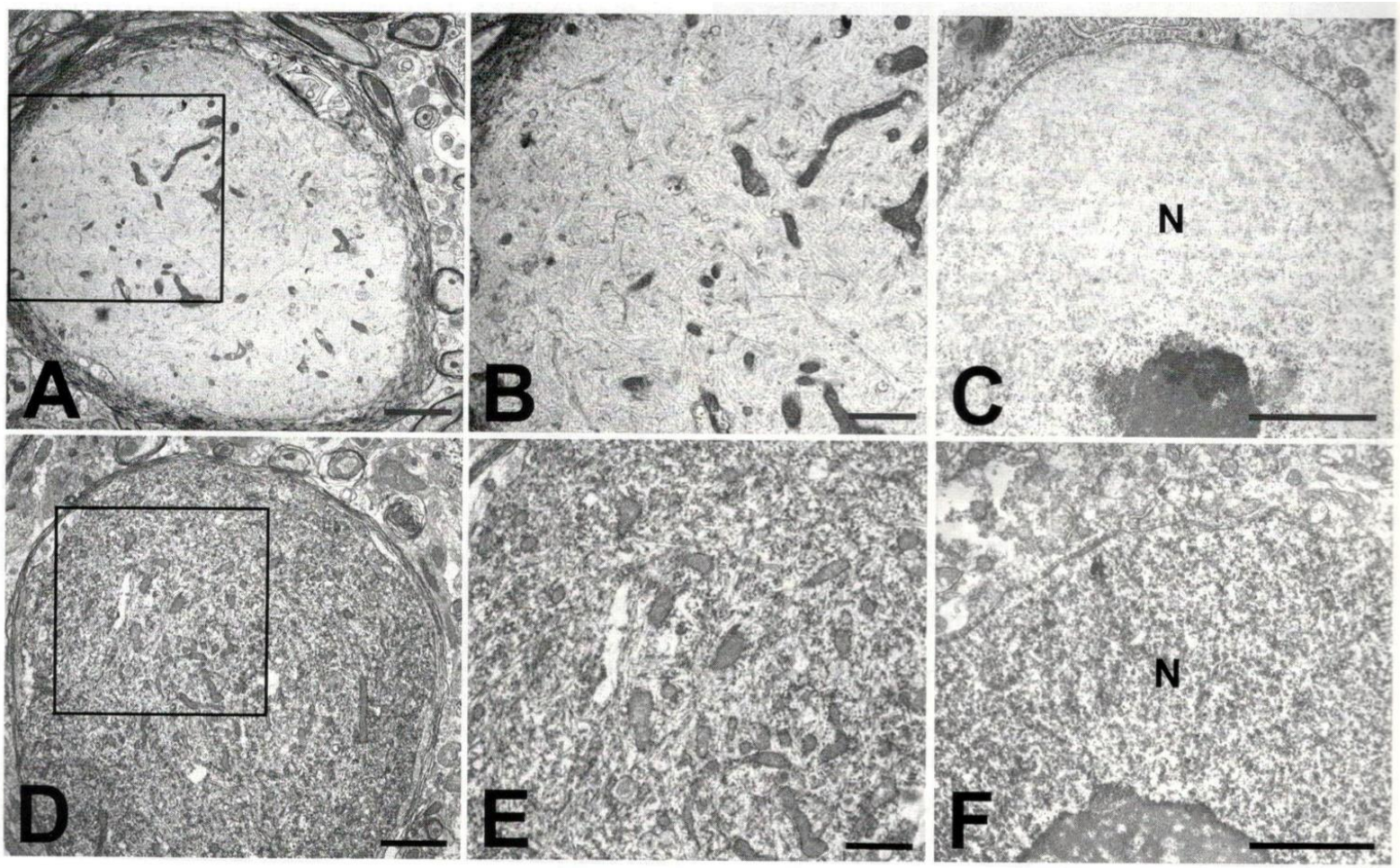


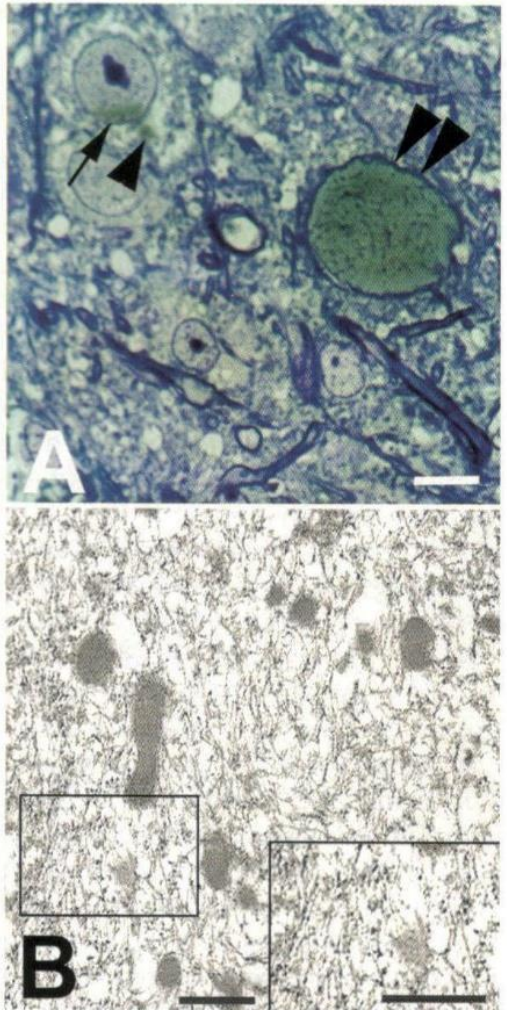
Fig. 6. Preembedding immunoelectron microscopy of the swelling axon and the nucleus of motor neuron from *dt/dt* mutant. Ultrathin sections obtained from *dt/dt* spinal cords were stained with uranyl acetate and lead citrate (**A–C**), or stained with antibody to α-internexin (**D–F**), and examined by electron microscopy. Randomly orientated intermediate filaments and axonal organelles were found in the swelling axons (A,B), yet the morphology of the cell nucleus looked normal in the spinal neuron of the *dt/dt* (C). α-Internexin (dark DAB reaction products) was detected in the swelling axon (D,E) and also in the nucleus (F) of spinal motor neuron by immunoelectron microscopy. N, nucleus. Scale bars=1 μm in A,D; 500 nm in B,C,E,F.

