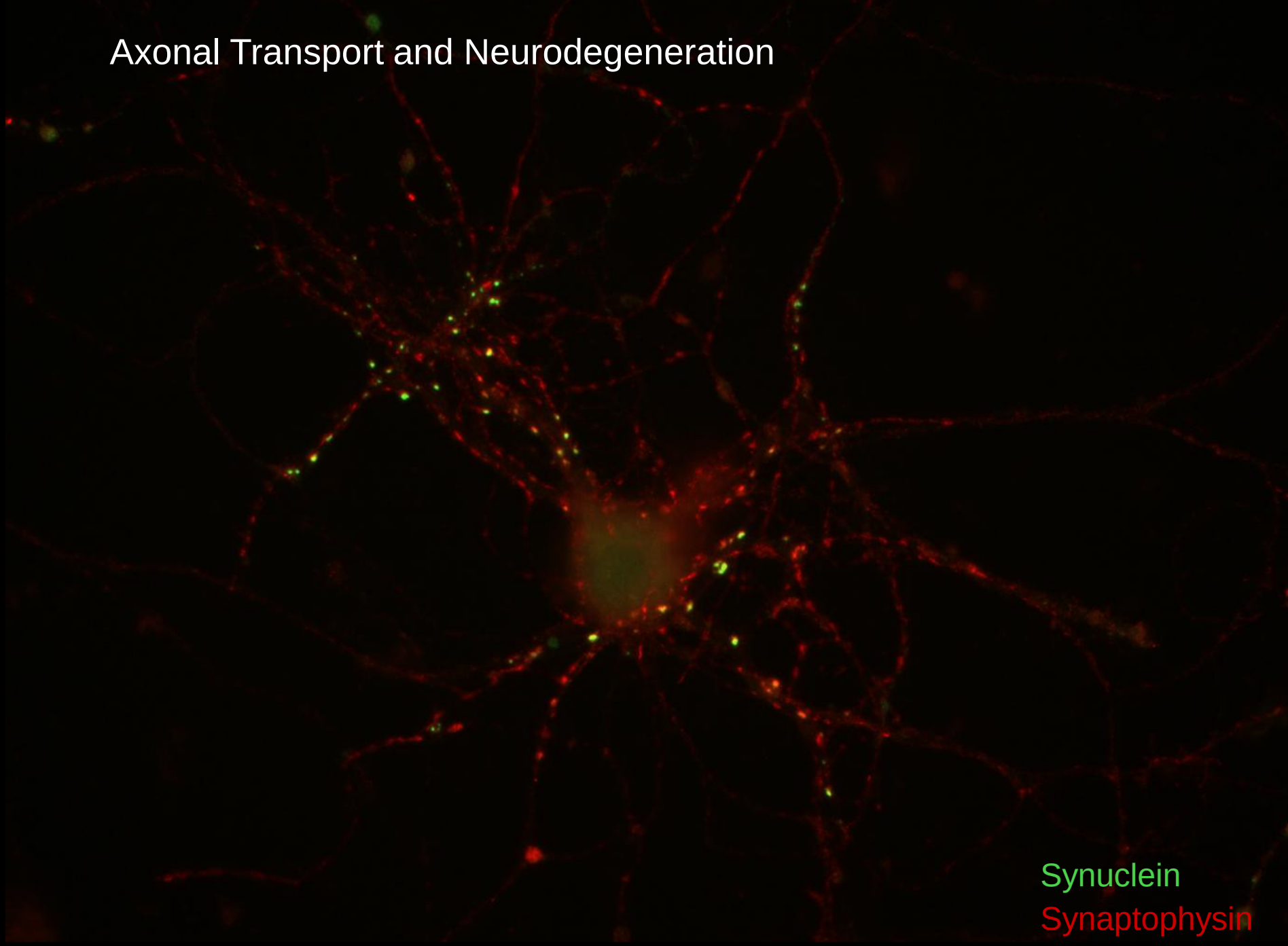
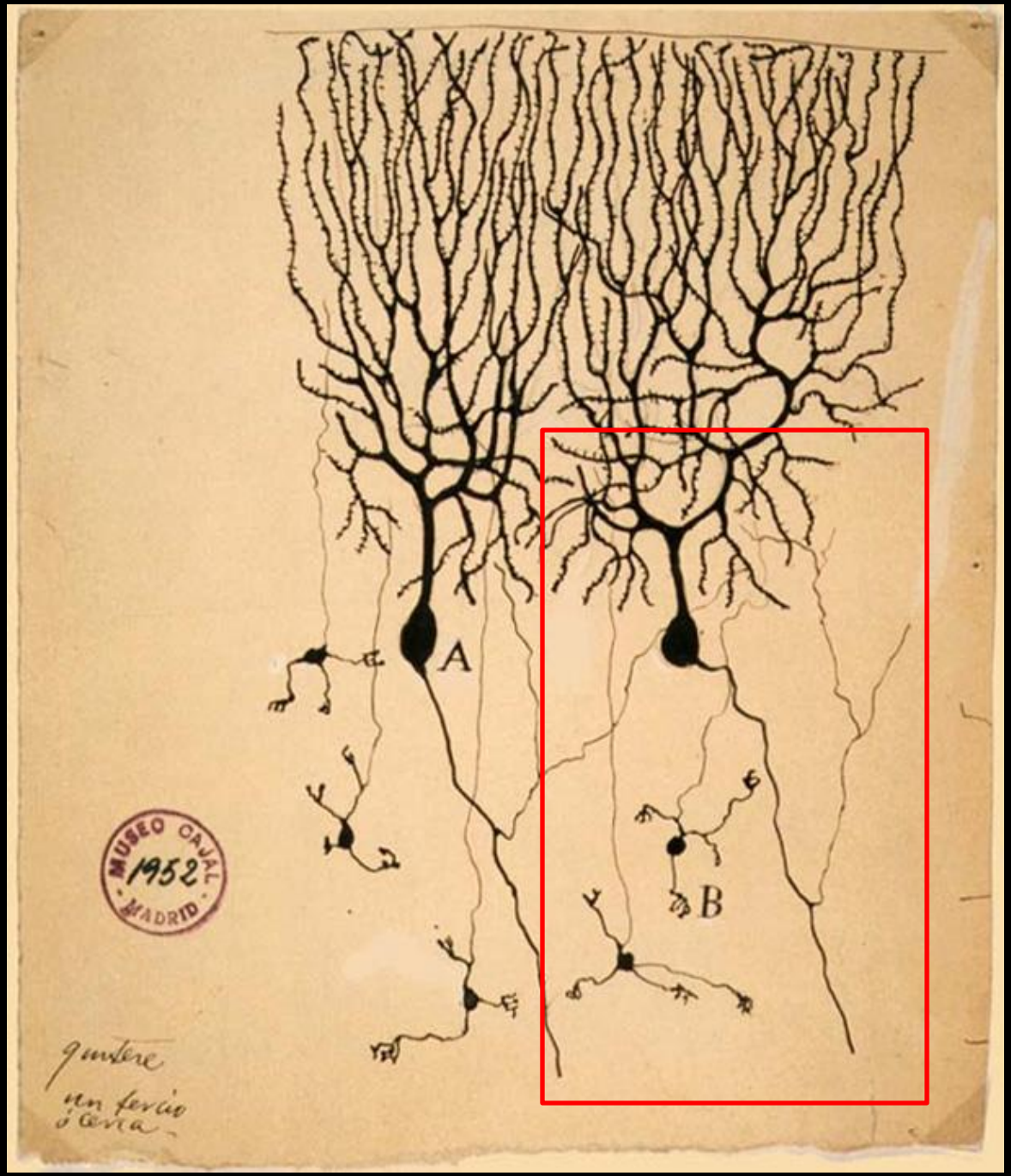
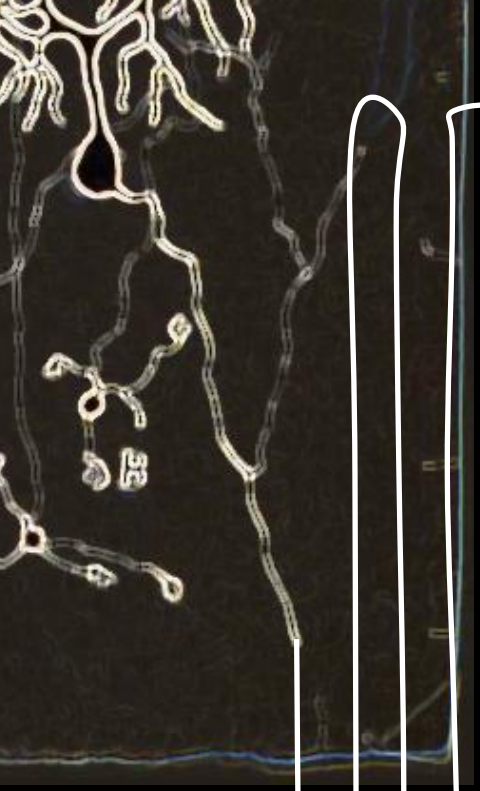
Axonal Transport and Neurodegeneration




A
B
MUSEO CAJAL
1952
MADRID



Simple scaling experiment

- Cell body=10 μm---- Axon=100,000 μm (1m)
- Cell body=50 m-------- Axon=500,000 m
- Cell body=Conference room ----Axon=300+miles

ends in Phoenix

0270-6474/84/0408-1944$02.00/0
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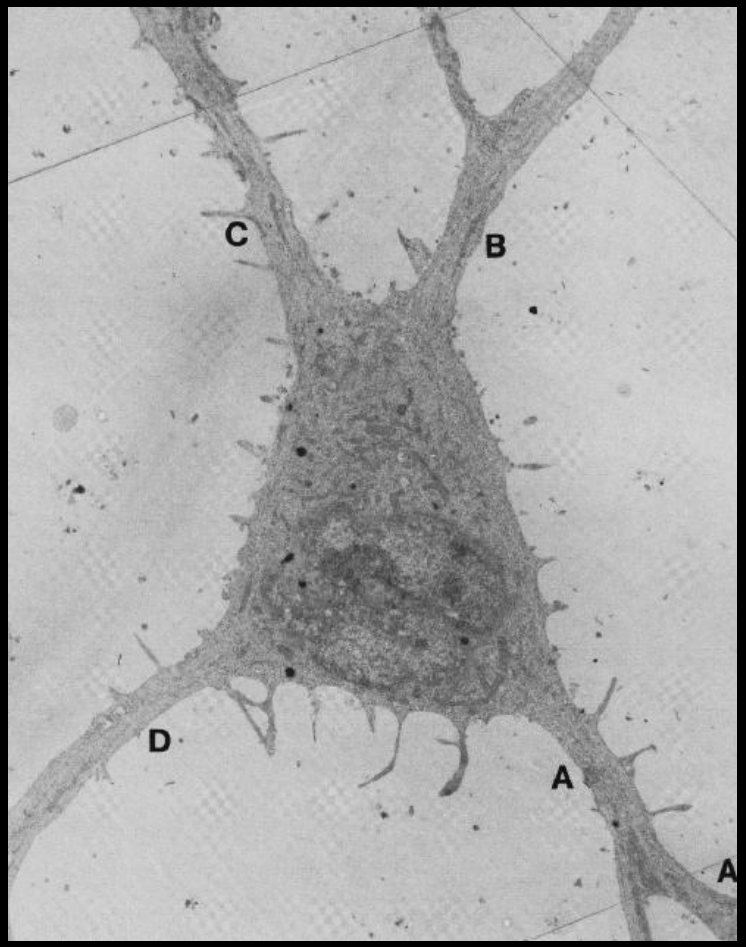
The Journal of Neuroscience
Vol. 4, No. 8, pp. 1944–1953
August 1984

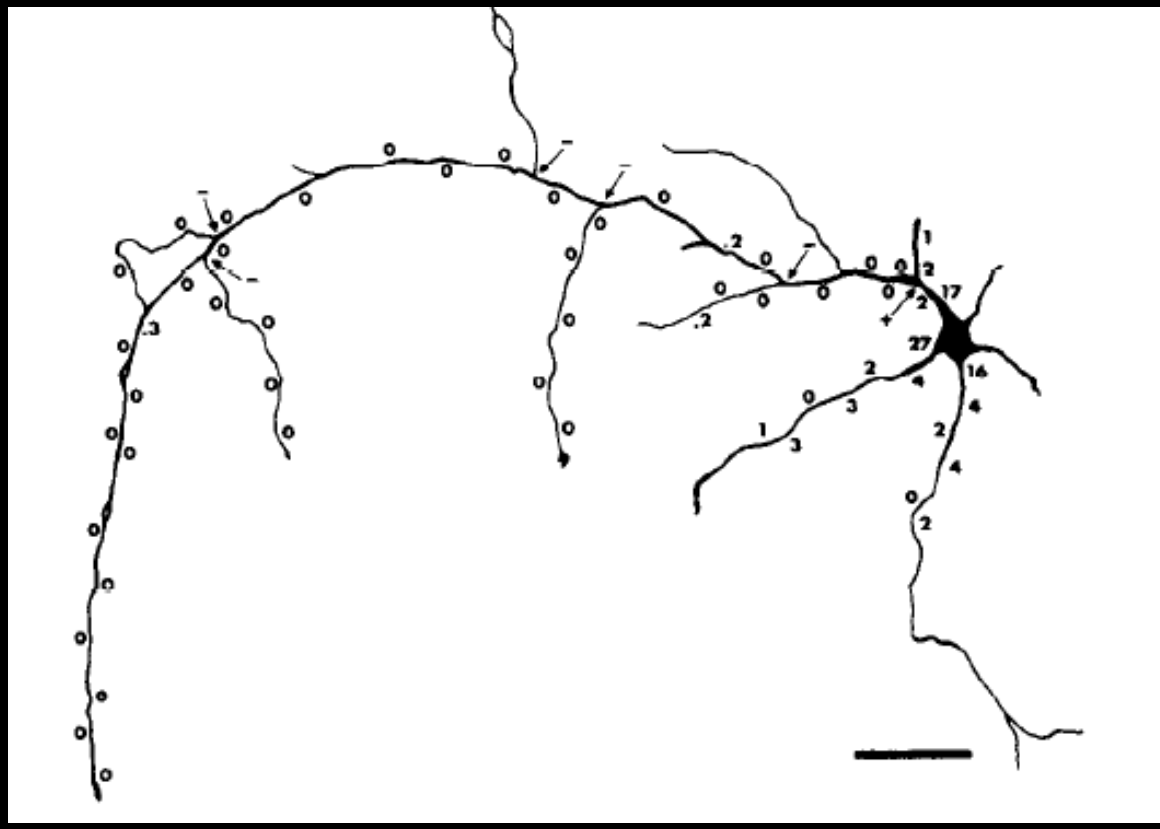
AN ELECTRON MICROSCOPIC STUDY OF THE DEVELOPMENT OF AXONS AND DENDRITES BY HIPPOCAMPAL NEURONS IN CULTURE

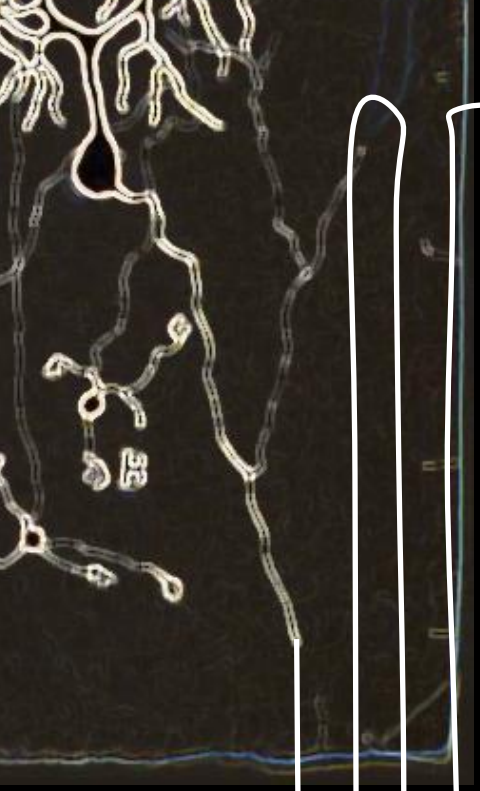
I. Cells Which Develop Without Intercellular Contacts[1]

WILLIAM P. BARTLETT[2] AND GARY A. BANKER[3]

Department of Anatomy, Albany Medical College, Albany, New York 12208







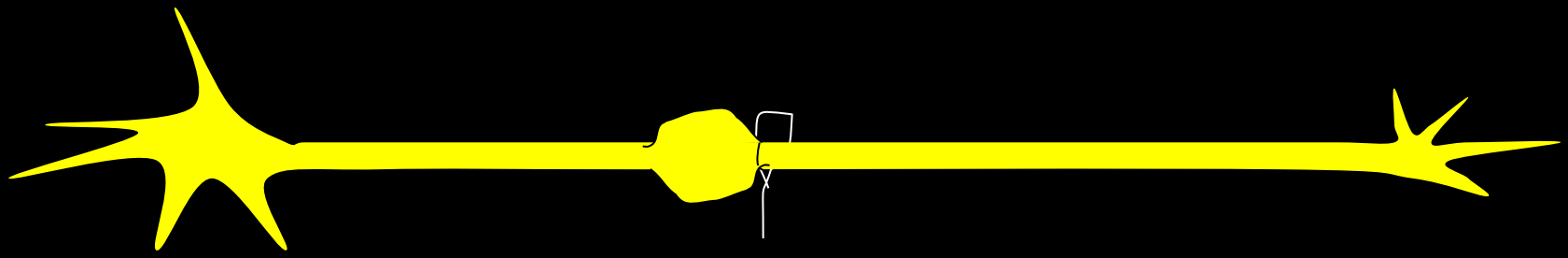
Why study axonal transport?