Post-embedding immunoEM

Tseng et al.,
J. Comp. Neurol. 2008

The Journal of Comparative Neurology. DOI 10.1002/cne

ABNORMAL TRANSLOCATION OF A-INTERNEXIN



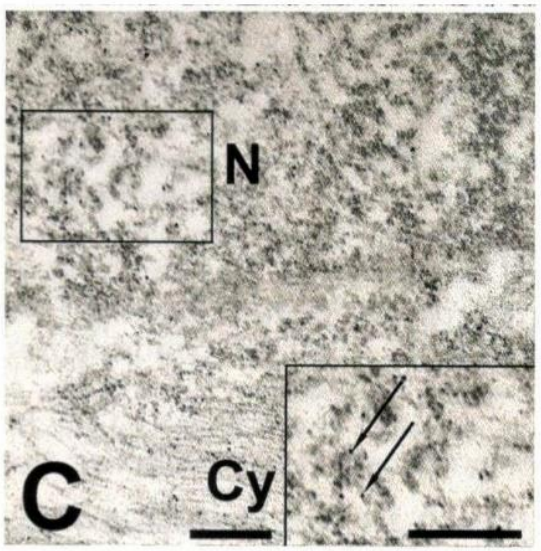
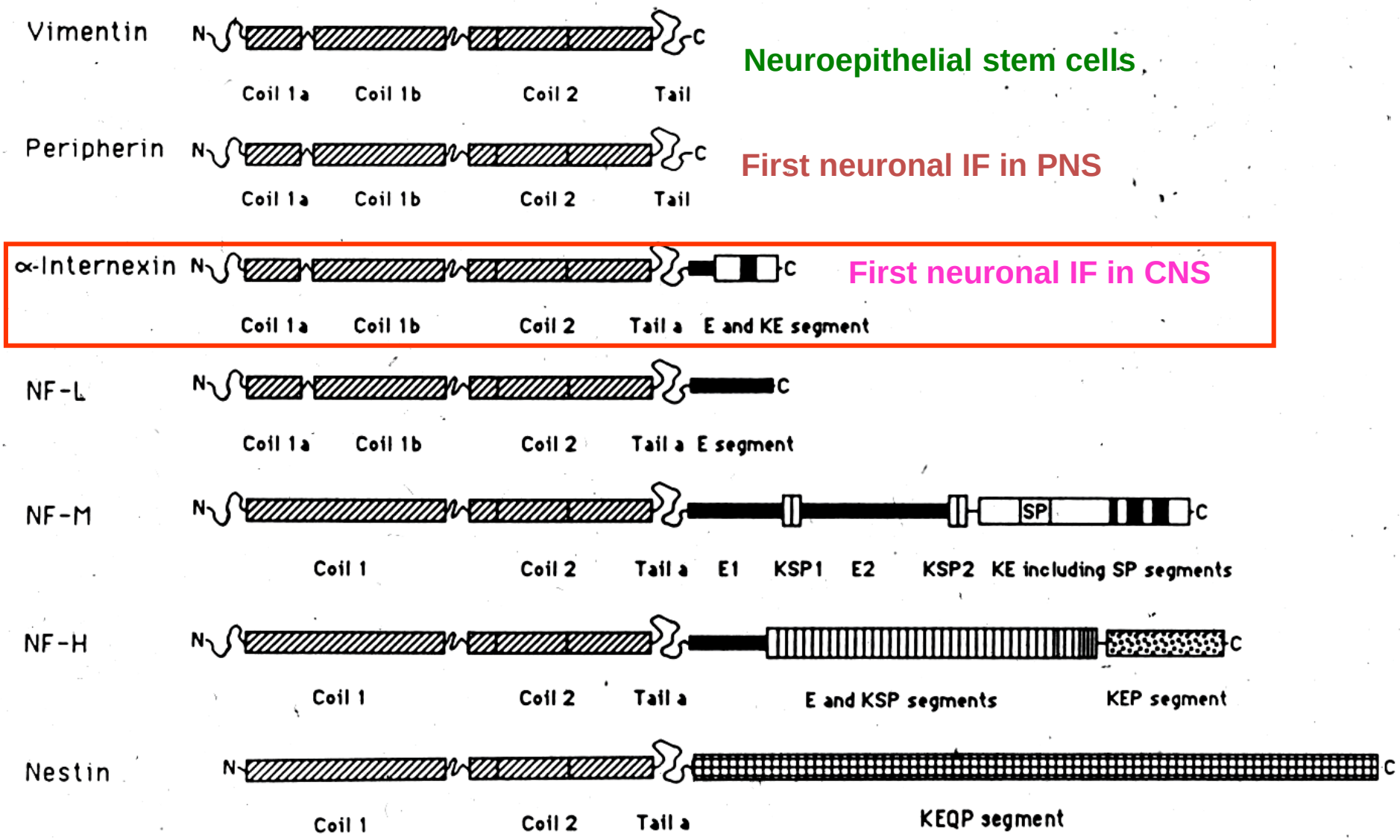
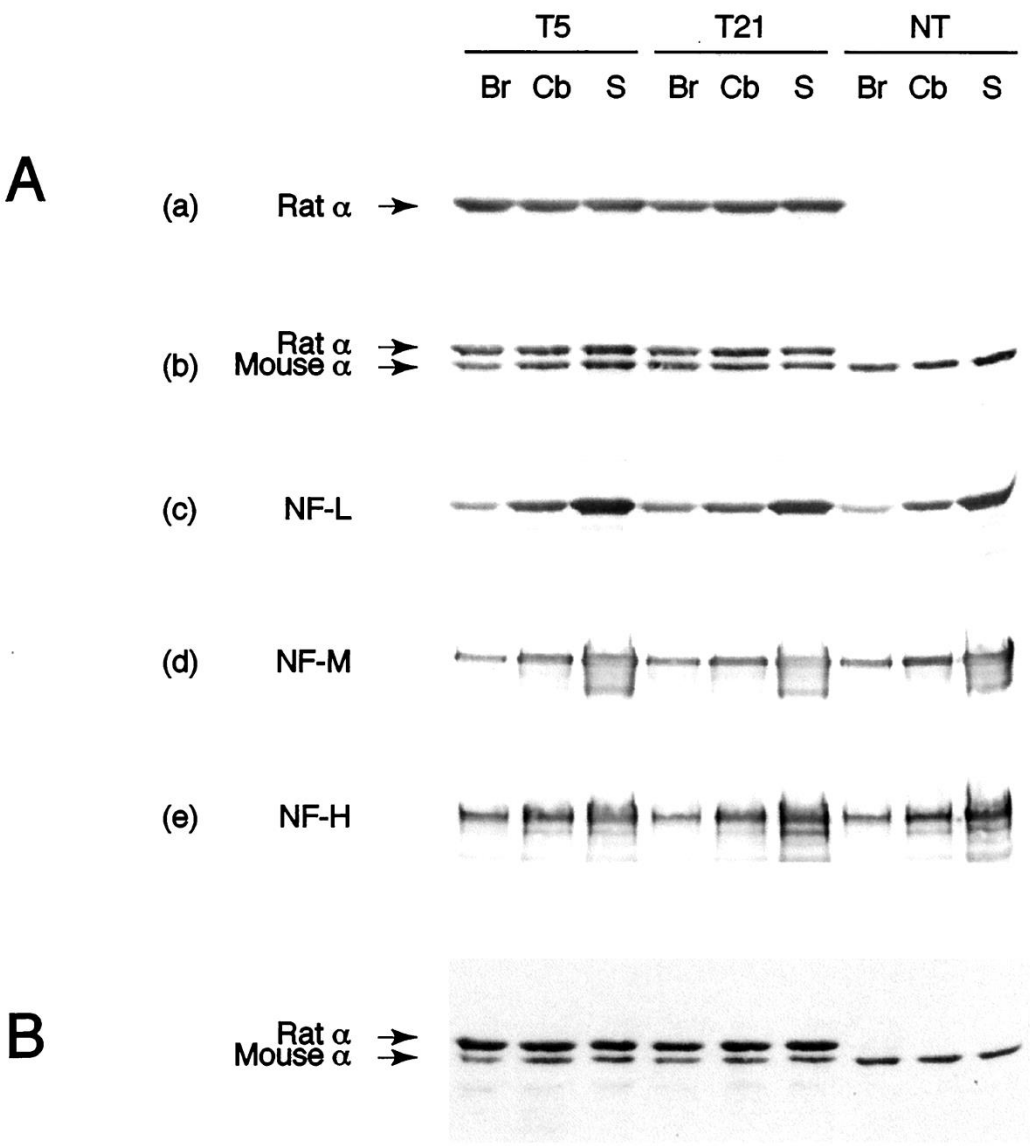


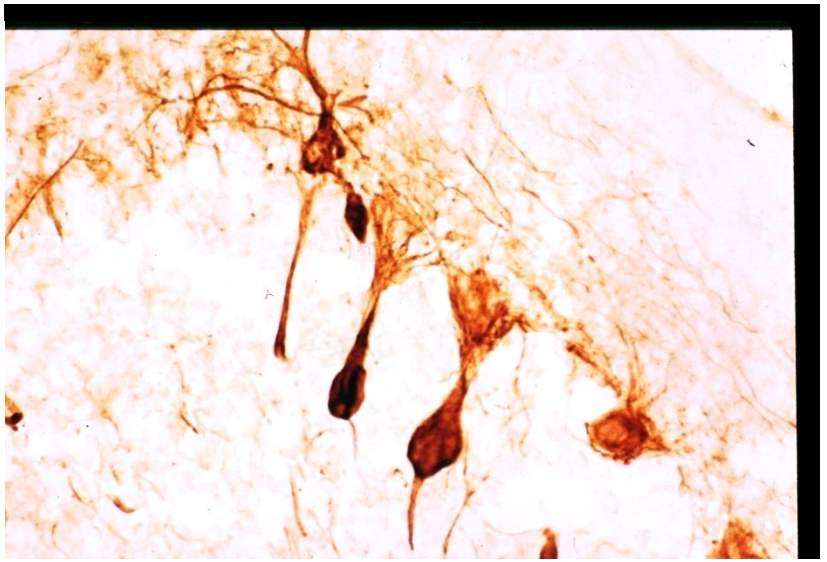
Fig. 7. Postembedding immunoelectron microscopy of swelling axon and the nucleus of motor neuron from *dt/dt* mutant. Sections were stained with antibody to α-internexin and then revealed by DAB reaction (**A**) or gold particles (**B,C**). In the semithin section, immunopositive α-internexin was found in the cytoplasm (arrowhead) and also in the nucleus (arrow) of spinal motor neuron. Further, immunopositive α-internexin was also observed in the myelinated swelling axon (double arrowheads) in the ventral horn of *dt/dt* mice (A). In the ultrathin section, filamentous α-internexin labeled with 10 nm gold particles was found in the swelling axon (B) and cytoplasm (C). Note that some immunopositive gold particles (arrows) were found in the cell nucleus (C). Cy, cytoplasm; N, nucleus. Scale bars=20 μm in A; 500 nm in B,C.

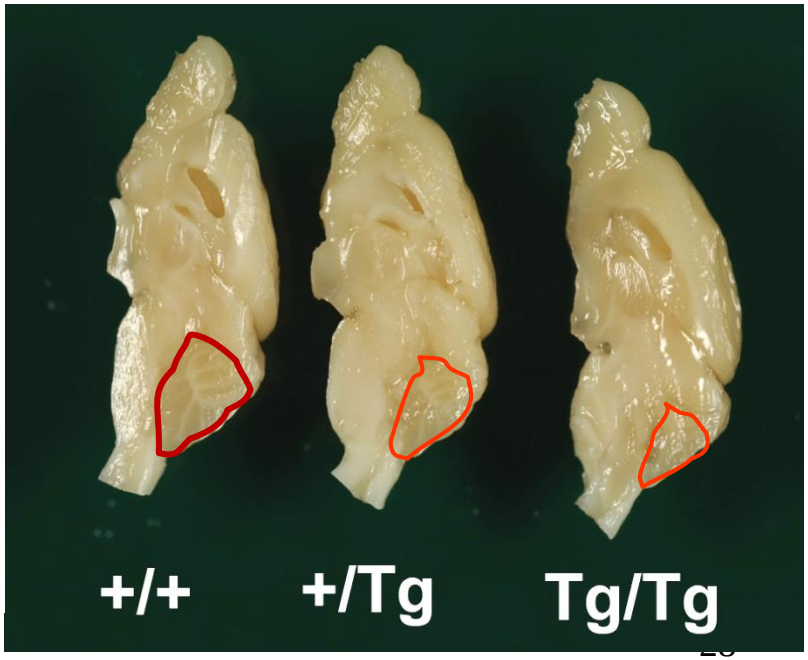
Seven Intermediate Filament Proteins in Neural Differentiation

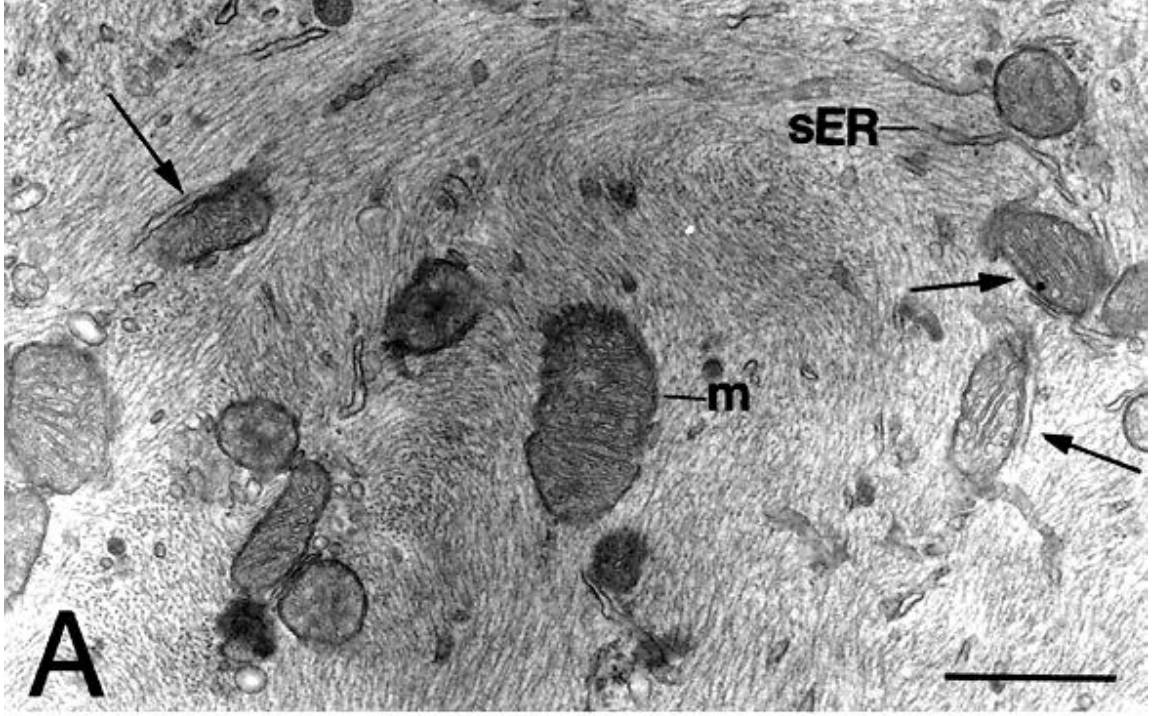


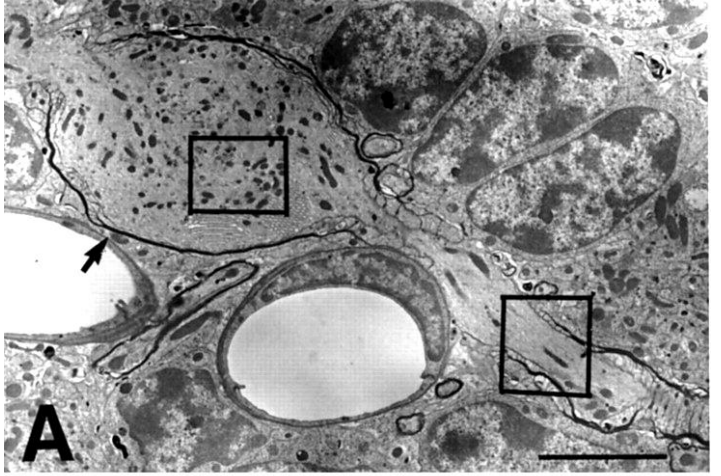
Animal model for cerebellar atrophy (J. Neurosci. 19:2974-2986, 1999)