- Fundamental mechanism responsible for axonal and pre-synaptic homeostasis

- Axonal and presynaptic neuropathology in neurodegenerative diseases, mutations in motor proteins

- Axonal transport – a brief historical perspective
- An potential example of axonal transport impairment in Alzheimer’s disease

Paul Alfred Weiss H. Hiscoe. Experiments on the mechanism of cell outgrowth.
J. Exp. Zool. 107: 315-395. 1948



Fast Axonal Transport in Squid Giant Axon

Abstract. *Video-enhanced contrast–differential interference contrast microscopy has revealed new features of axonal transport in the giant axon of the squid, where no movement had been detected previously by conventional microscopy. The newly discovered dominant feature is vast numbers of "submicroscopic" particles, proba-*

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SCIENCE, VOL. 218, 10 DECEMBER 1982

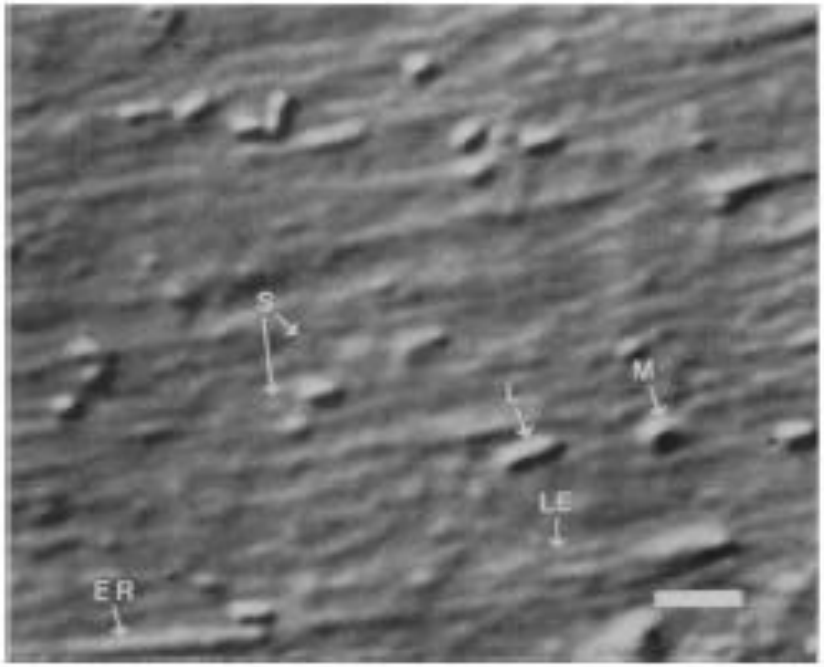
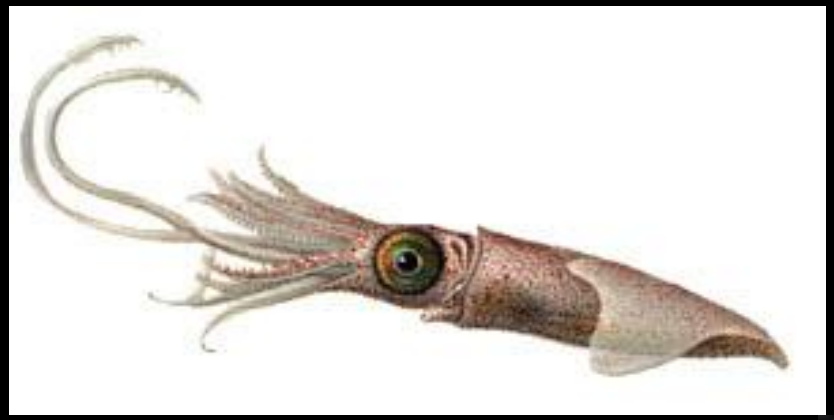
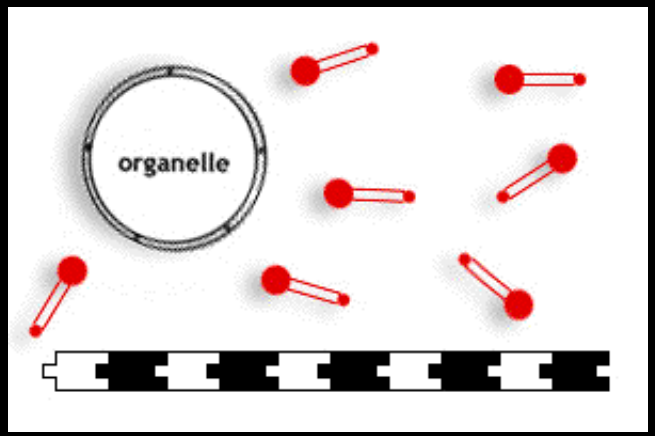
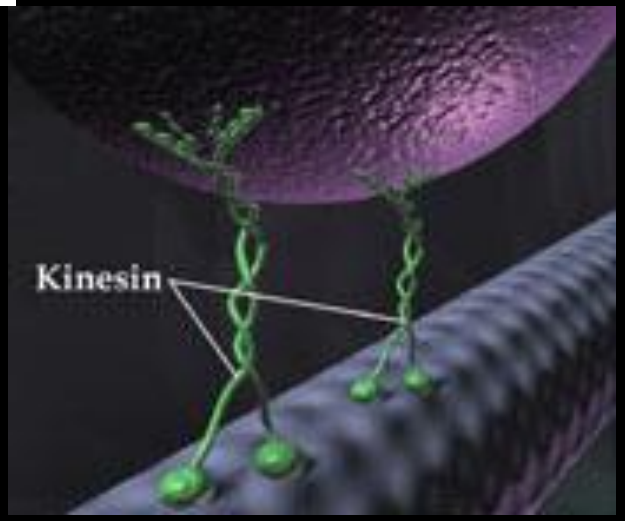


Fig. 1. A video micrograph of an optical section about 200 nm thick and 10 μm deep in the axoplasm of a squid giant axon showing examples of large (*L*), medium (*M*), and small (*S*) particles and linear elements (*LE*). Studies with the stain Fast Green suggest that structures such as that labeled *ER* may represent a segment of the smooth endoplasmic reticulum of the axon (*15*). In video records, many of these particles are moving (as described in text). Scale bar, 2 μm.

Discovery of motor proteins (kinesins and dyneins)





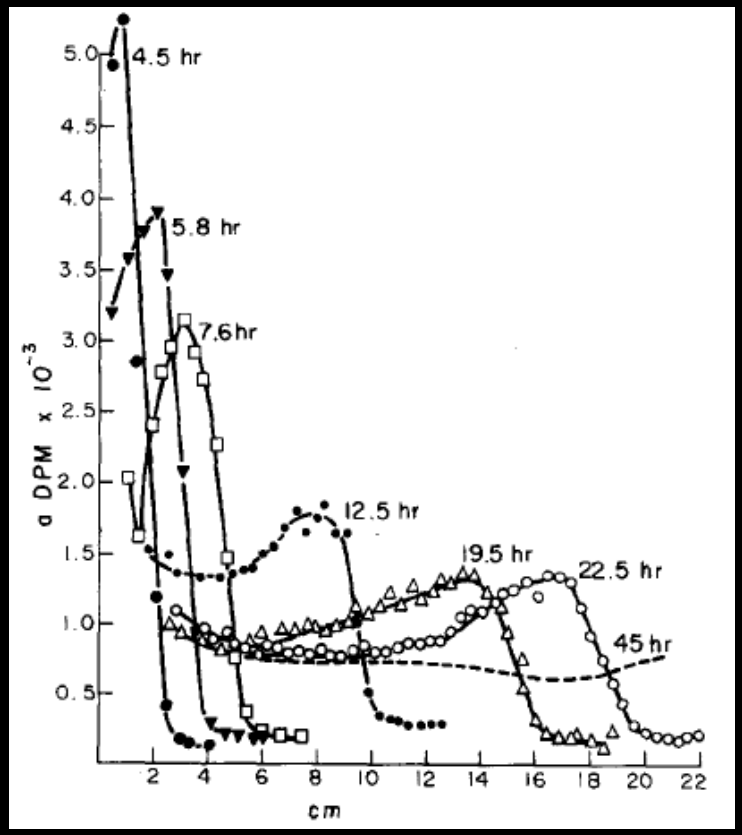


Brady ST, Nature (317) 1985, 73-75

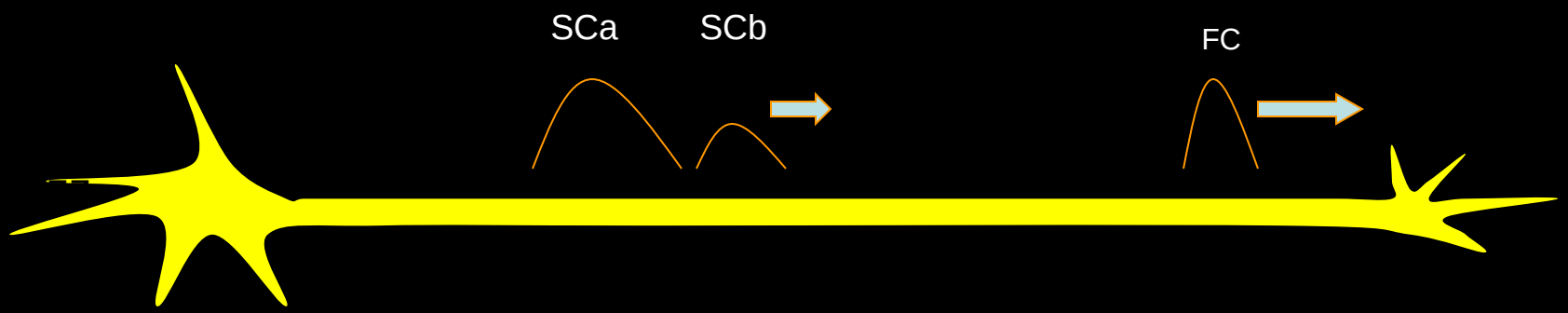
Classical paradigm demonstrating axonal transport

Inject radiolabeled amino acids

TIME



Fast and slow axonal transport



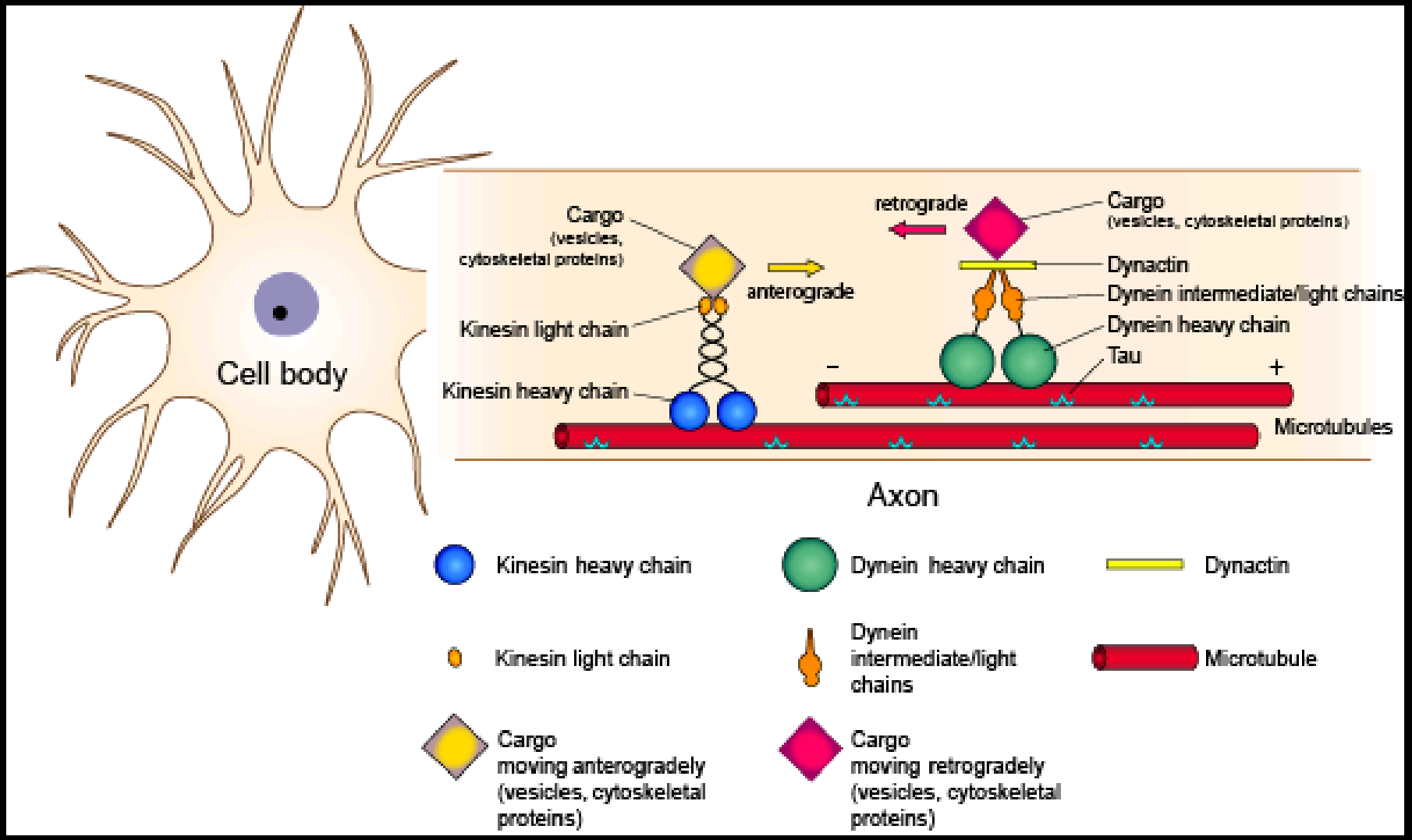
• Classical “pulse-chase” radiolabeling revealed two major populations:

- Fast axonal transport
  (vesicles, mitochondria)-
  100-400mm/day (1-5μ/s)
- Slow axonal transport

  **SCa- neurofilaments and microtubules

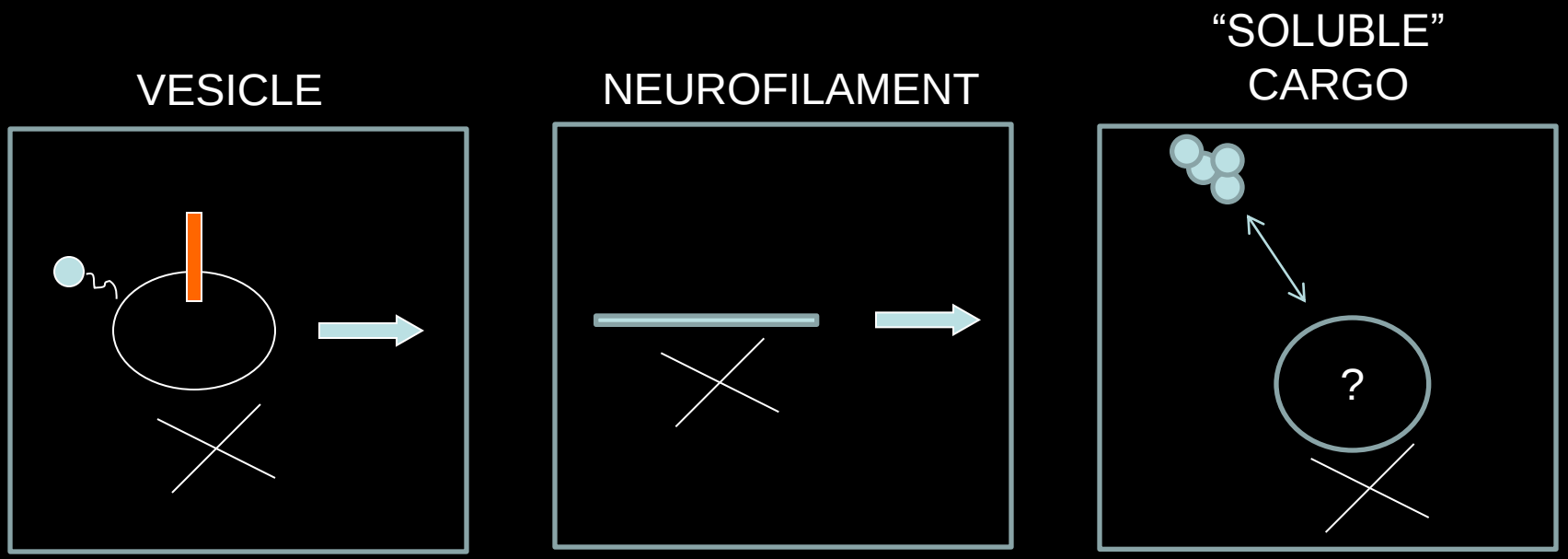
  **SCb- ~200 “soluble” or “cytosolic” proteins

General principles of axonal transport

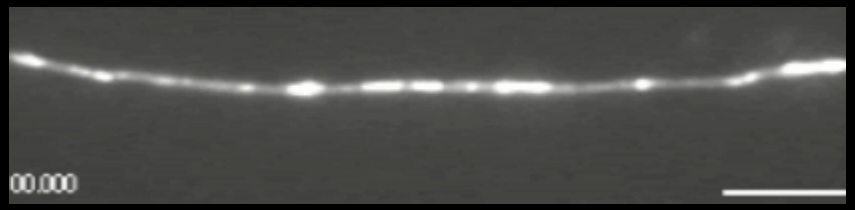


Roy, Zhang, Lee, Trojanowski. Acta Neuropathol (2005) 109:5-13

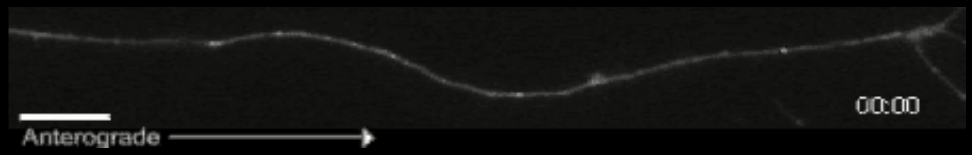
Take home message: Neurons adopt diverse strategies for moving cargoes



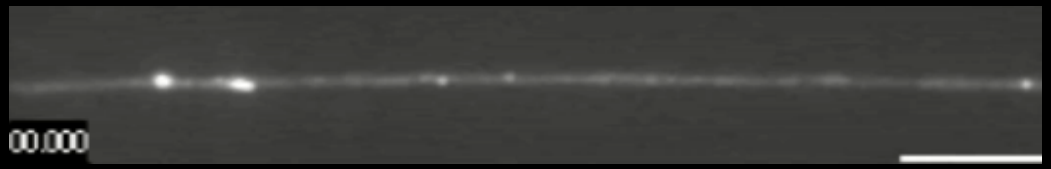
Synaptophysin



Amyloid precursor protein

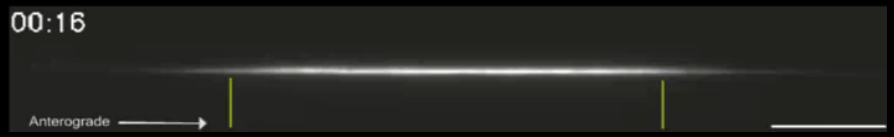


Bassoon – marker of dense-core vesicles

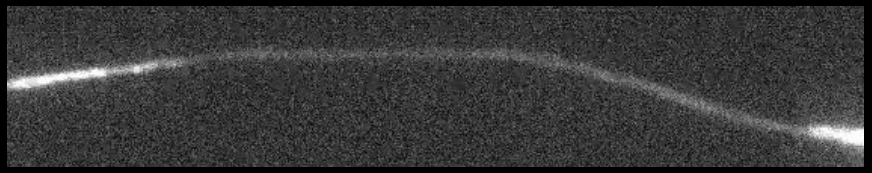


Mitochondria

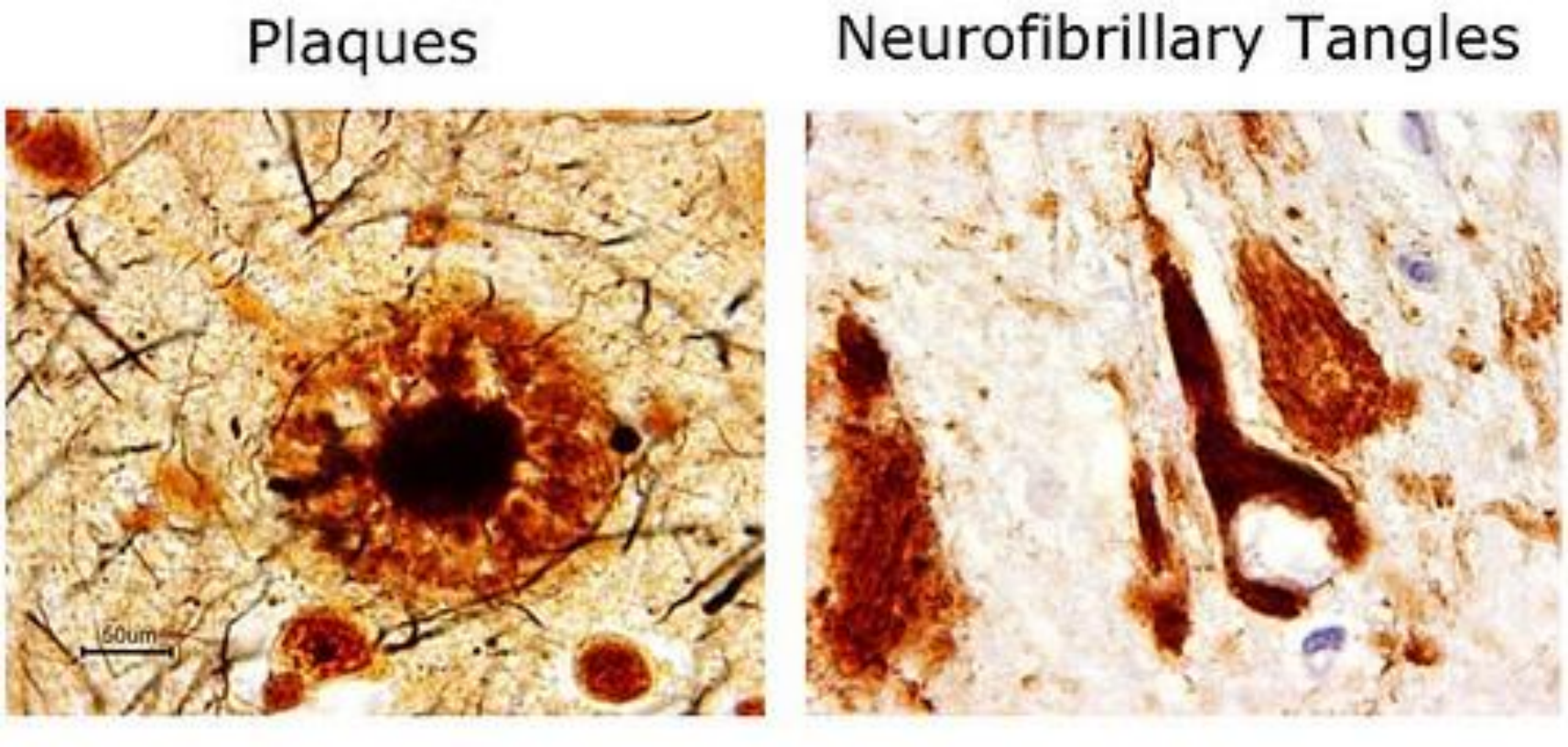
Synapsin – “soluble” protein

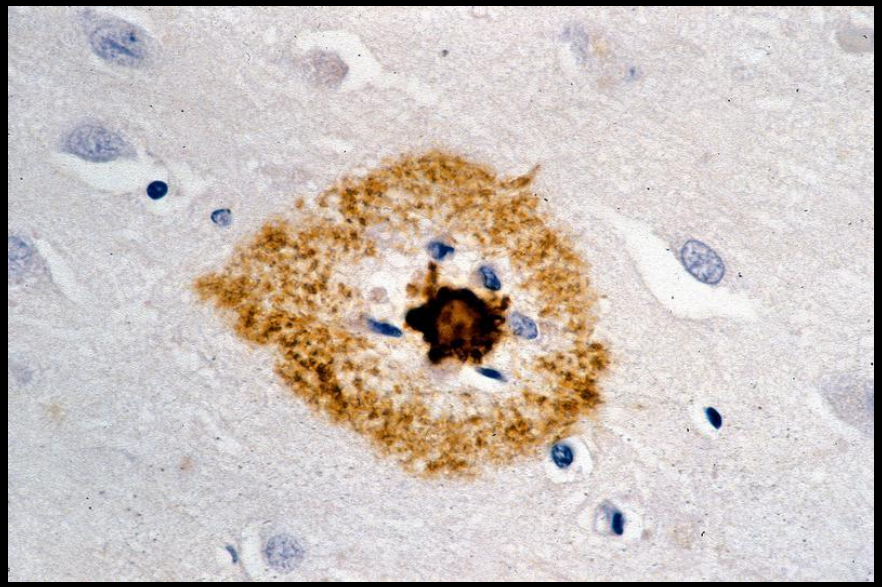


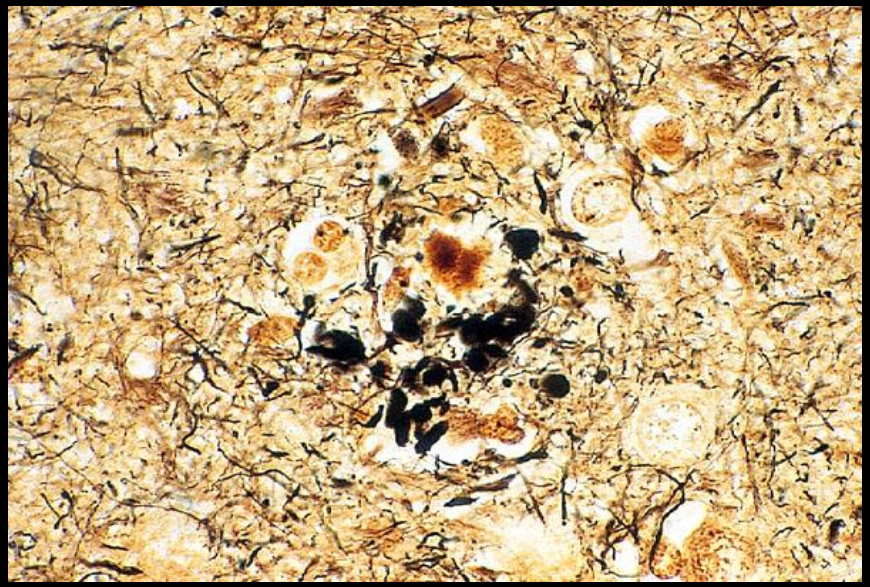
Neurofilament

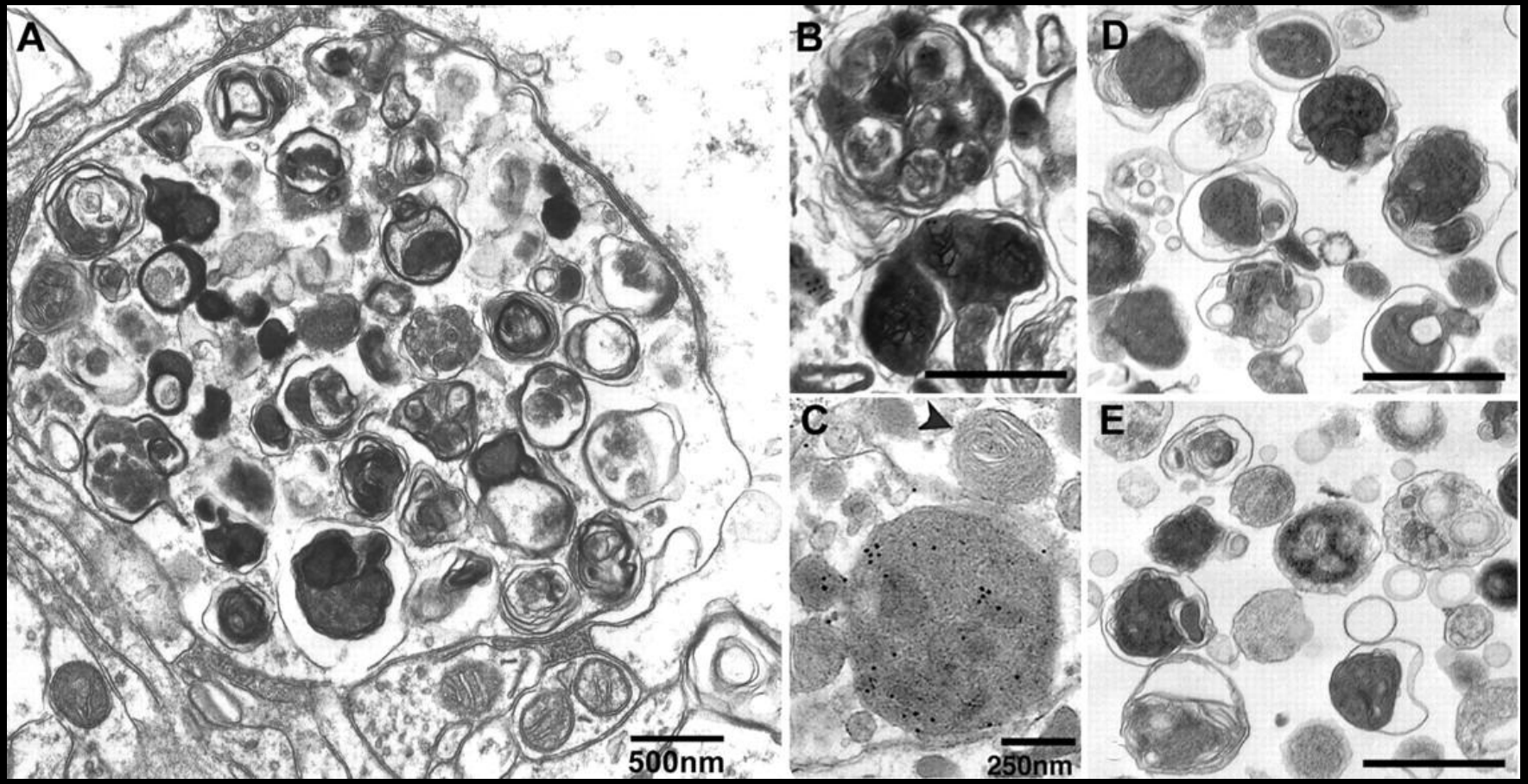


Axonal transport and Alzheimer's Disease

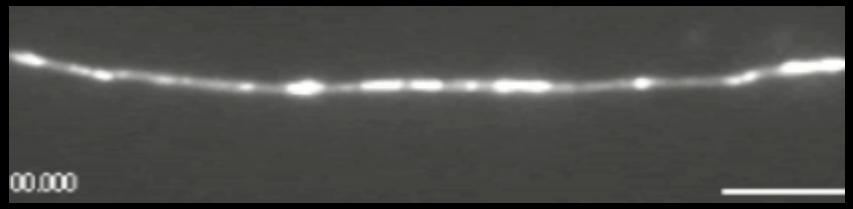


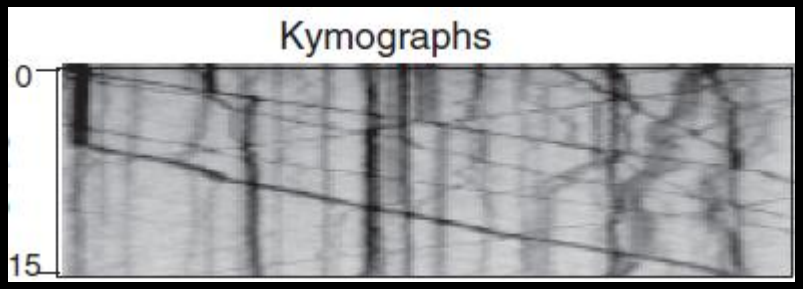




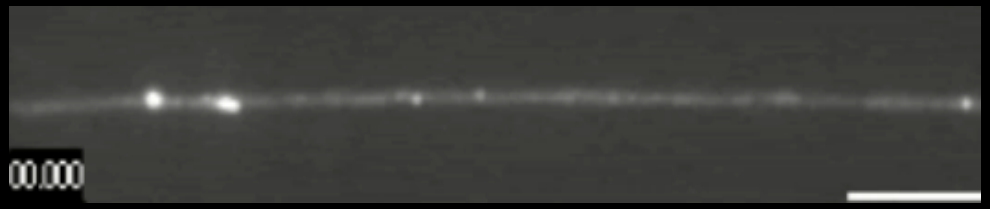

A
500nm
B
C
250nm
D
E

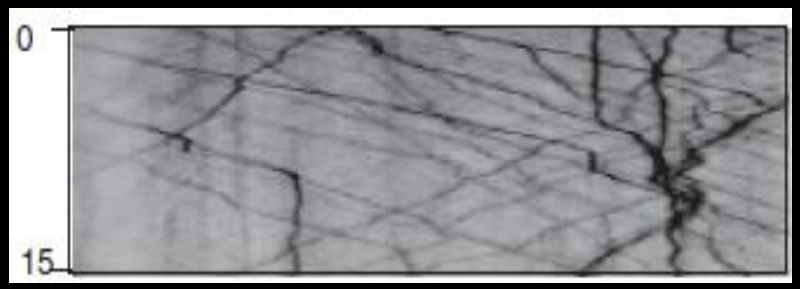
Synaptophysin:mRFP



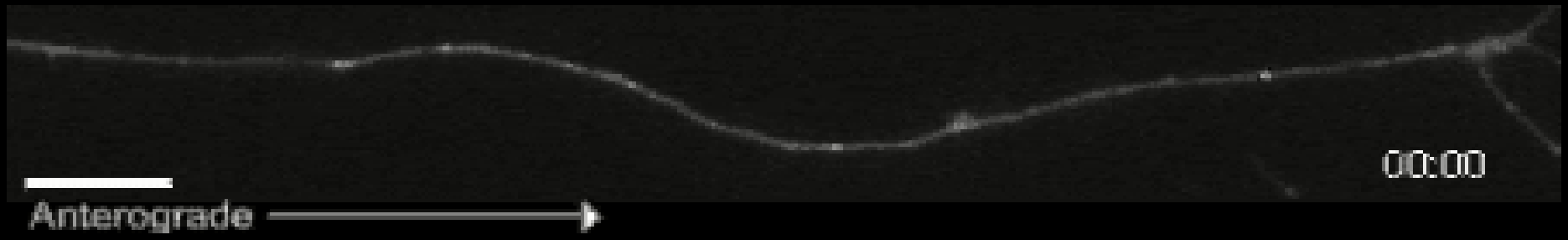


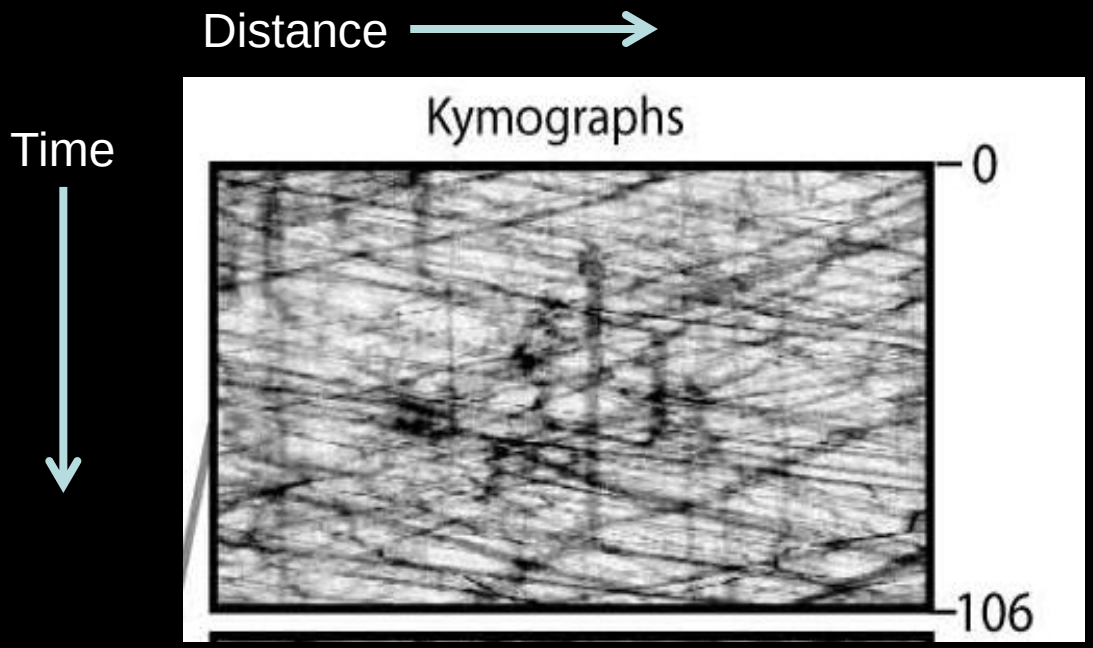
Bassoon:GFP

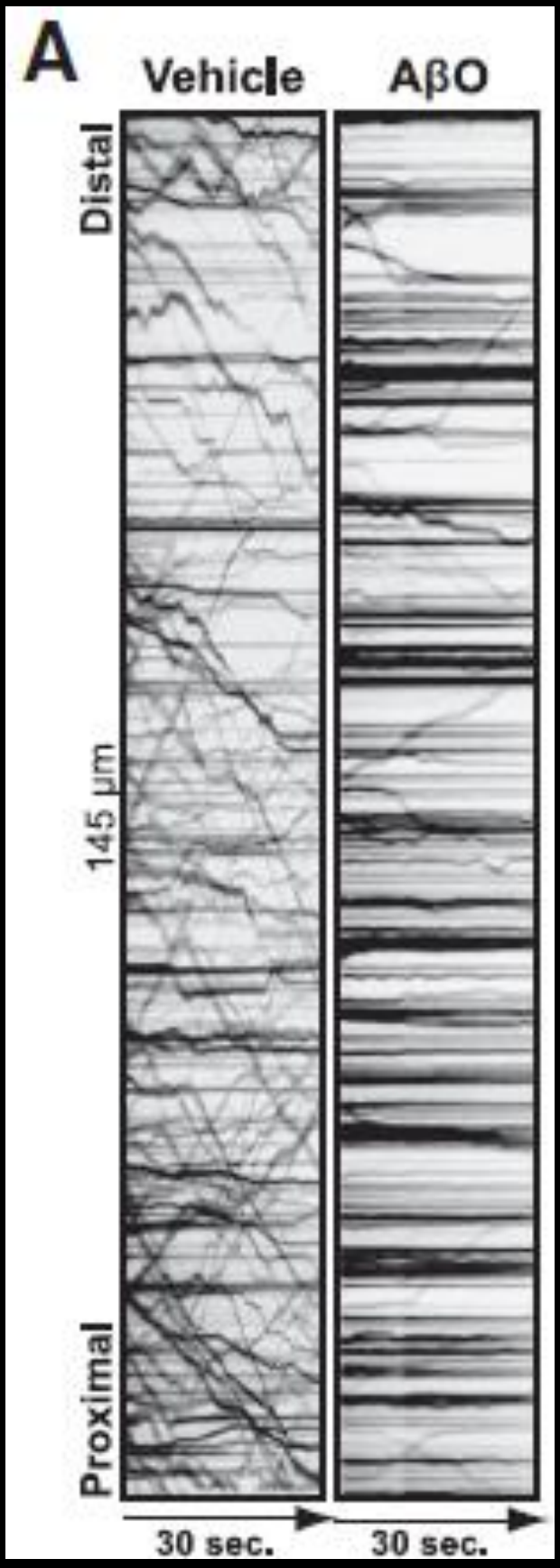




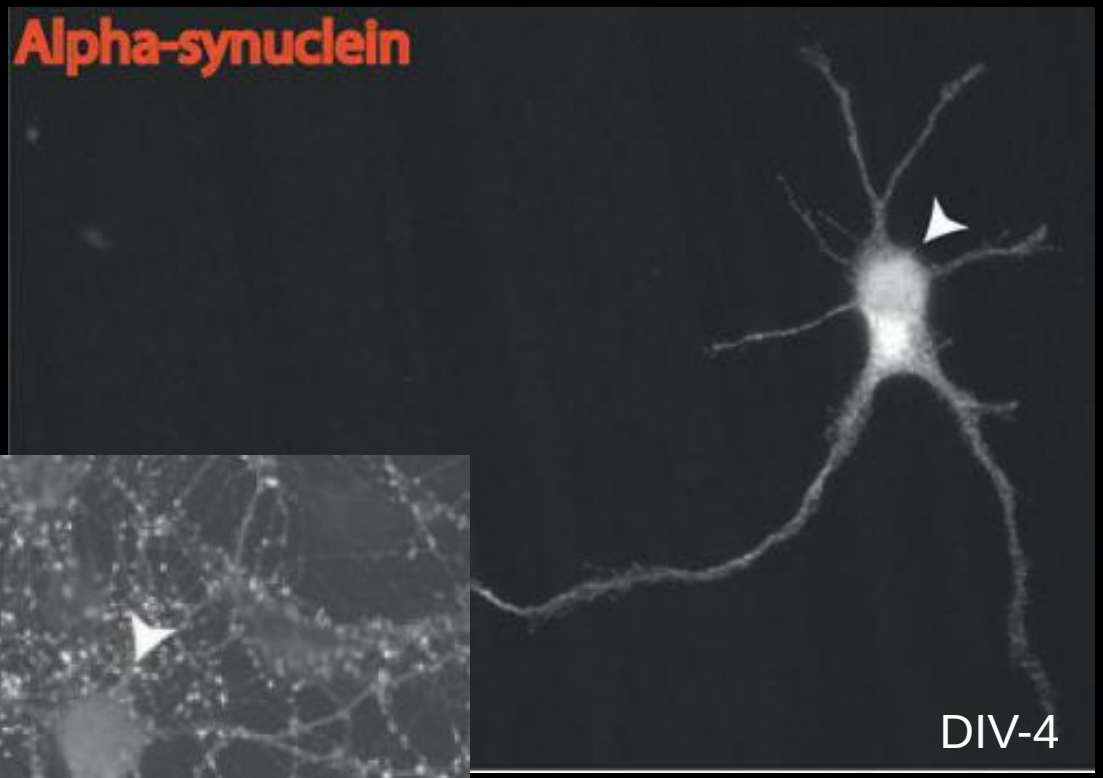
Kymographs

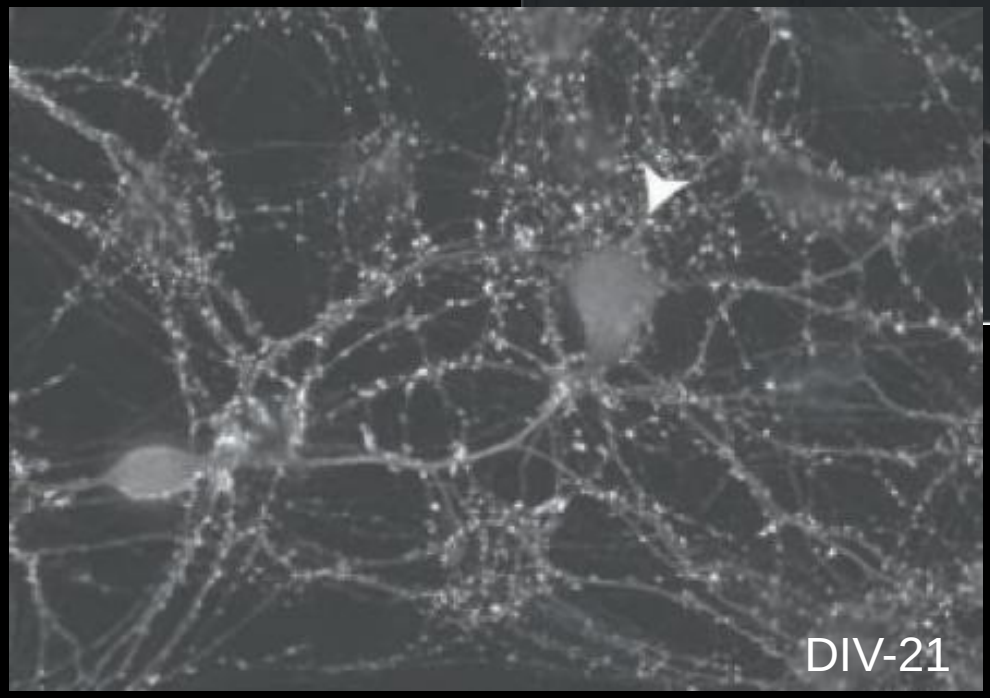





A
Vehicle
AβO
Distal
145 µm