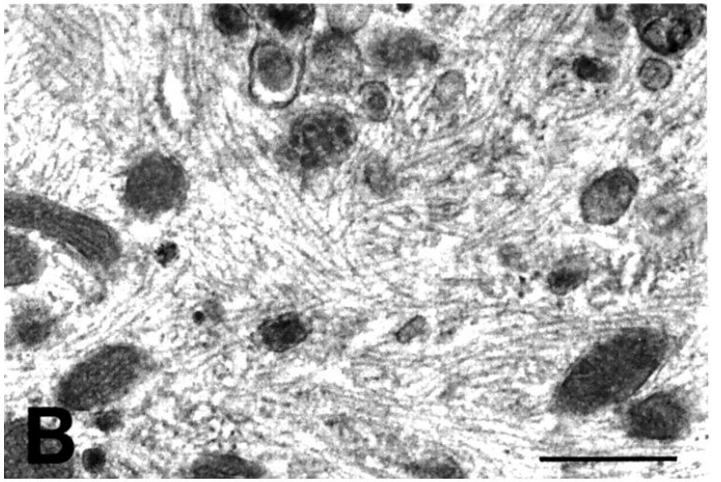


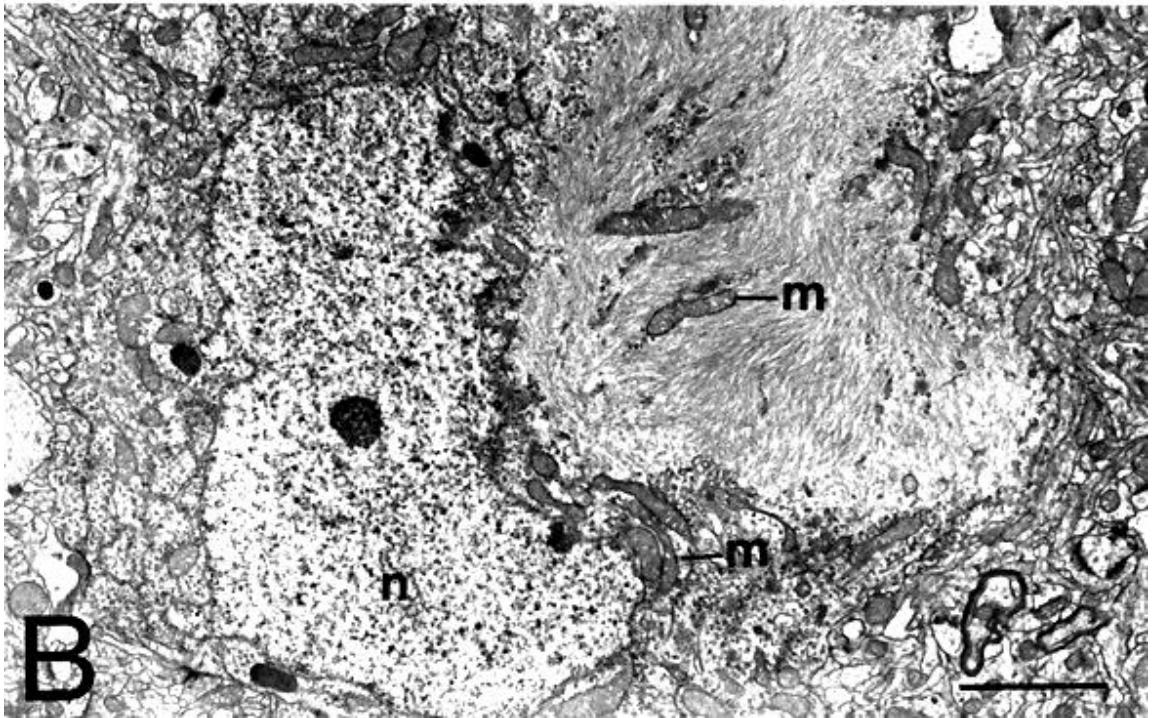


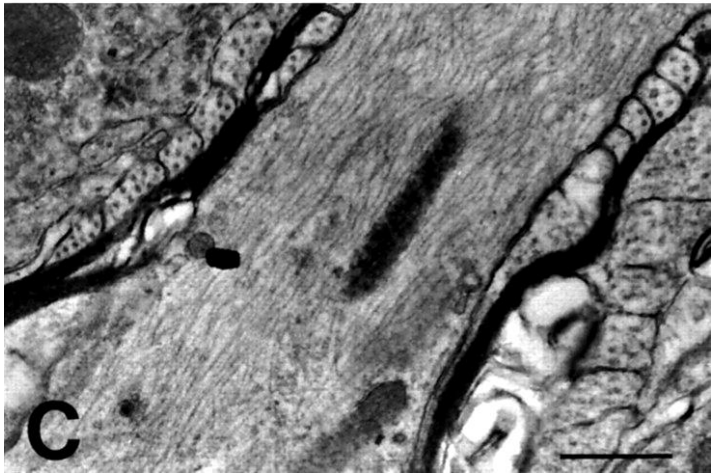



sER
m
A


A


B

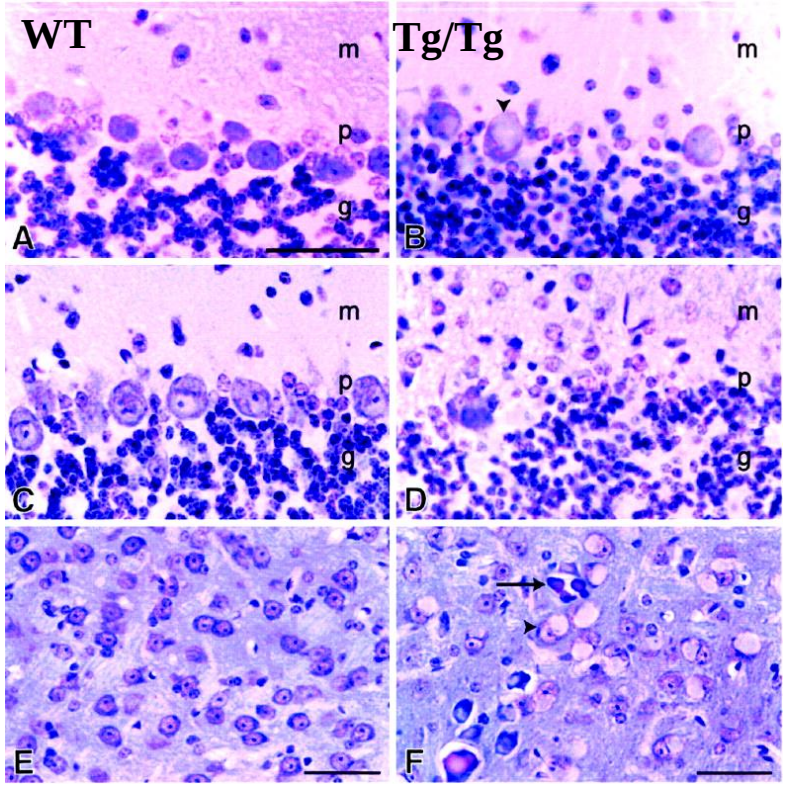

m
m
n
B

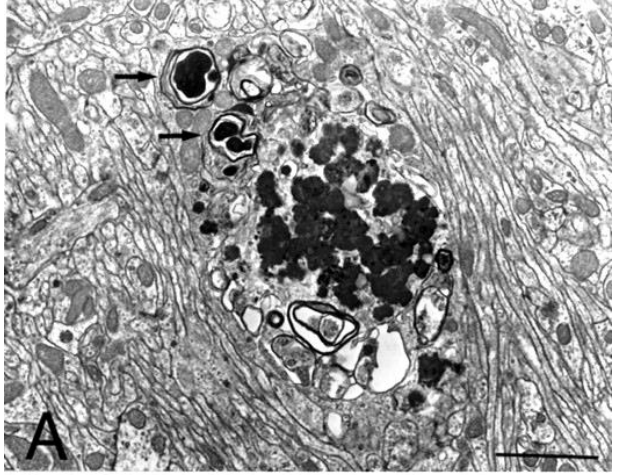

C

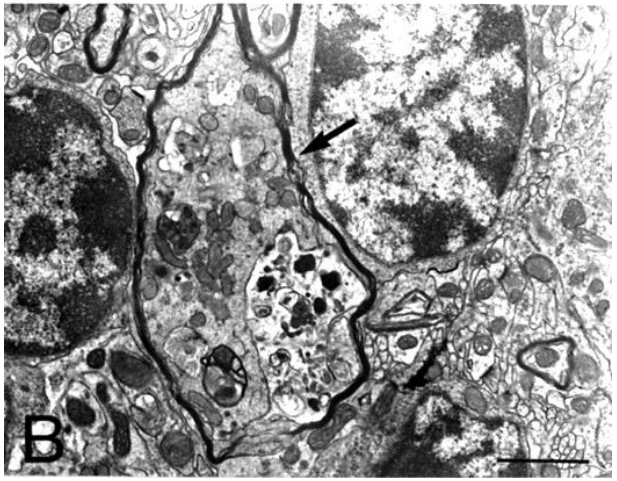
12 m cerebella

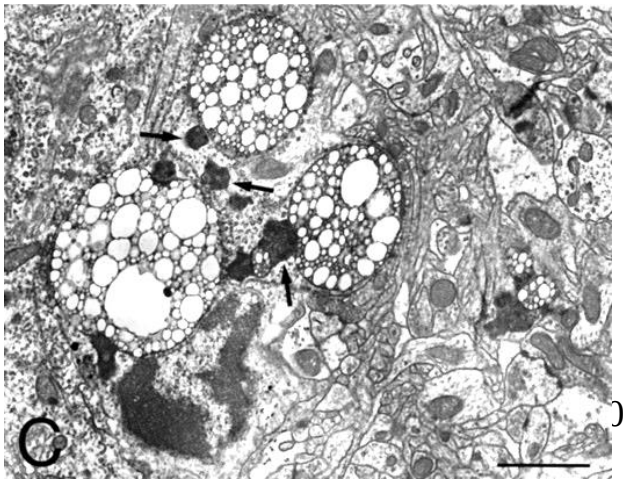
18 m cerebella

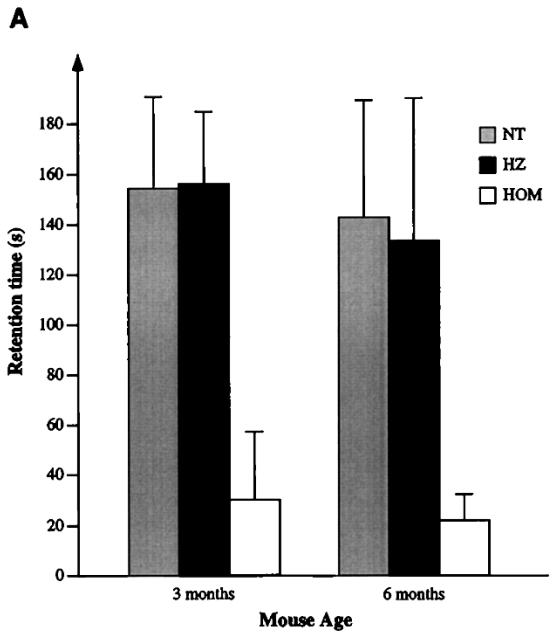