Proximal
30 sec.
30 sec.

Experiments in cultured hippocampal neurons





A Experimental strategy

Culture hippocampal neurons from P0-P2 pups

14d

Transfect desired fluorescent-tagged construct

17h

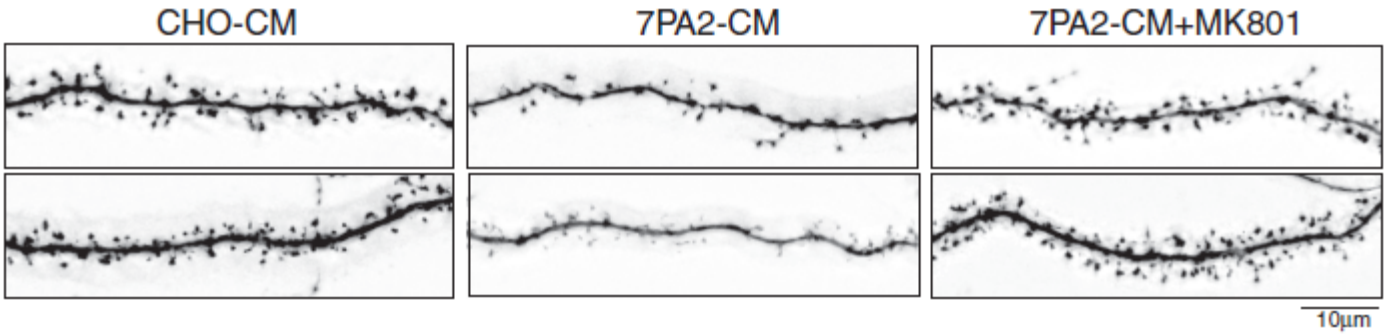
Add conditioned media from CHO (control) or 7PA2 cells (containing 200 pM Aβ42 oligomers)

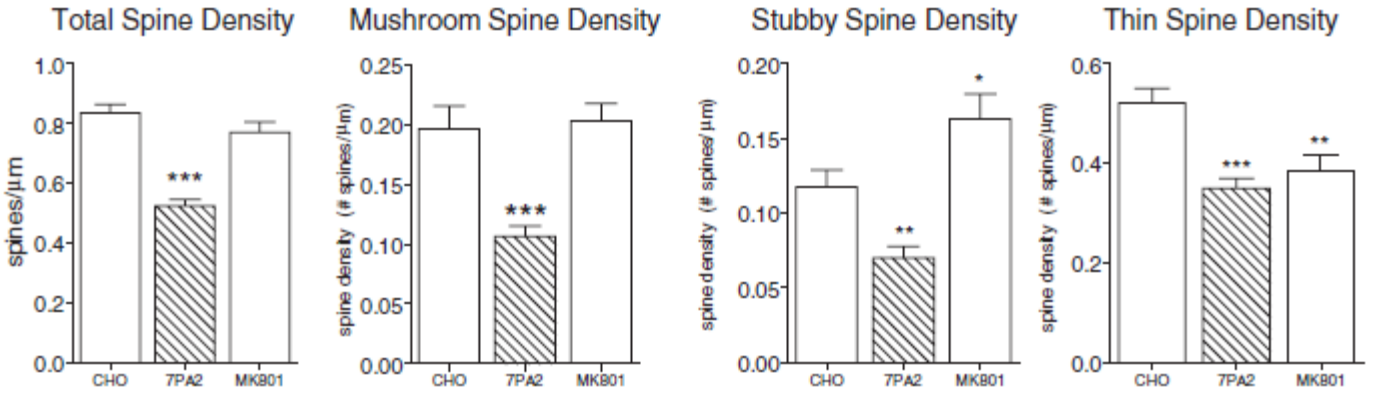
2 (or 24) h +/- MK801/DAP-5 +/- GSK-3β i

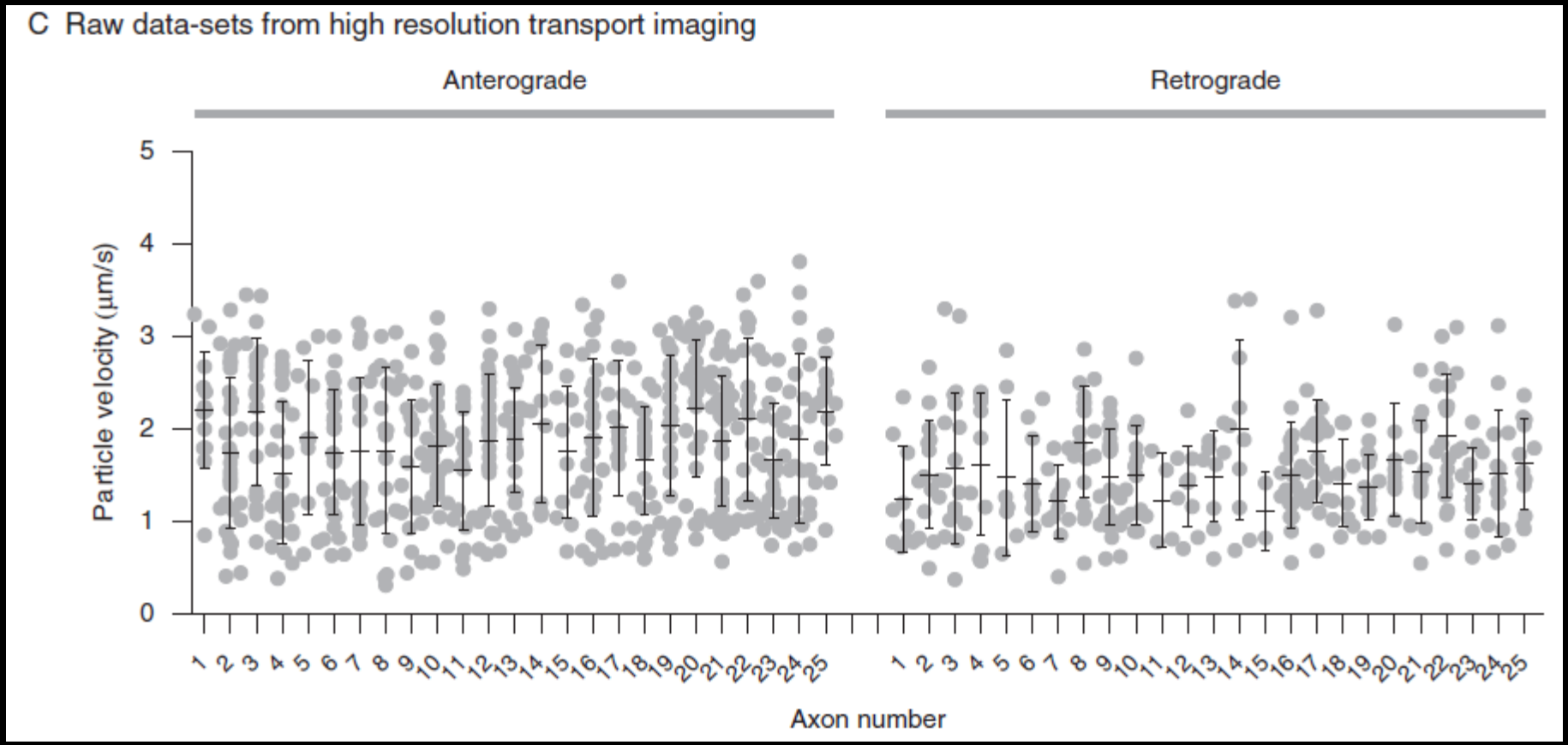
Evaluate axonal transport with high-resolution live imaging

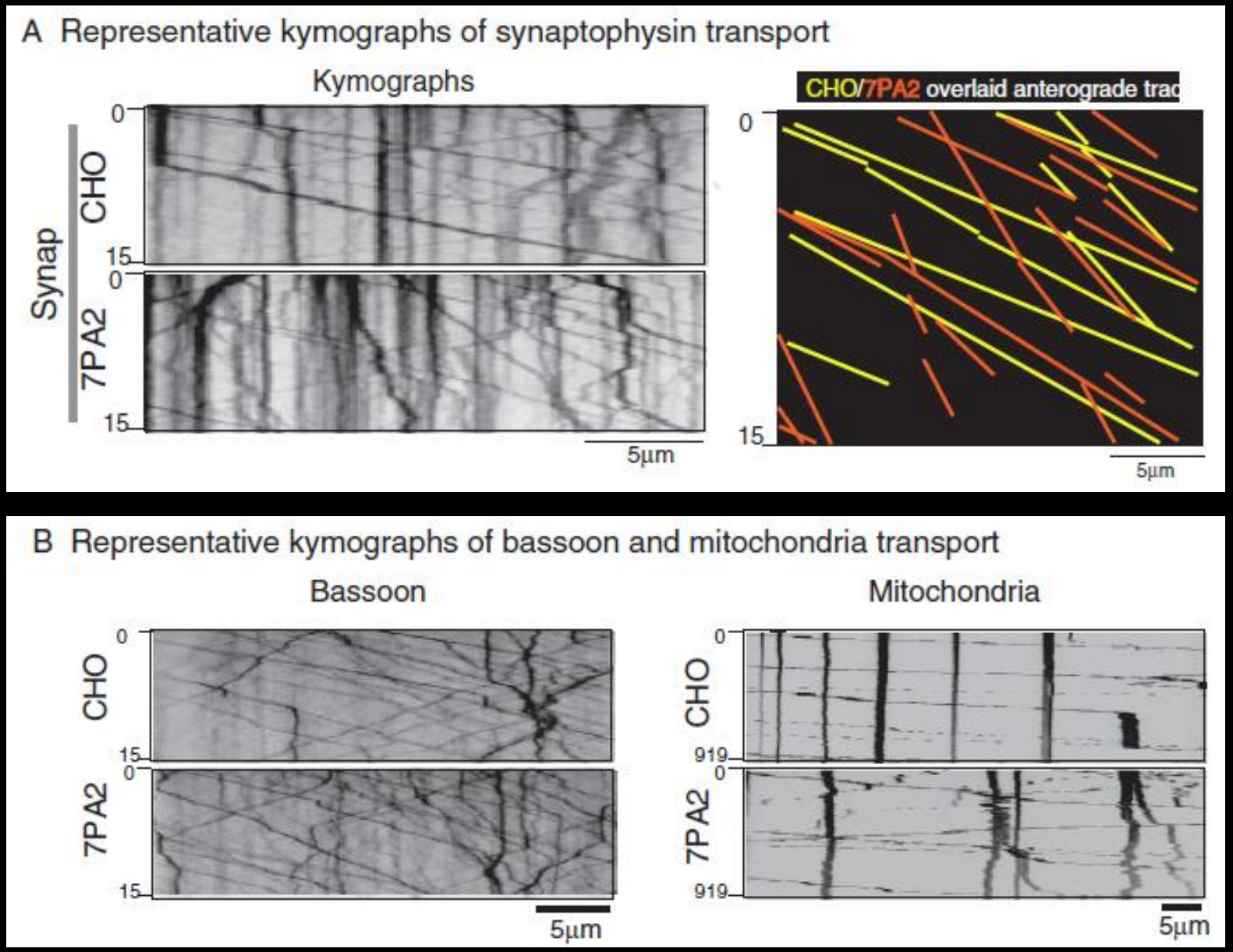
Blinded analyses of axonal transport

B Effects of cell-derived Aβ oligomers on dendritic spines

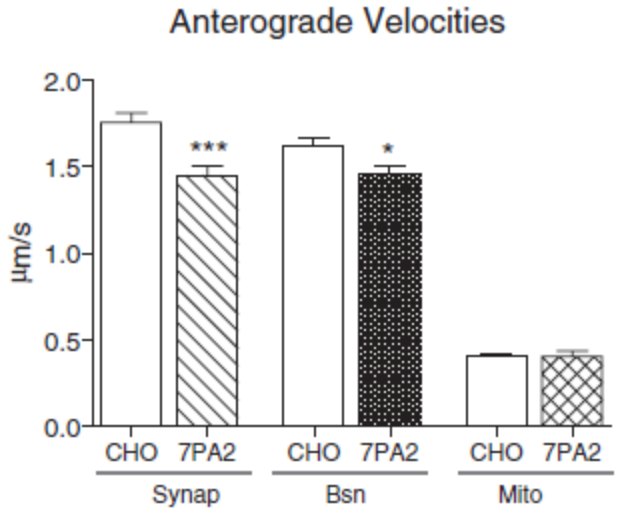


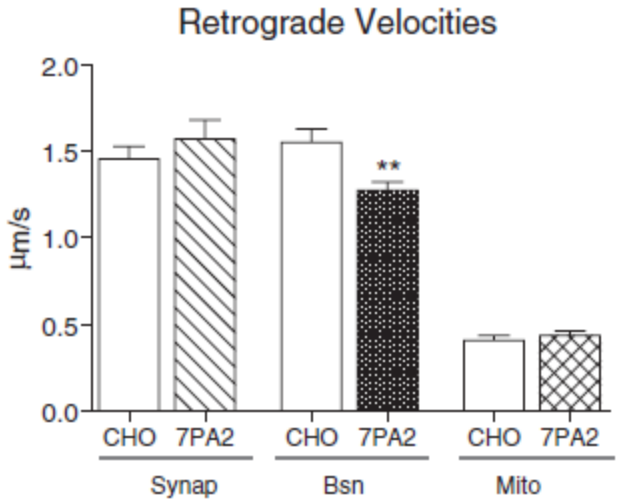


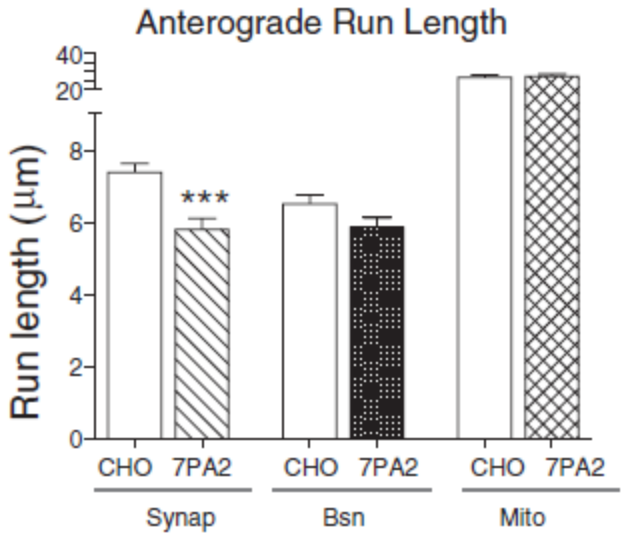

C Raw data-sets from high resolution transport imaging
Anterograde
Retrograde
Particle velocity (µm/s)
0
1
2
3
4
5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Axon number