18 m thalamus

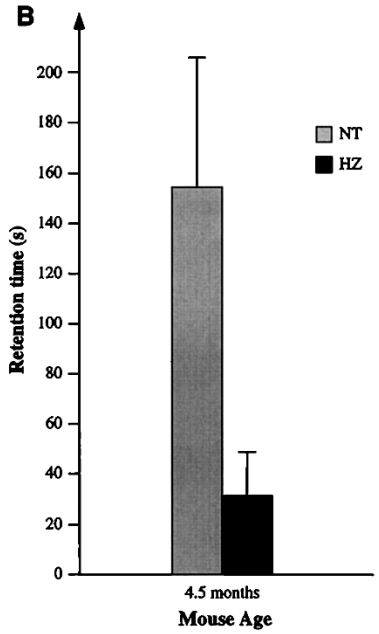


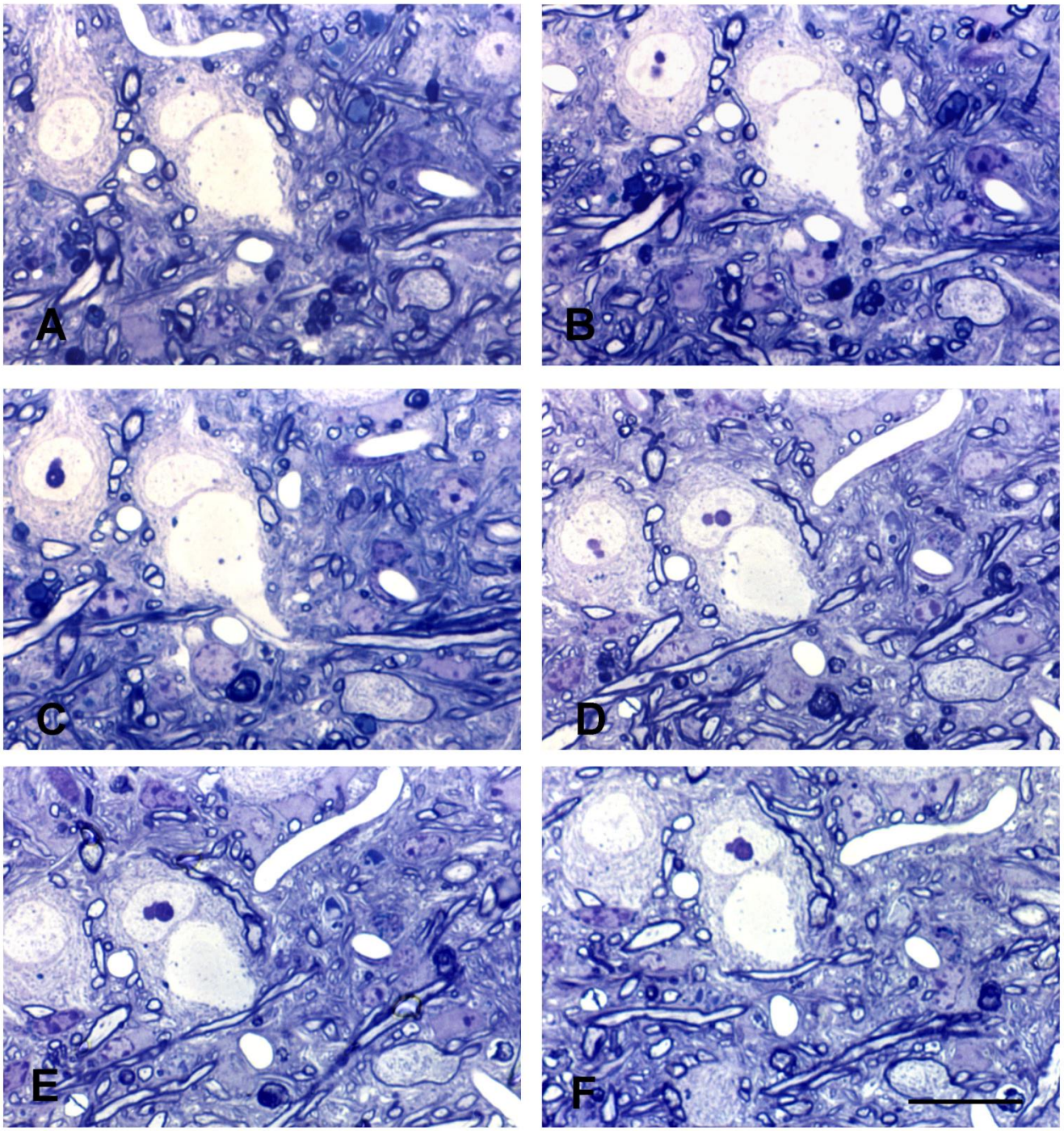


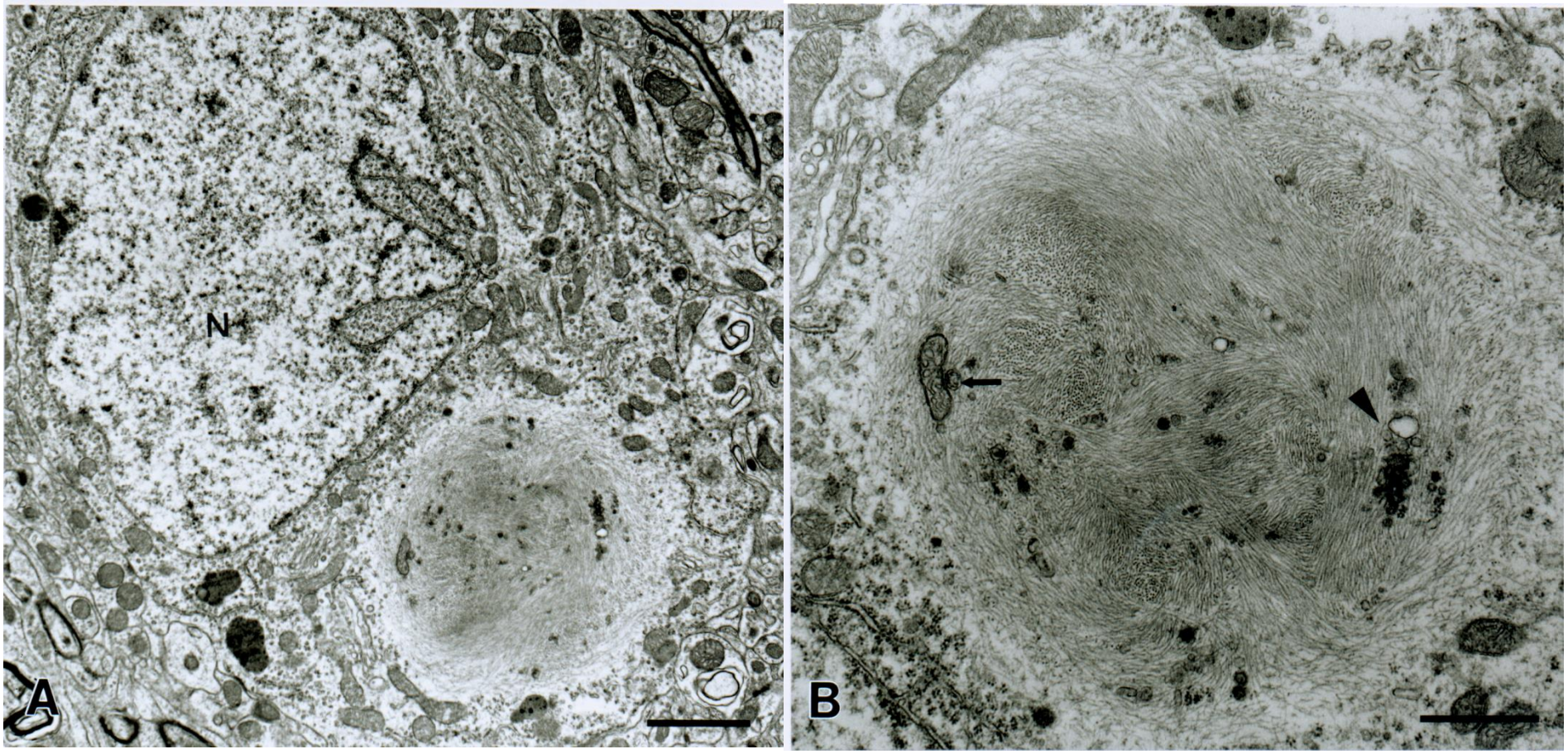









A
B
C
D
E
F


N
A
B

Journal of Neuroscience Research 80:693–706 (2005)

Overexpression of Neuronal Intermediate Filament Protein α-Internexin in PC12 Cells

Chung-Liang Chien,[1]* Tzu-Chiang Liu,[1] Chung-Liang Ho,[2] and Kuo-Shyan Lu[1]

[1]Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei, Taiwan
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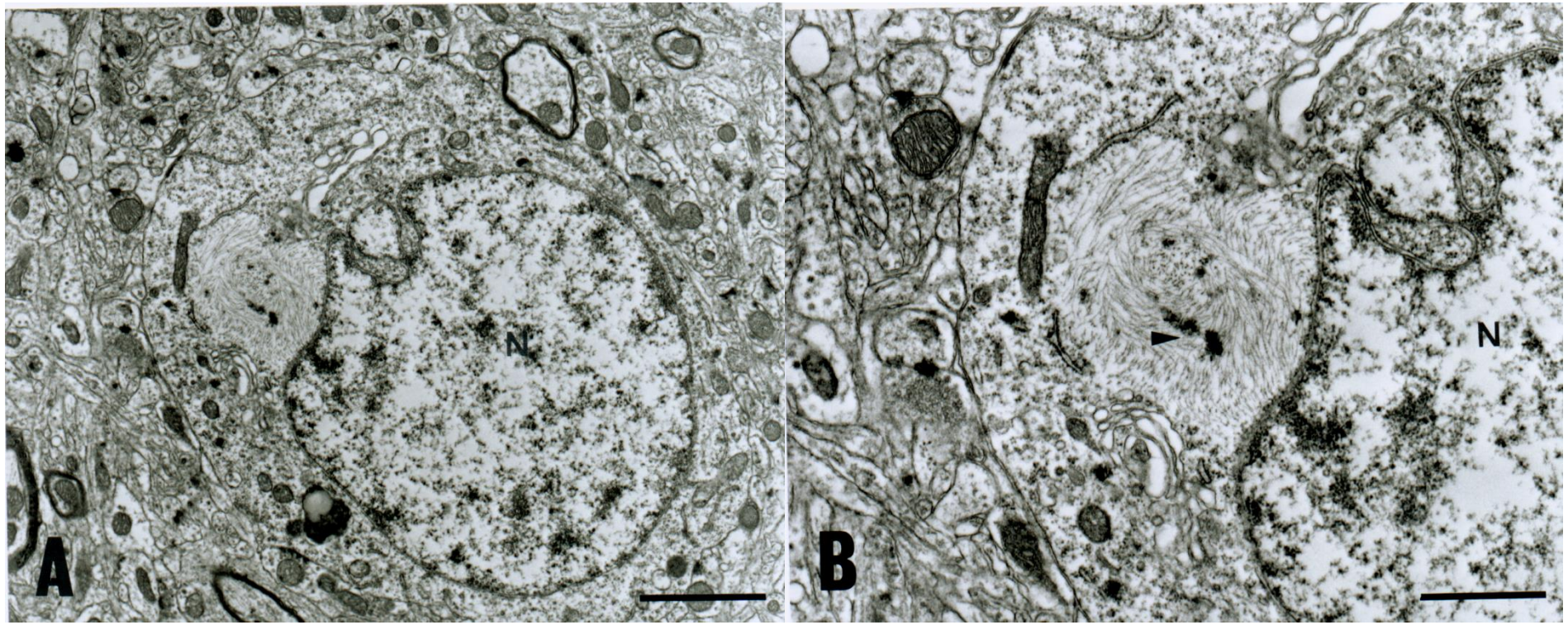
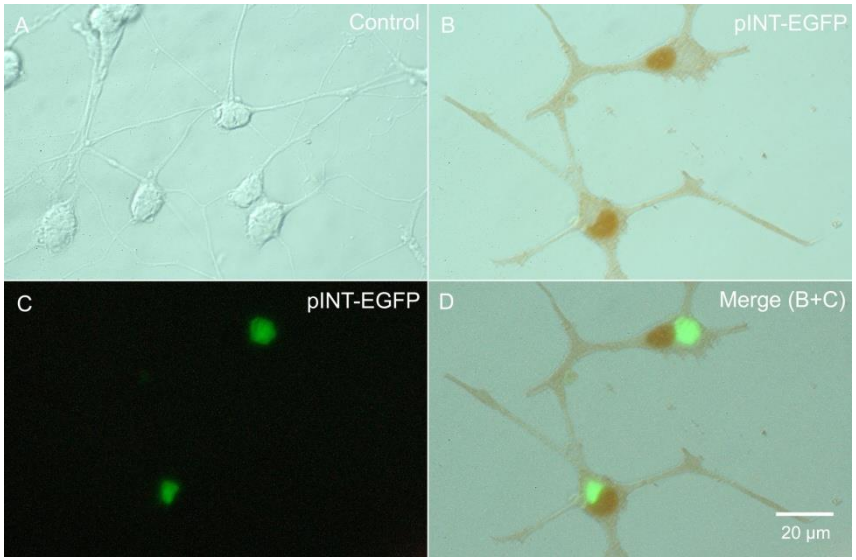
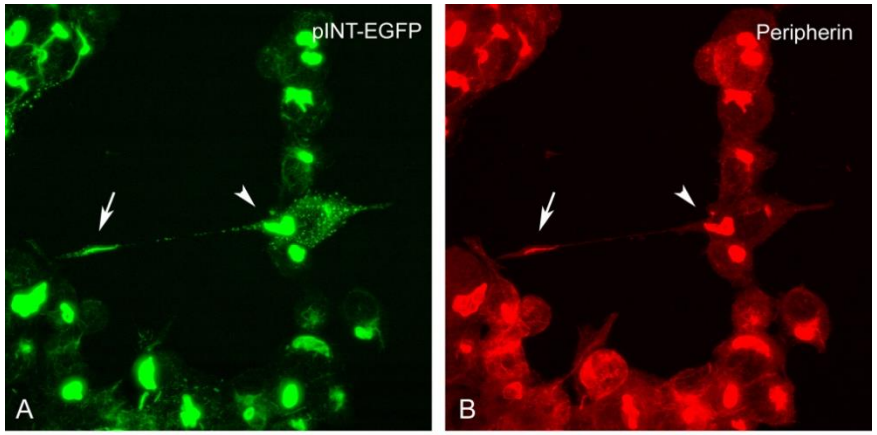
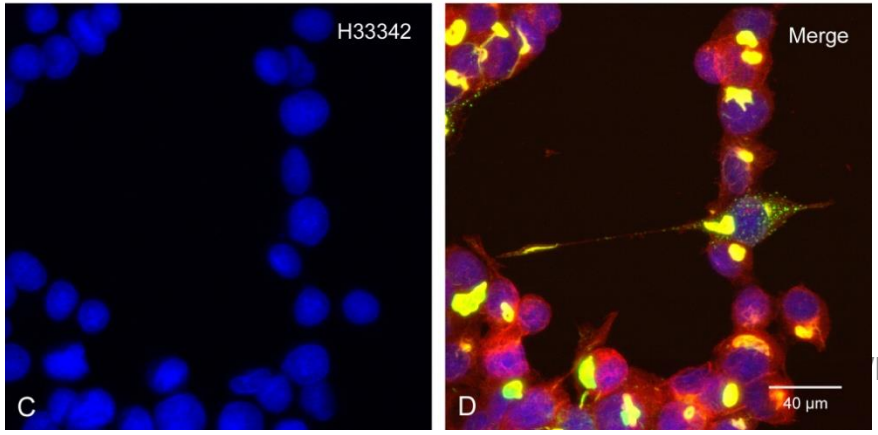
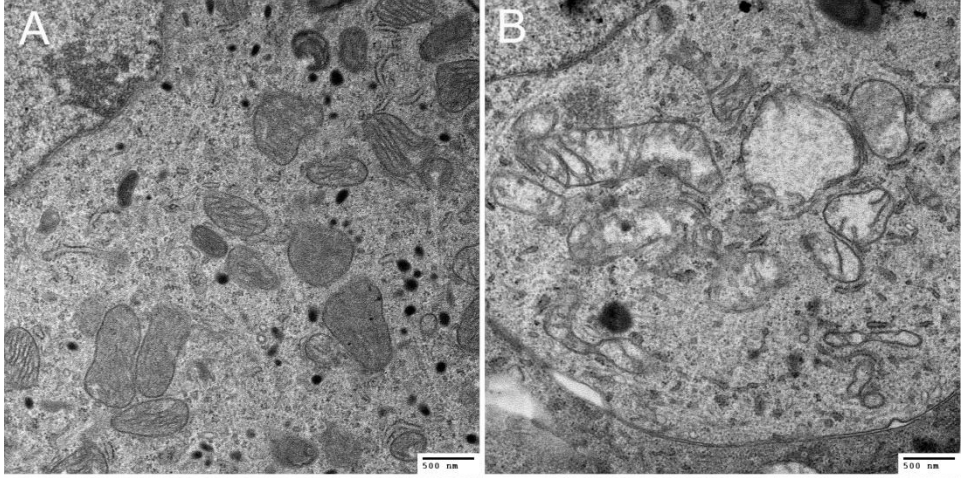
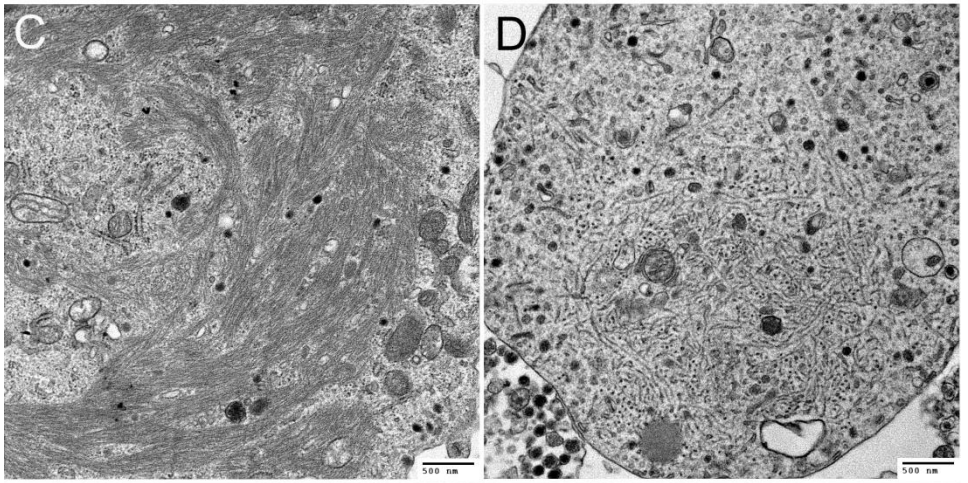