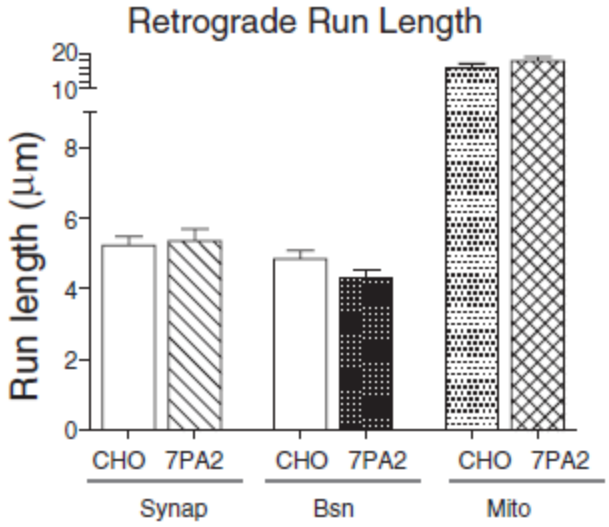

A Representative kymographs of synaptophysin transport
Kymographs
CHO/7PA2 overlaid anterograde trac
Synap
CHO
7PA2
0
15
0
15
0
15
5µm
5µm
B Representative kymographs of bassoon and mitochondria transport
Bassoon
Mitochondria
CHO
7PA2
0
15
0
15
0
919
0
919
5µm
5µm

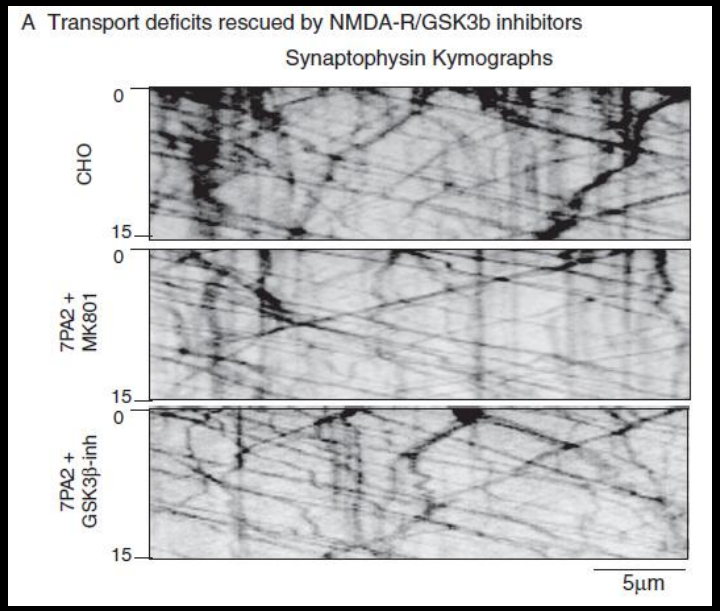
C Quantitative analyses

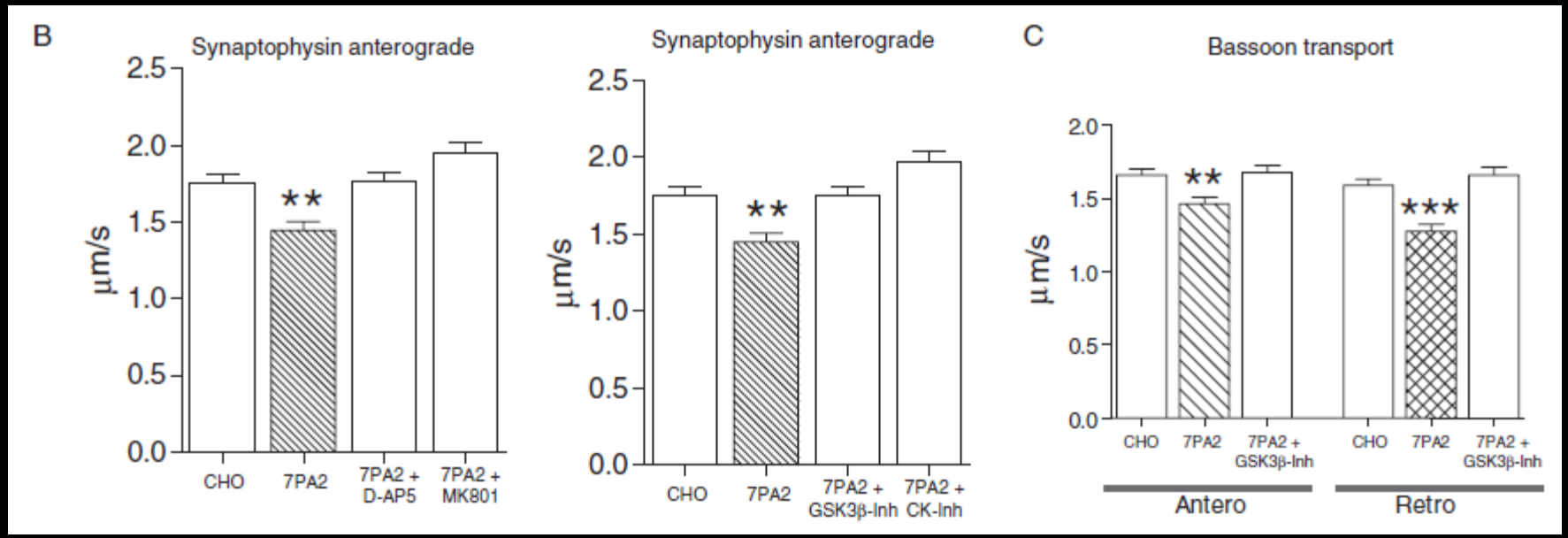


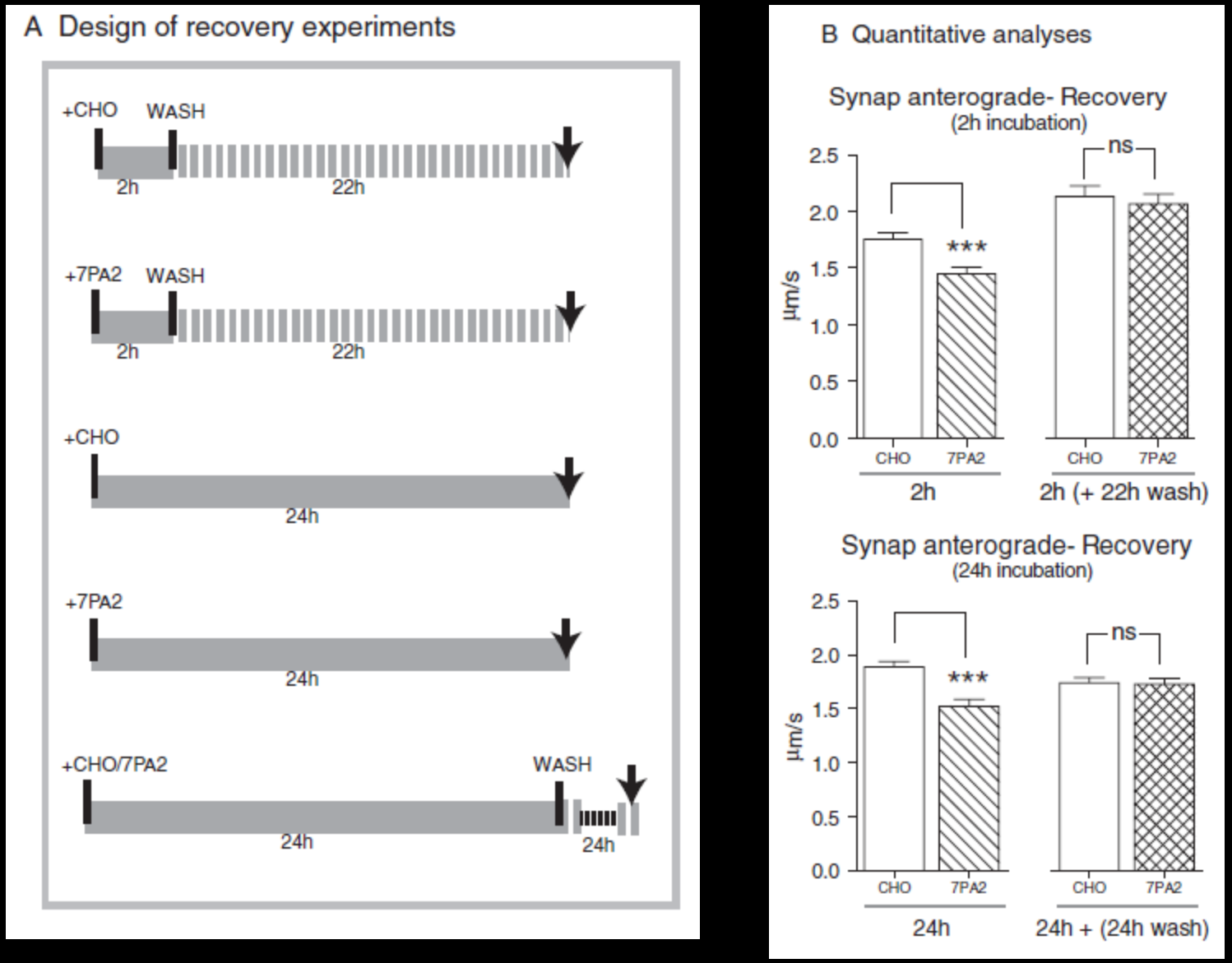




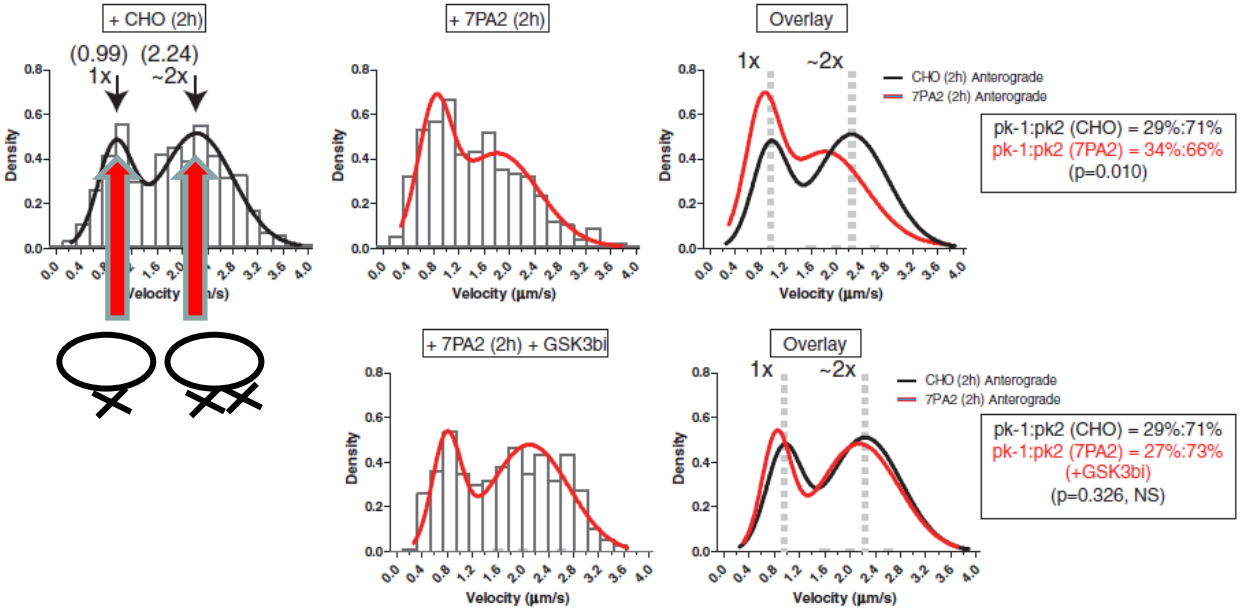



A Transport deficits rescued by NMDA-R/GSK3b inhibitors
Synaptophysin Kymographs
CHO
7PA2 + MK801
7PA2 + GSK3β-inh
0
15
5μm

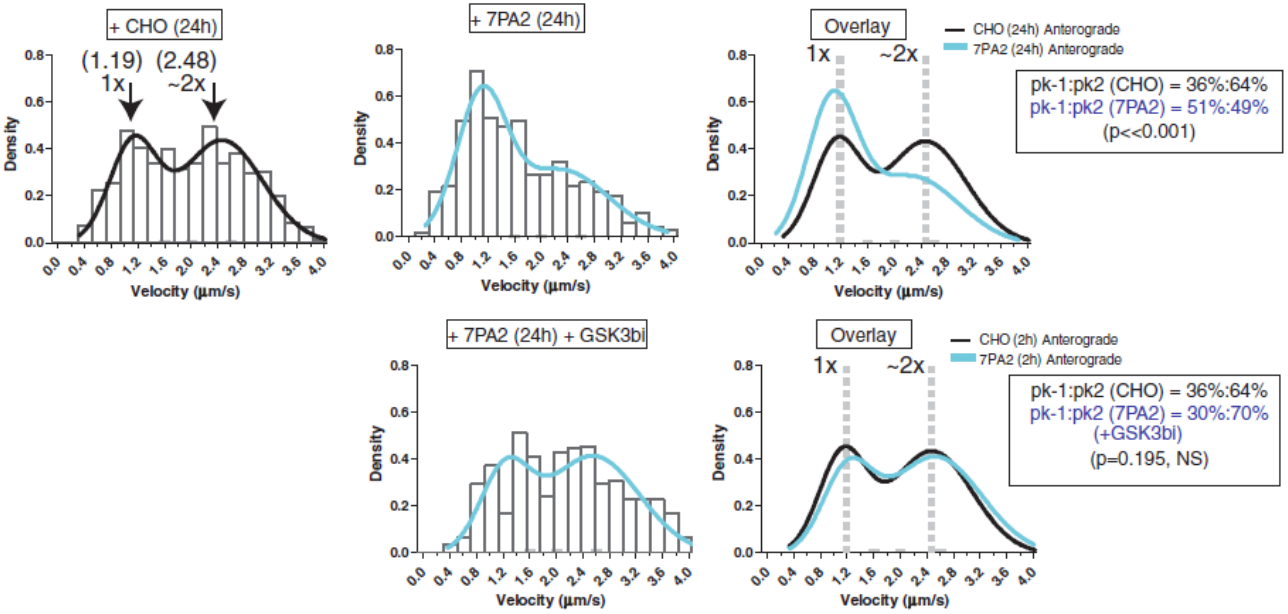

B
Synaptophysin anterograde
μm/s
2.5
2.0
1.5
1.0
0.5
0.0
**
CHO
7PA2
7PA2 + D-AP5
7PA2 + MK801
Synaptophysin anterograde
**
CHO
7PA2
7PA2 + GSK3β-Inh
7PA2 + CK-Inh
C
Bassoon transport
2.0
1.5
1.0
0.5
0.0
**
***
CHO
7PA2
7PA2 + GSK3β-Inh
CHO
7PA2
7PA2 + GSK3β-Inh
Antero
Retro