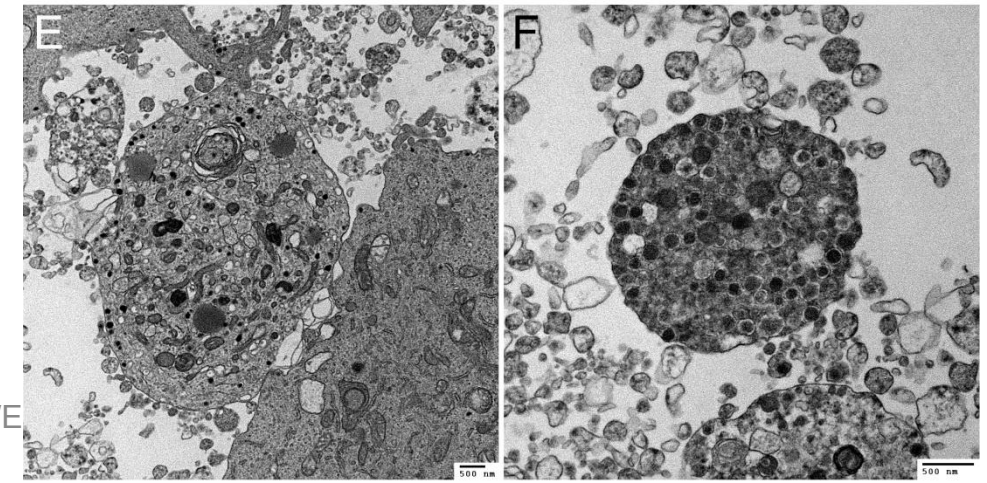

A
Control
B
pINT-EGFP
C
pINT-EGFP
D
Merge (B+C)
20 µm

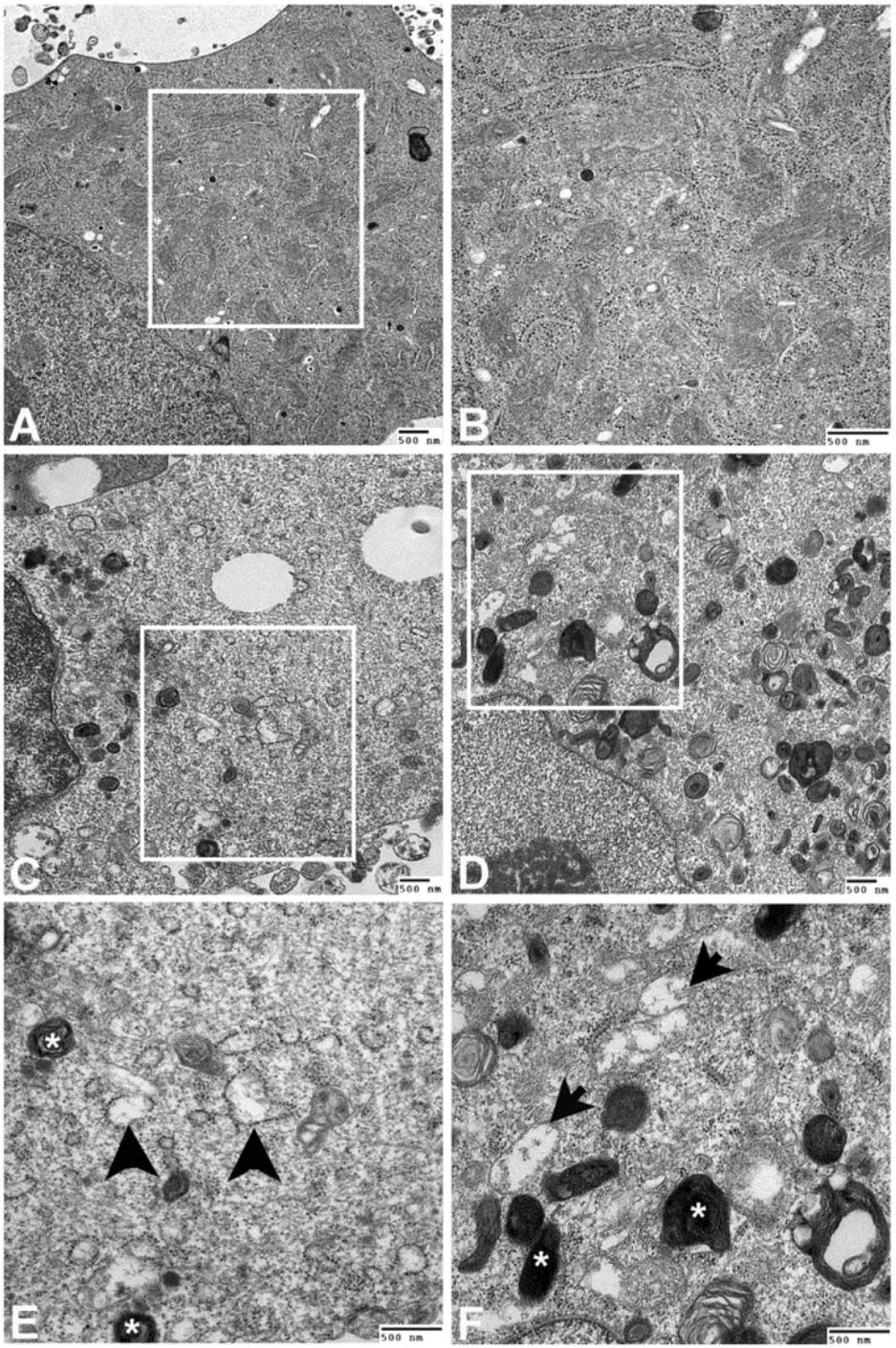

pINT-EGFP
Peripherin
A
B

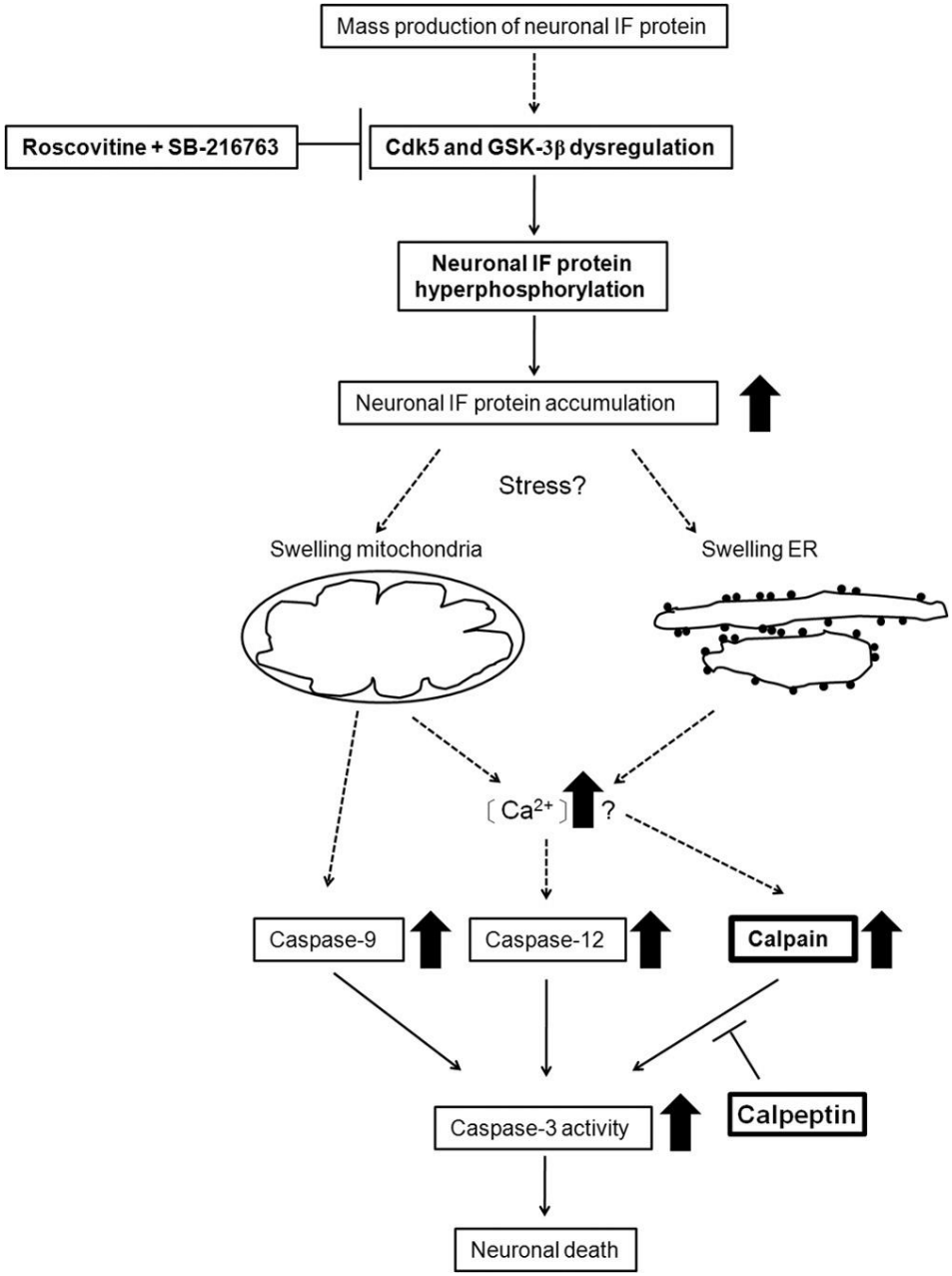

H33342
Merge
C
D
40 µm

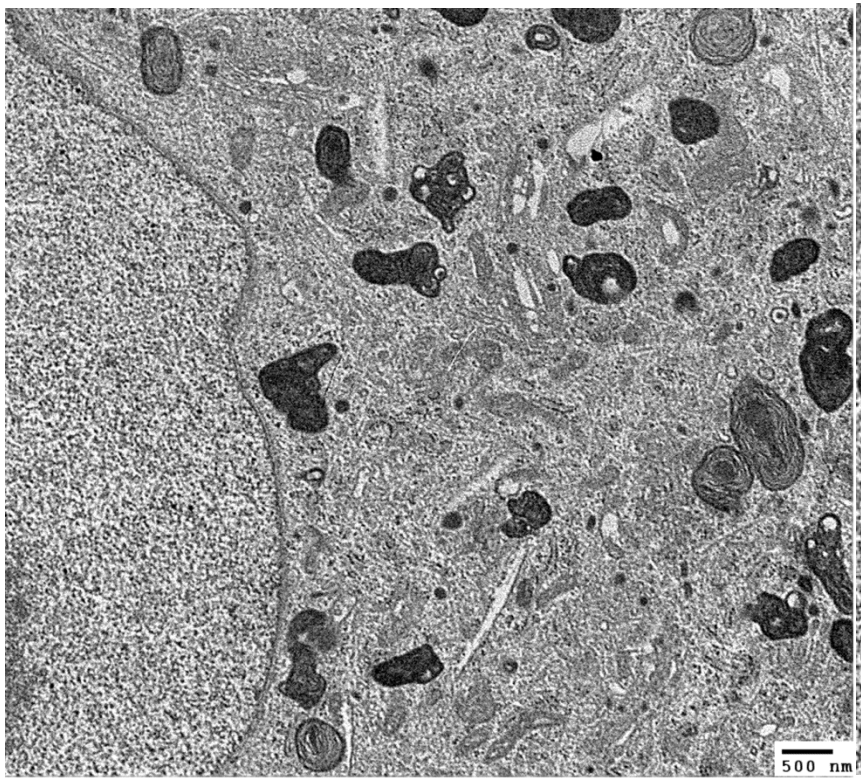

A
B
500 nm
500 nm

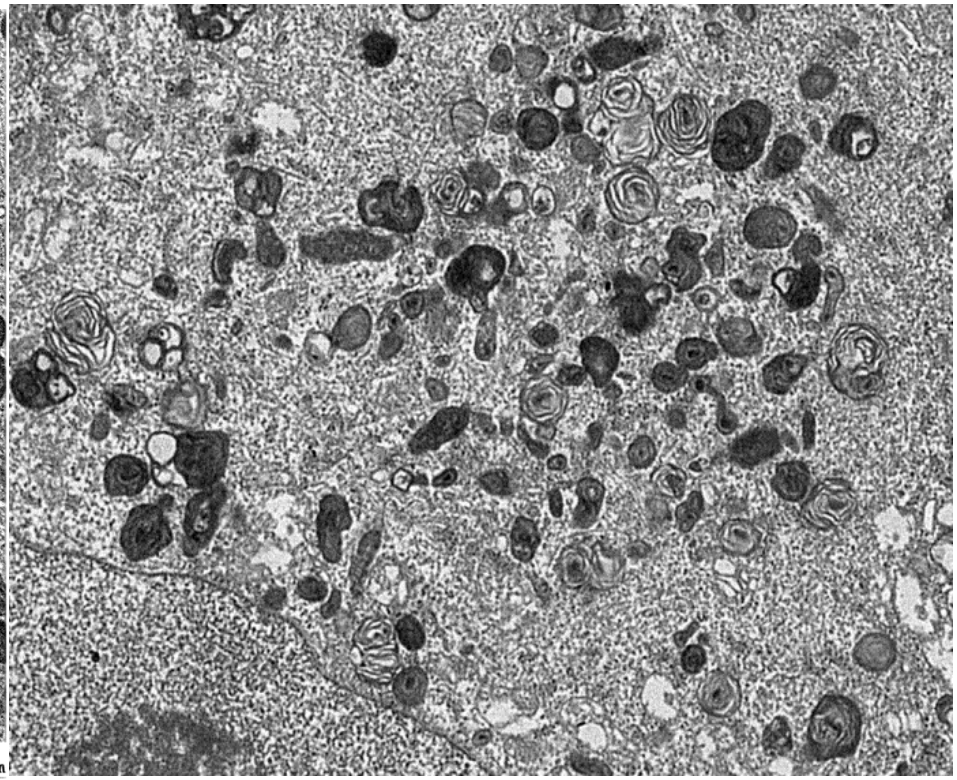

C
D
500 nm
500 nm


E
F
500 nm
500 nm


A
500 nm
B
500 nm
C
500 nm
D
500 nm
E
500 nm
F
500 nm

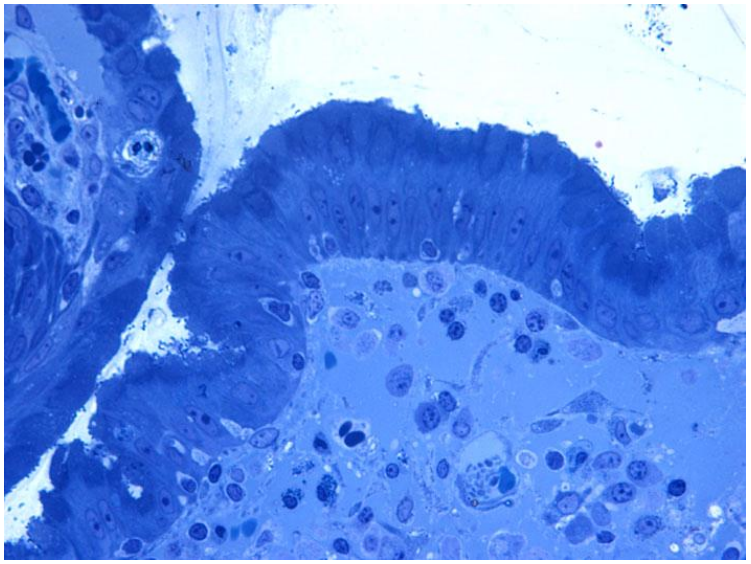

Mass production of neuronal IF protein
Roscovitine + SB-216763
Cdk5 and GSK-3β dysregulation
Neuronal IF protein hyperphosphorylation
Neuronal IF protein accumulation
Stress?
Swelling mitochondria
Swelling ER
〔Ca2+〕?
Caspase-9
Caspase-12
Calpain
Caspase-3 activity
Calpeptin
Neuronal death

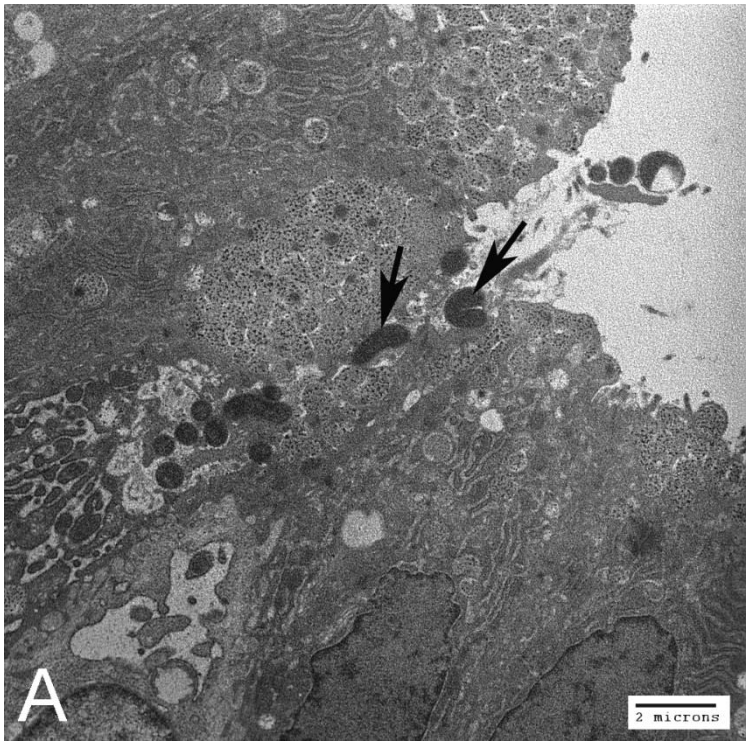

500 nm

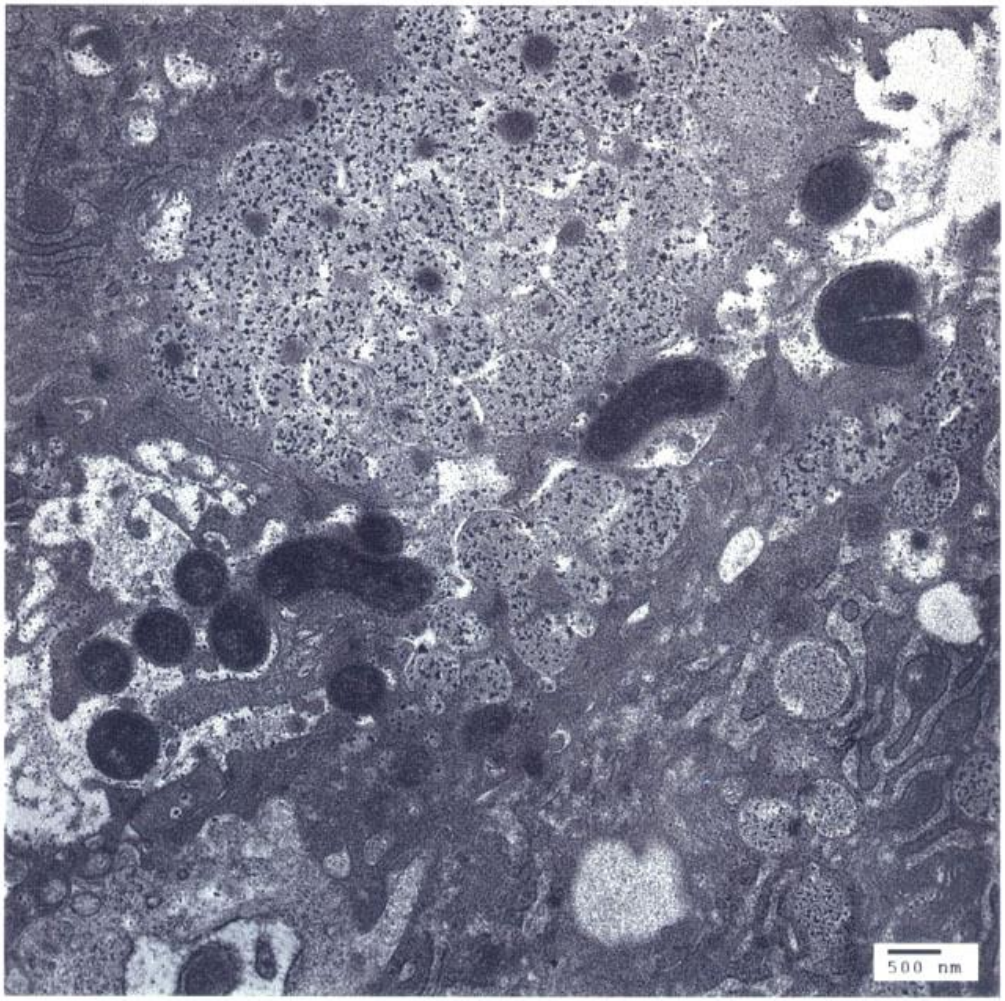


EM Sectioning (Gastric epithelia)

H. pylori between epithelial cells.







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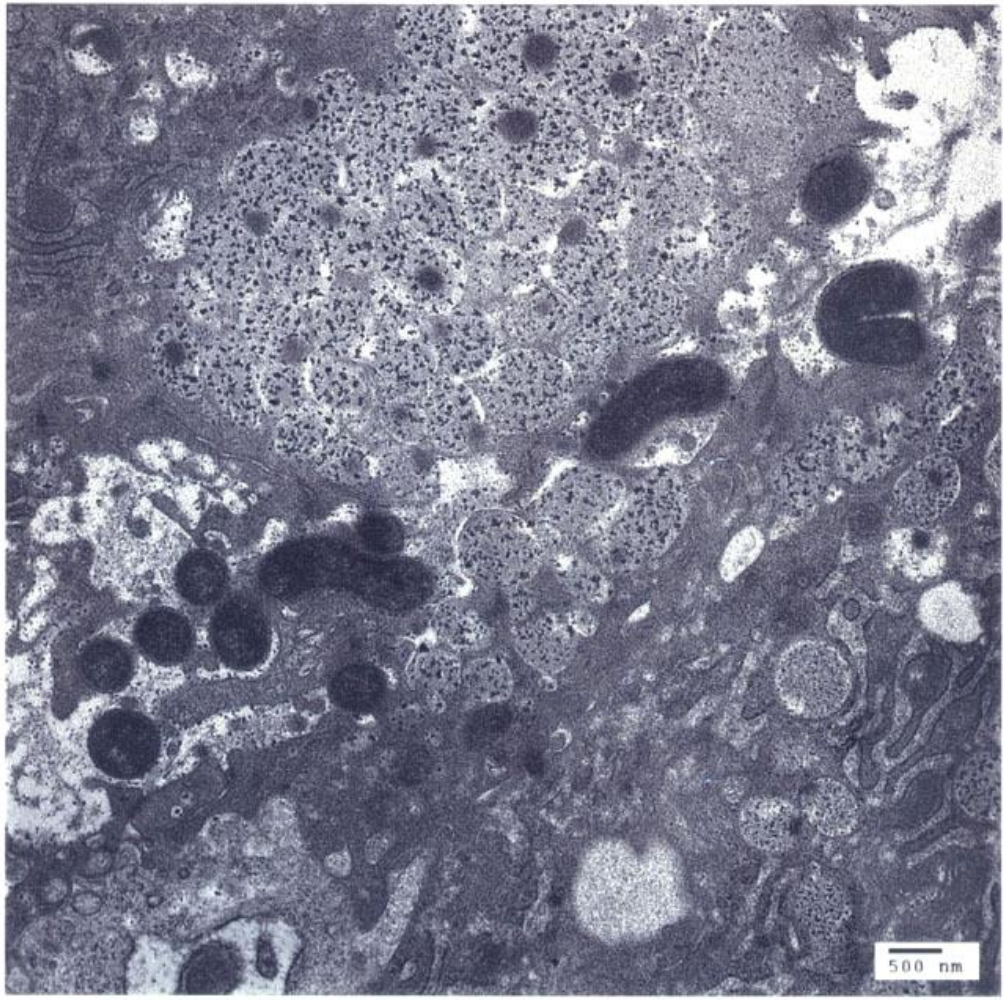
H. pylori between epithelial cells.



Barry Marshall Nobel Laureate

Dr. Barry Marshall
The 2005 in Medicine