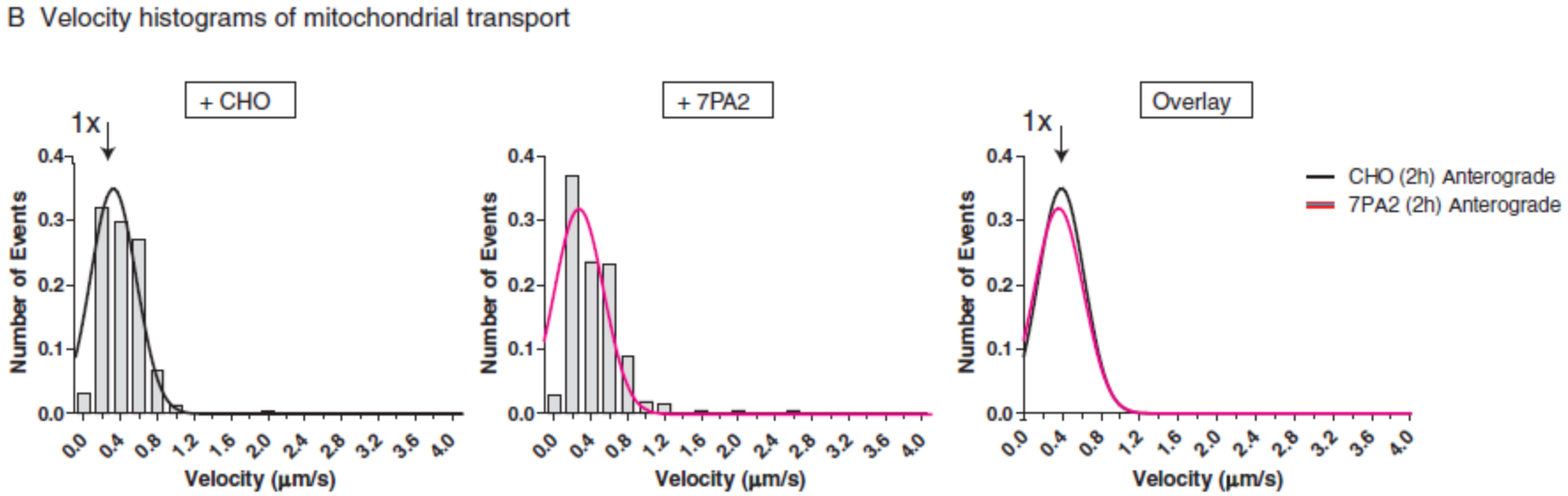

A Design of recovery experiments
+CHO
WASH
2h
22h
+7PA2
WASH
2h
22h
+CHO
24h
+7PA2
24h
+CHO/7PA2
WASH
24h
24h
B Quantitative analyses
Synap anterograde- Recovery
(2h incubation)
µm/s
2.5
2.0
1.5
1.0
0.5
0.0
***
ns
CHO
7PA2
CHO
7PA2
2h
2h (+ 22h wash)
Synap anterograde- Recovery
(24h incubation)
µm/s
2.5
2.0
1.5
1.0
0.5
0.0
***
ns
CHO
7PA2
CHO
7PA2
24h
24h + (24h wash)

A Cluster-mode analyses of synaptophysin transport (2h 7PA2 treatment)



B Cluster-mode analyses of synaptophysin transport (24h 7PA2 treatment)





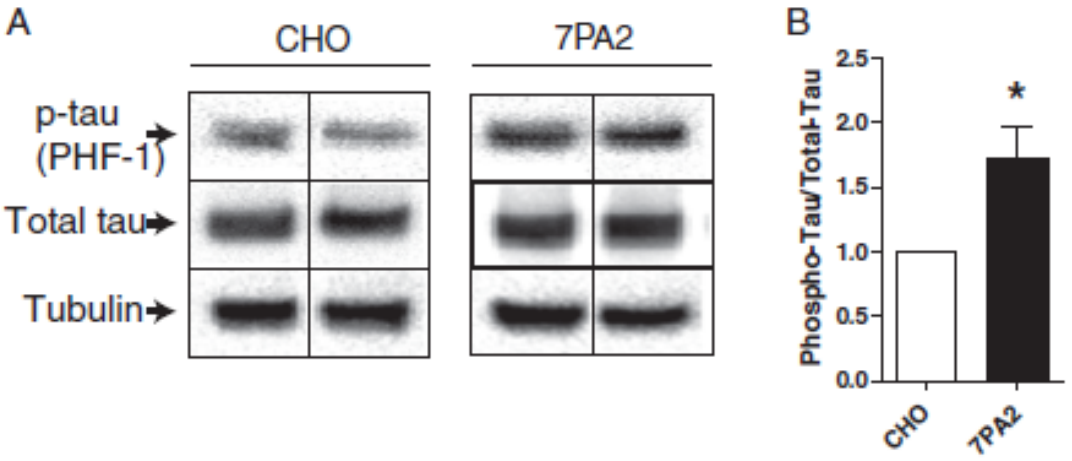


Figure 7: Biochemical analyses. Cultured neurons (DIV 14) were incubated with CHO or 7PA2 (1 nM Aβ-42) media for 24 h, and levels of total tau and phospho-tau were detected by

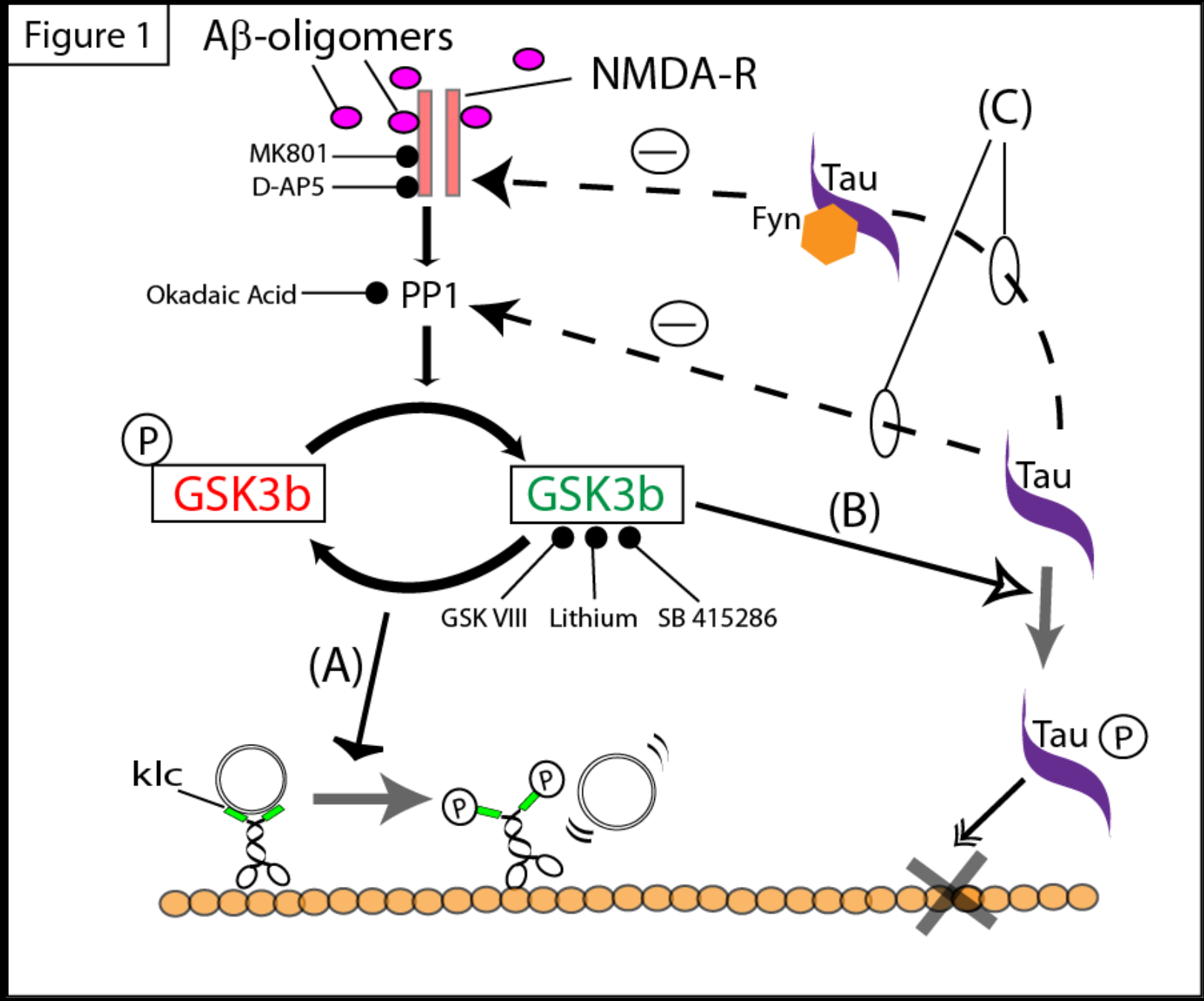


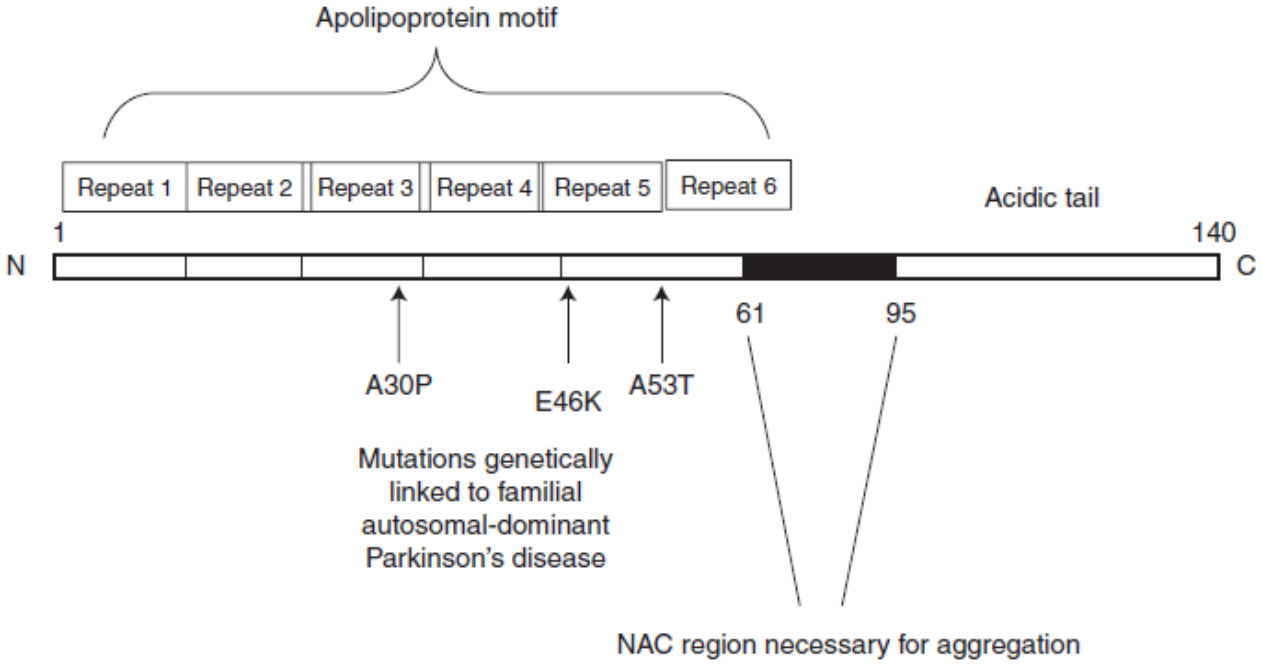
Figure 1
Aβ-oligomers
NMDA-R
MK801
D-AP5
(C)
Tau
Fyn
Okadaic Acid
PP1
P
GSK3b
GSK3b
(B)
Tau
GSK VIII
Lithium
SB 415286
(A)
Tau P
klc
P
P

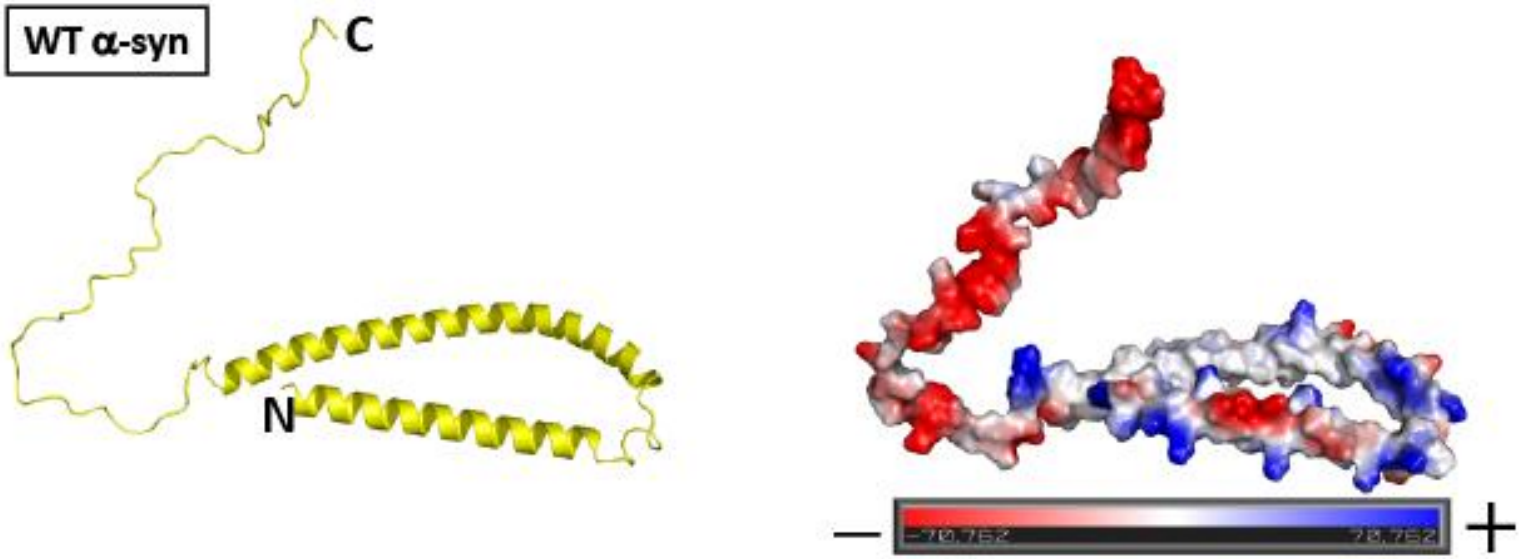
α-synuclein.....

Chair: Subhojit Roy, MD, PhD (UCSD)

Co-chair: Sreeganga Chandra, PhD (Yale)

ALPHA SYNUCLEIN


Apolipoprotein motif
Repeat 1
Repeat 2
Repeat 3
Repeat 4
Repeat 5
Repeat 6
Acidic tail
1
140
N
C
61
95
A30P
E46K
A53T
Mutations genetically linked to familial autosomal-dominant Parkinson's disease
NAC region necessary for aggregation


WT α-syn
C
N
−
+

Intracellular aggregates of a-syn in LB diseases

SNCA variation is the most important genetic risk factor in four independent GWAS studies

Consider the evidence

Autosomal-dominant mutations of a-syn in familial LB diseases

Neuronal loss in mouse, yeast, fly and worm a-syn models

Gene-multiplications of a-syn in familial LB diseases

Subtle increases in WT a-synuclein levels can manifest phenotypes

- Multiplication patients show that excessive protein *can* cause disease
- Increased a-syn mRNA in sporadic disease
- A sporadic polymorphism (Rep1) increases a-syn expression (Chiba-Falek/Nussbaum and colleagues)
- GWAS studies in sporadic cases
- MPTP/LPS models show attenuated pathology in the absence of endogenous a-syn (Dauer et. al. PNAS, 2002/Gao et al., J Neurosci 2008)

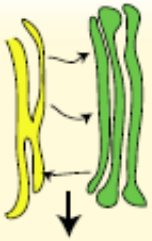
Devine et. al., *Movement* Disorders, Vol. 26, No. 12, 2011

Mark R. Cookson Current Biology 22, R753–R761, September 11, 2012

Leonidas Stefanis *Cold Spring Harb Perspect Med* 2012;4:a009399

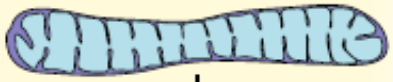
toxicity

toxic species?

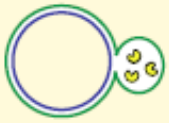


Blocked ER-golgi transport
ER stress and golgi fragmentation

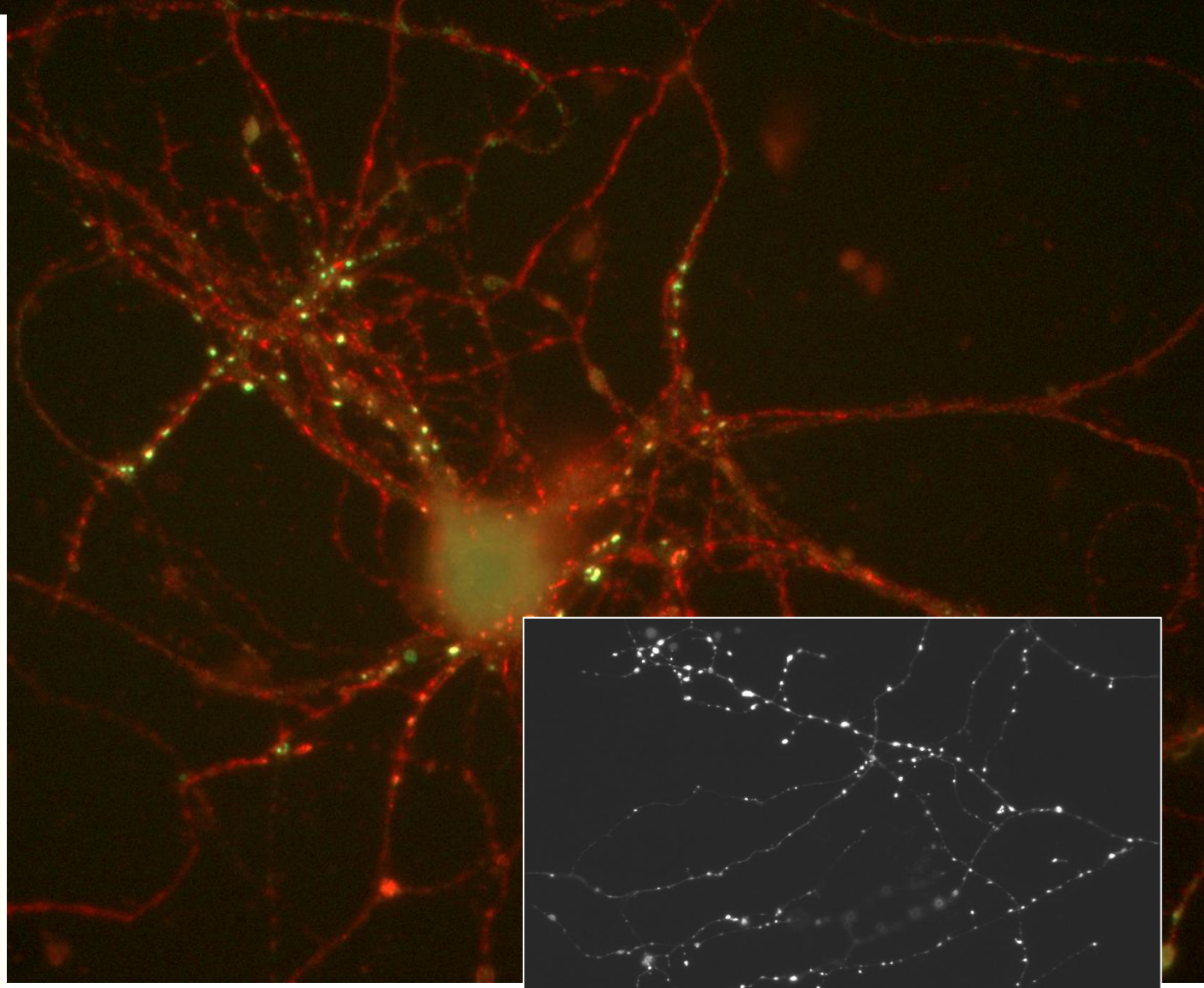
Decreased Synaptic vesicle release



Impaired energy production
Apoptosis induction

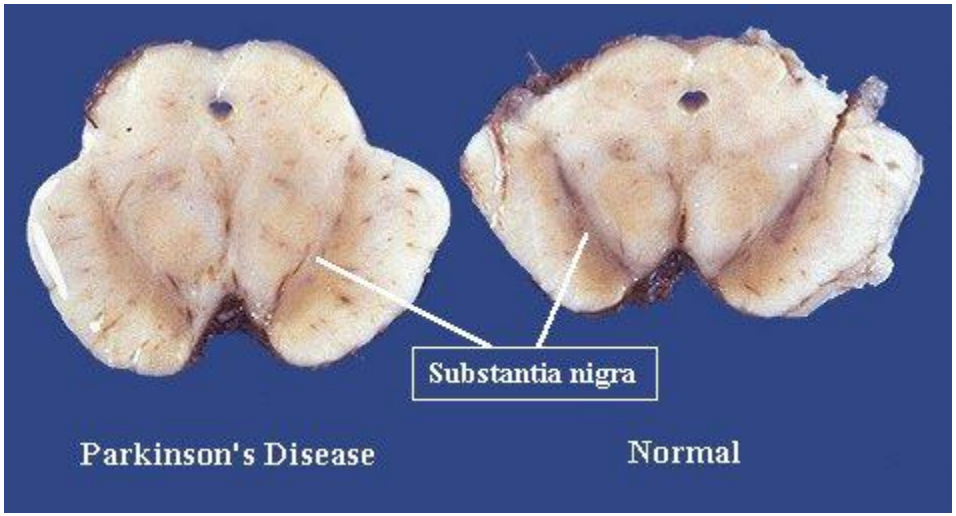


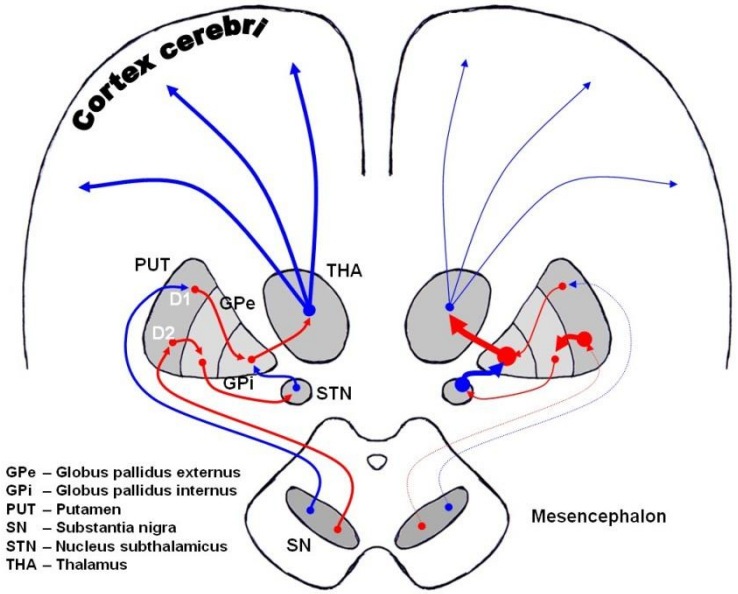
Accumulation of CMA substrates
?Proteasome impairment



Mark R. Cookson *Molecular Neurodegeneration* 2009, **4**:9

Parkinson’s disease





ELSEVIER

Neurobiology of Aging 24 (2003) 197–211

NEUROBIOLOGY OF AGING

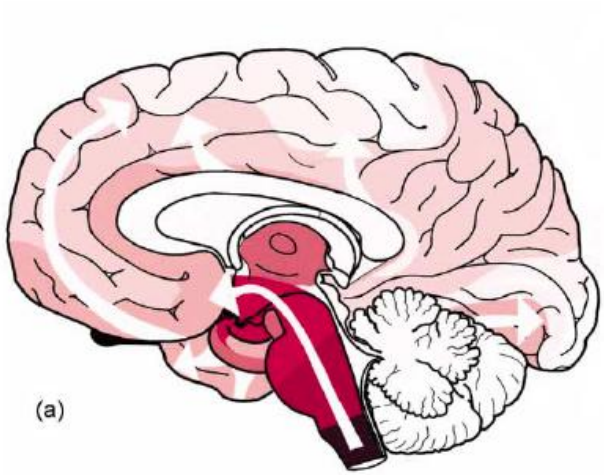
www.elsevier.com/locate/neuaging

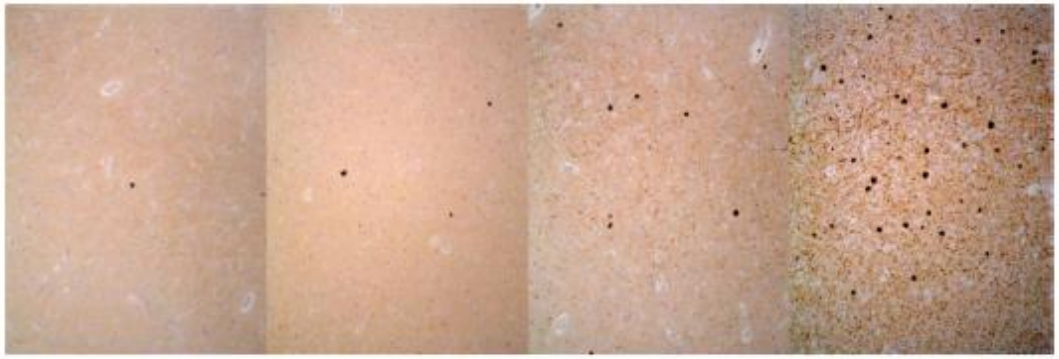
“Braak staging” for PD

Staging of brain pathology related to sporadic Parkinson’s disease

Heiko Braak[a,*], Kelly Del Tredici[a], Udo Rüb[a], Rob A.I. de Vos[b], Ernst N.H. Jansen Steur[b], Eva Braak[a,†]

[a] Department of Clinical Neuroanatomy, J.W. Goethe University, Theodor Stern Kai 7, D-60590 Frankfurt/Main, Germany

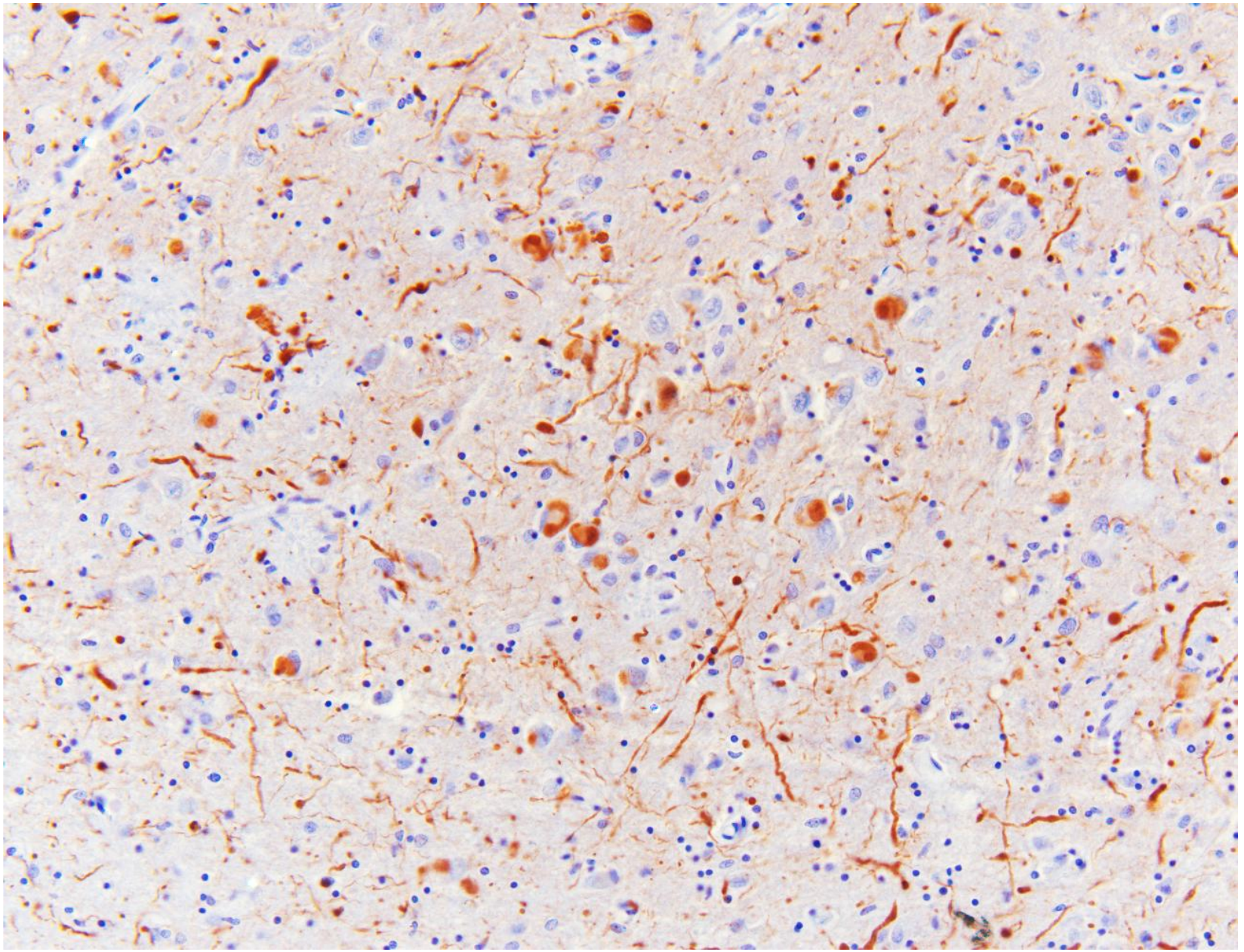




Stages in the evolution of PD-related pathology

Stage 1 $N = 21$; medulla oblongata	Lesions in the dorsal IX
Stage 2 $N = 13$; medulla oblongata and pontine tegmentum	Pathology of stage 1 plu coeruleus–subcoeruleus c
Stage 3 $N = 24$; midbrain	Pathology of stage 2 plu
Stage 4 $N = 24$; basal prosencephalon and mesocortex	Pathology of stage 3 plu (transentorhinal region) a
Stage 5 $N = 17$; neocortex	Pathology of stage 4 plu
Stage 6 $N = 11$; neocortex	Pathology of stage 5 plu occasionally mild change

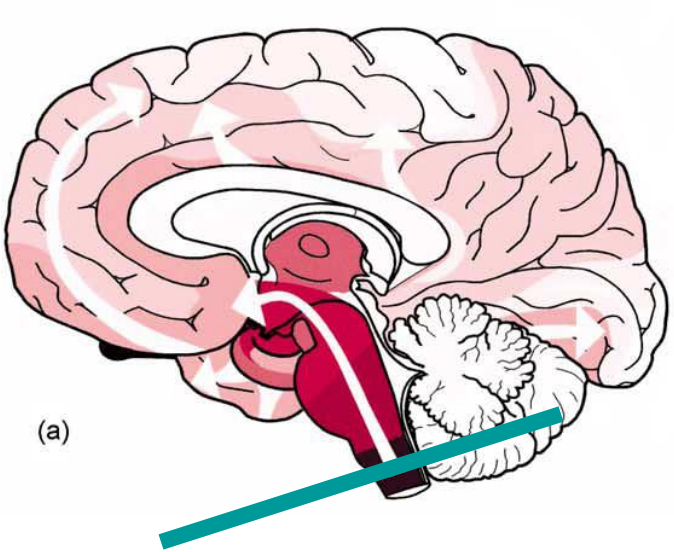
McKeith et al. Neurology, 2005

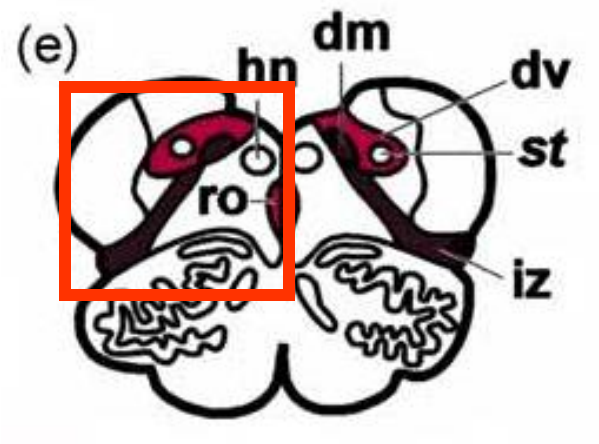


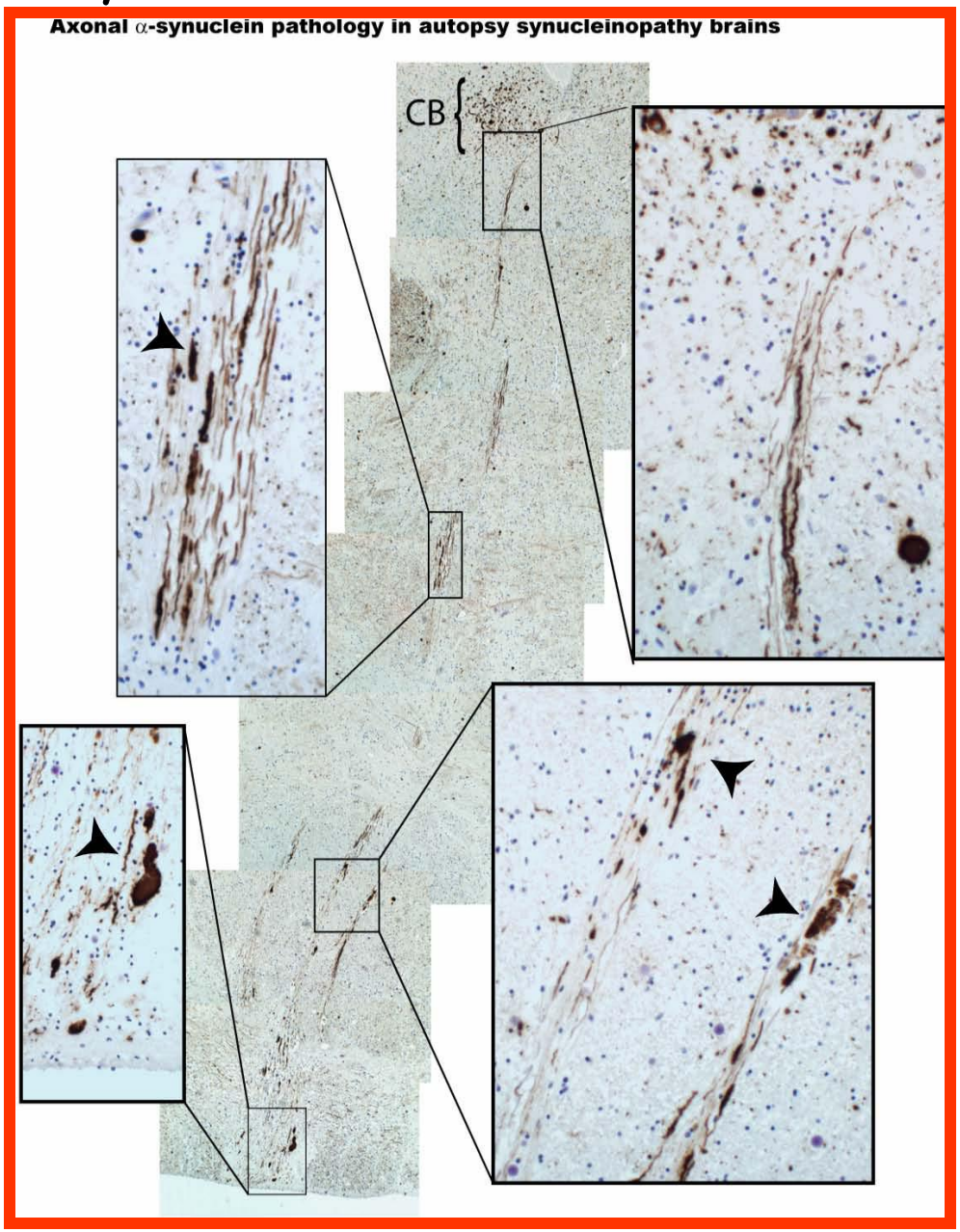
Alpha-synuclein staining of a DLB case

Axonal aggregates of a-synuclein in PD brains

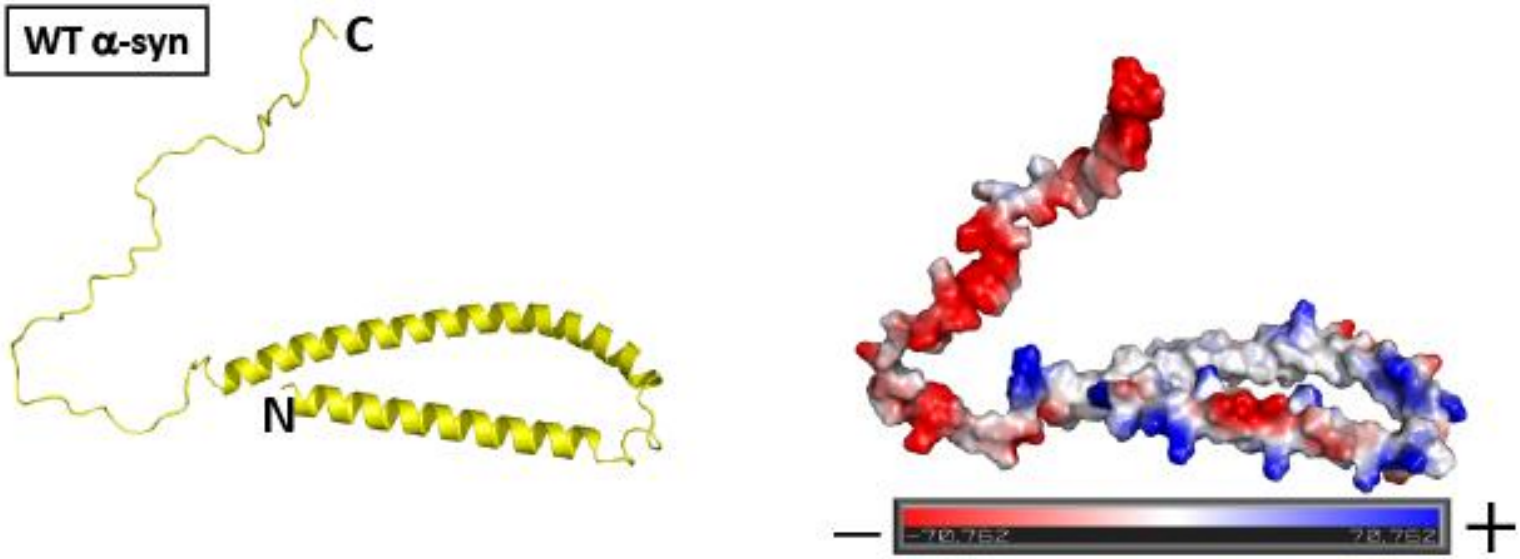
Progression of LB pathology







Unpublished observations

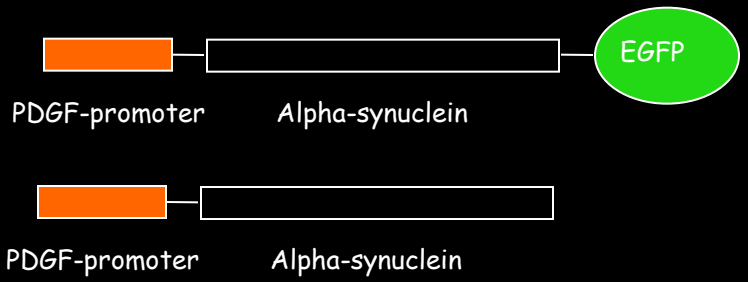

WT α-syn
C
N
−70.752
70.752
−
+

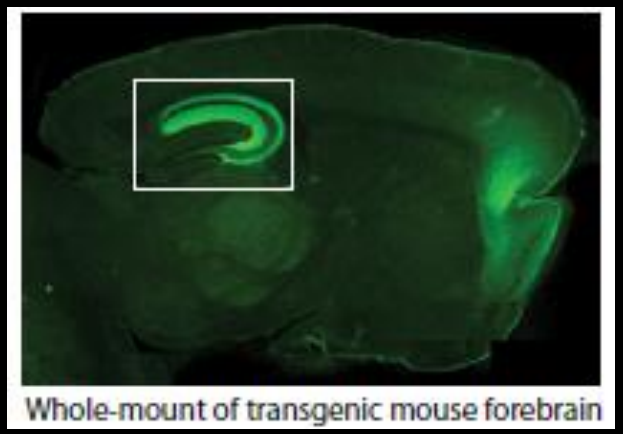
What are the cell-biologic events following a-synuclein elevation?

- Modestly elevated a-synuclein in a neuron

- Synaptic physiology/function

Quantitative model system using cultured hippocampal neurons from α-synuclein transgenic mice




Whole-mount of transgenic mouse forebrain

Advantages of PDGF promoter:

- Neuron-specific expression
- Modest over-expression

Advantages of EGFP tag:

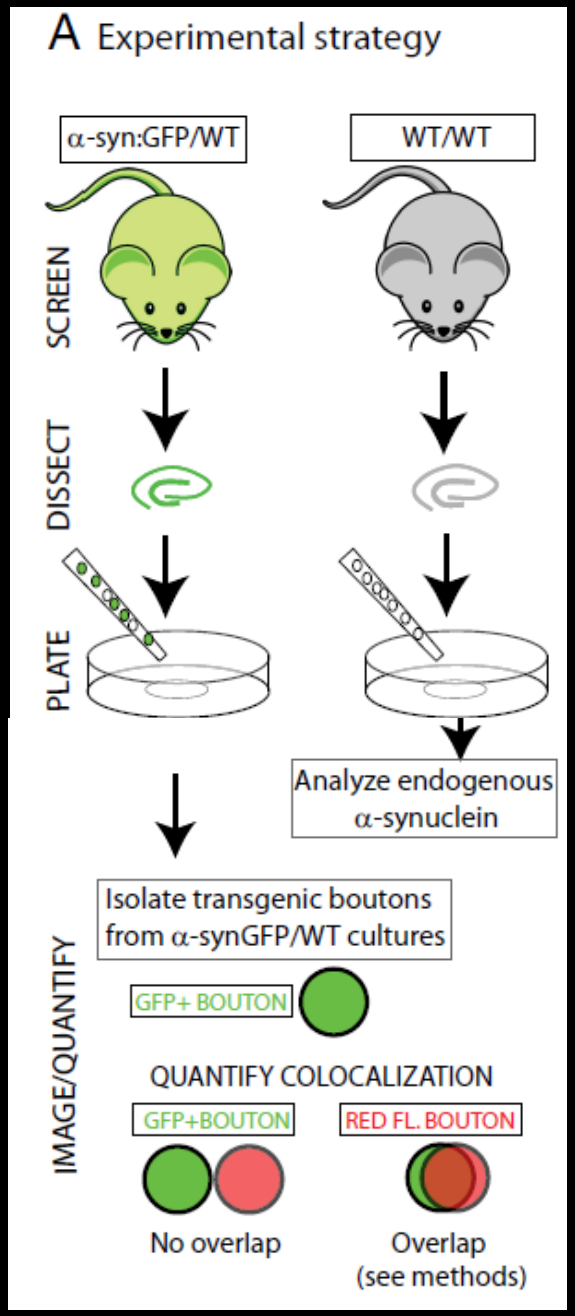
- Precise identification of over-expressing neurons
- Alpha-synuclein expression can be followed over time

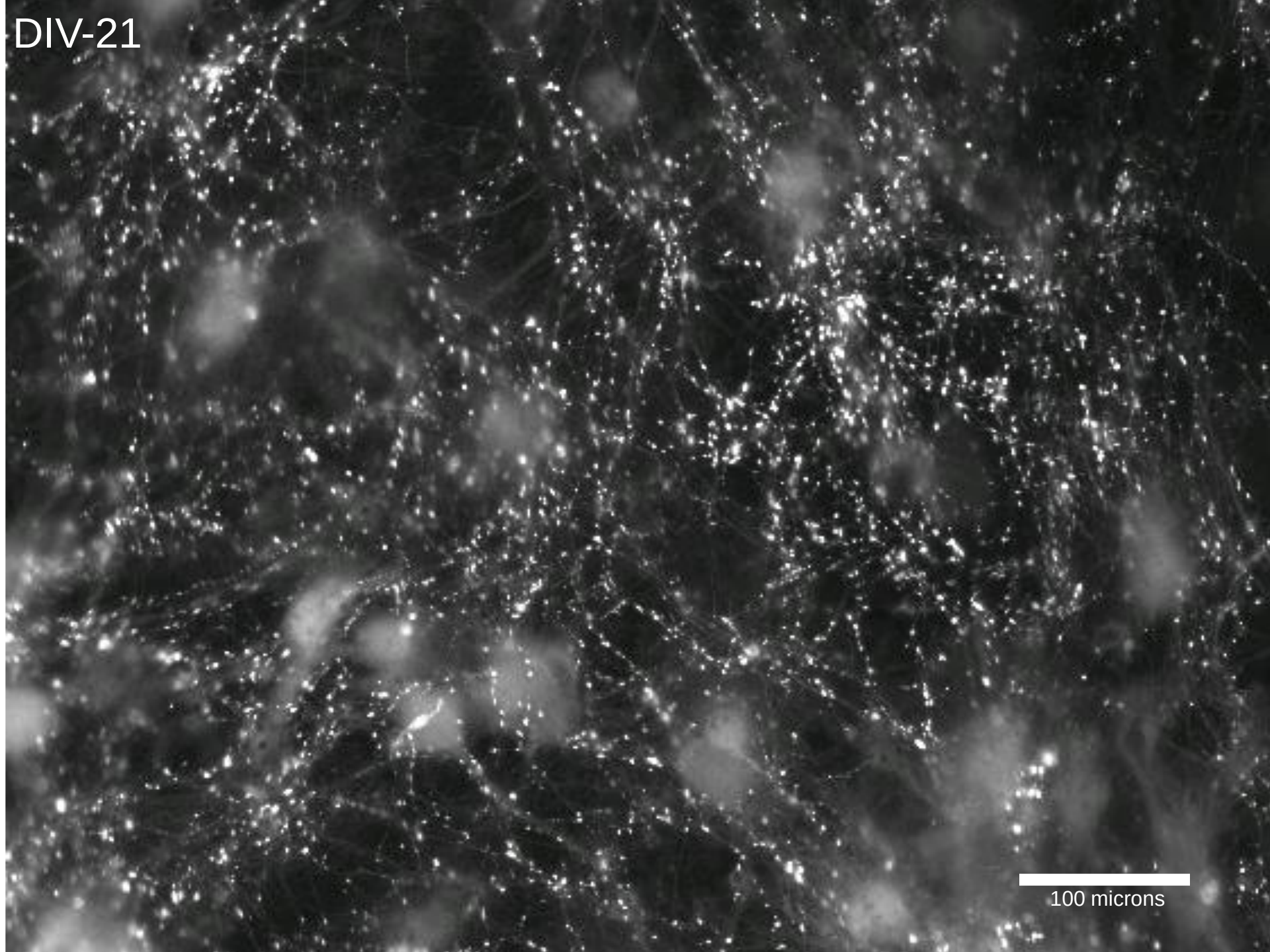
Lysosomal Pathology Associated With α-Synuclein Accumulation in Transgenic Models Using an eGFP Fusion Protein

Edward Rockenstein,[1] Gert Schwach,[3] Elisabeth Ingolic,[5] Anthony Adame,[1] Leslie Crews,[1] Michael Mante,[1] Roswitha Pfragner,[4] Edith Schreiner,[3] Manfred Windisch,[3] and Eliezer Masliah,[1,2]*

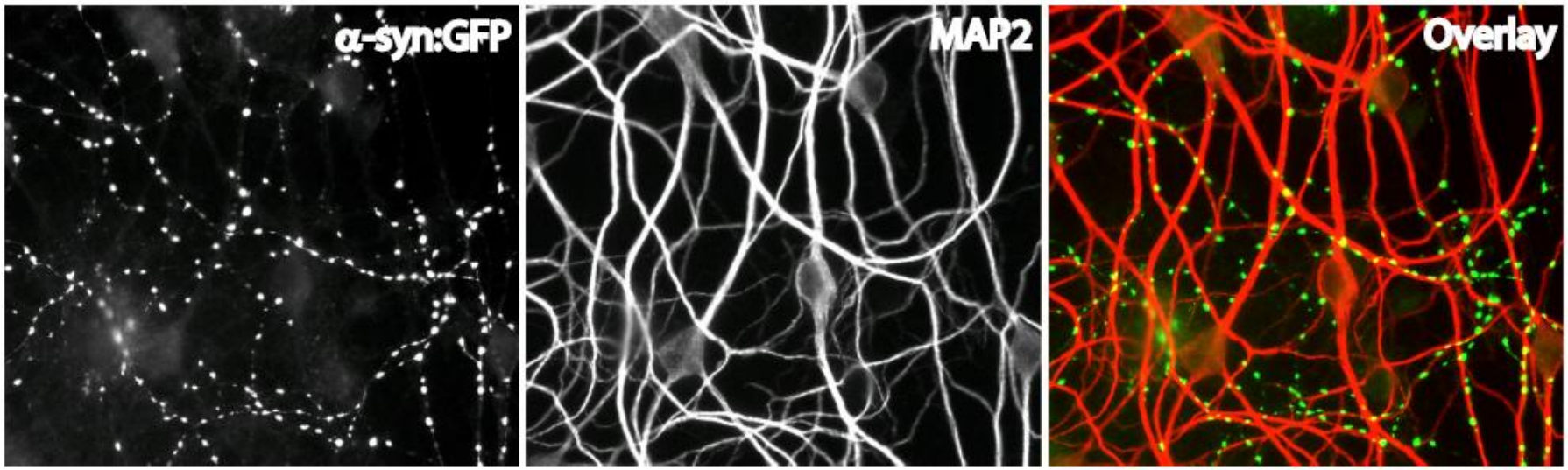
[1]Department of Neurosciences, University of California San Diego, School of Medicine,

Journal of Neuroscience Research 80:247–259 (2005)

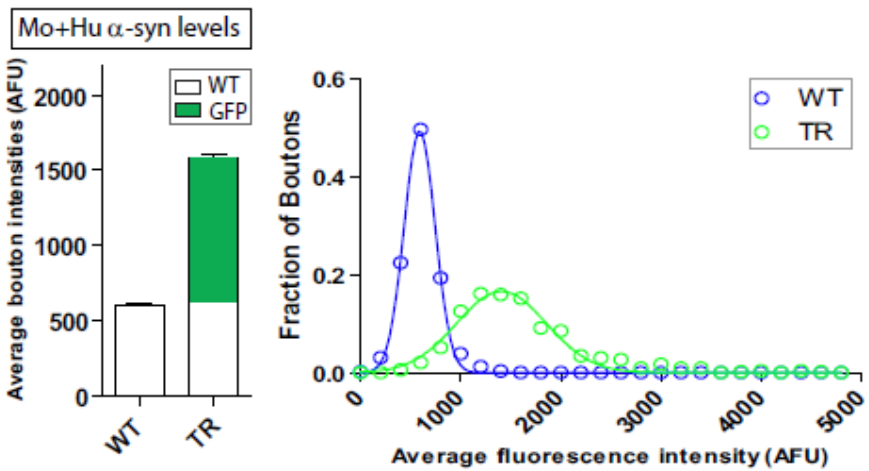



DIV-21
100 microns

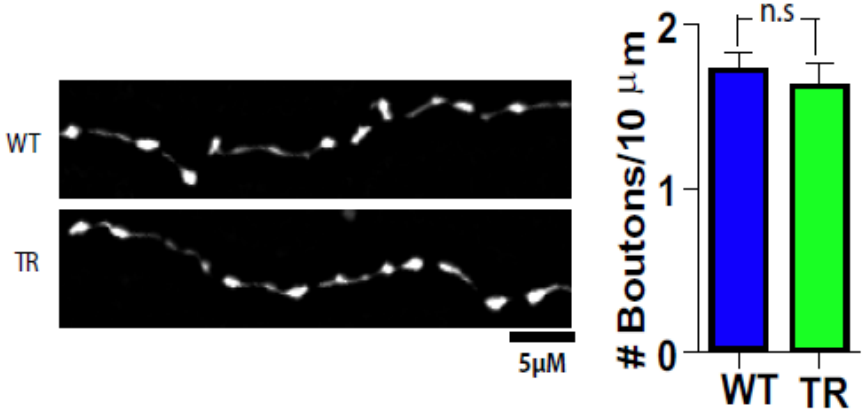
A Presynaptic targeting of α-synuclein:GFP fusion protein



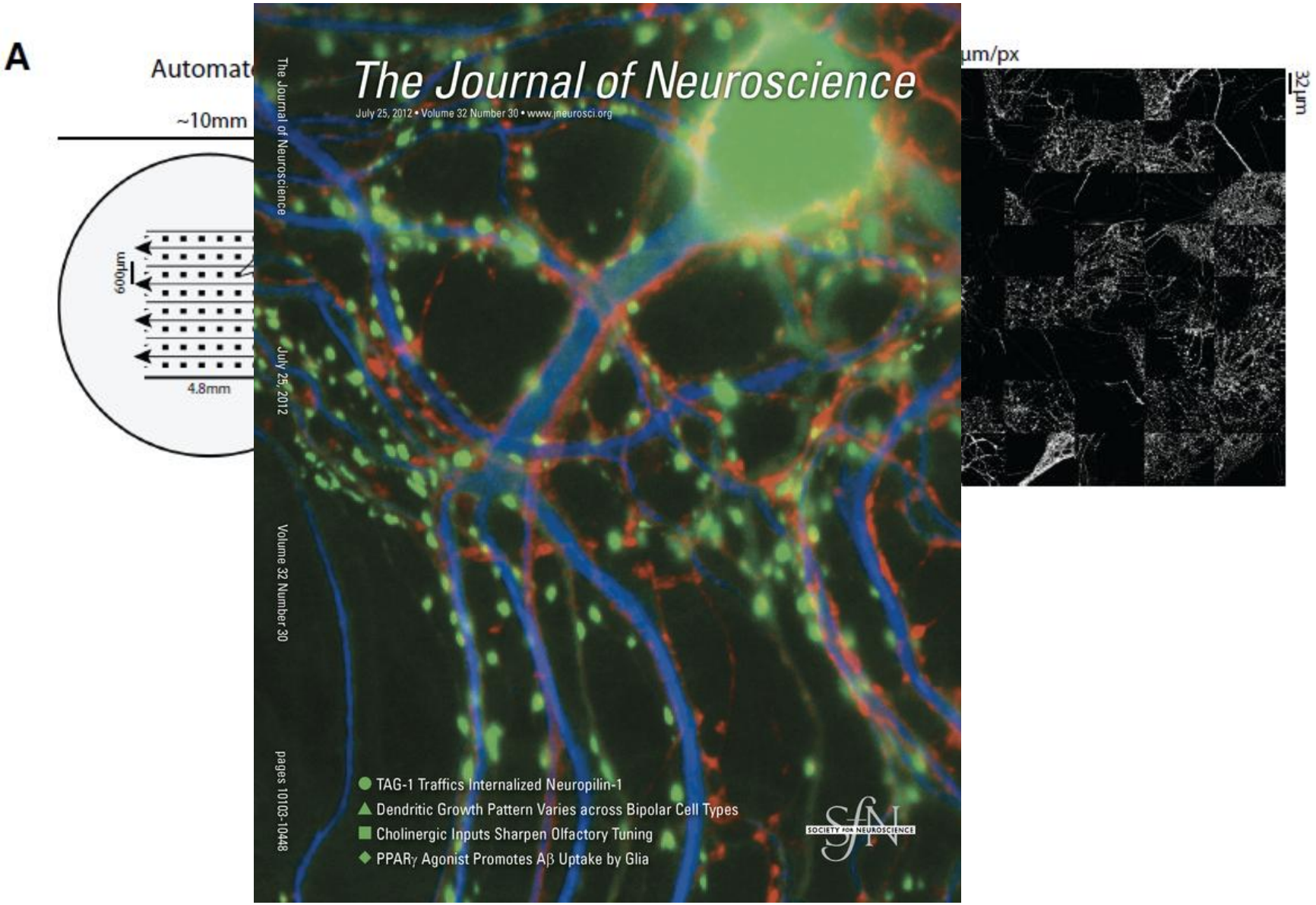
B Over-expression levels in cultured transgenic neurons



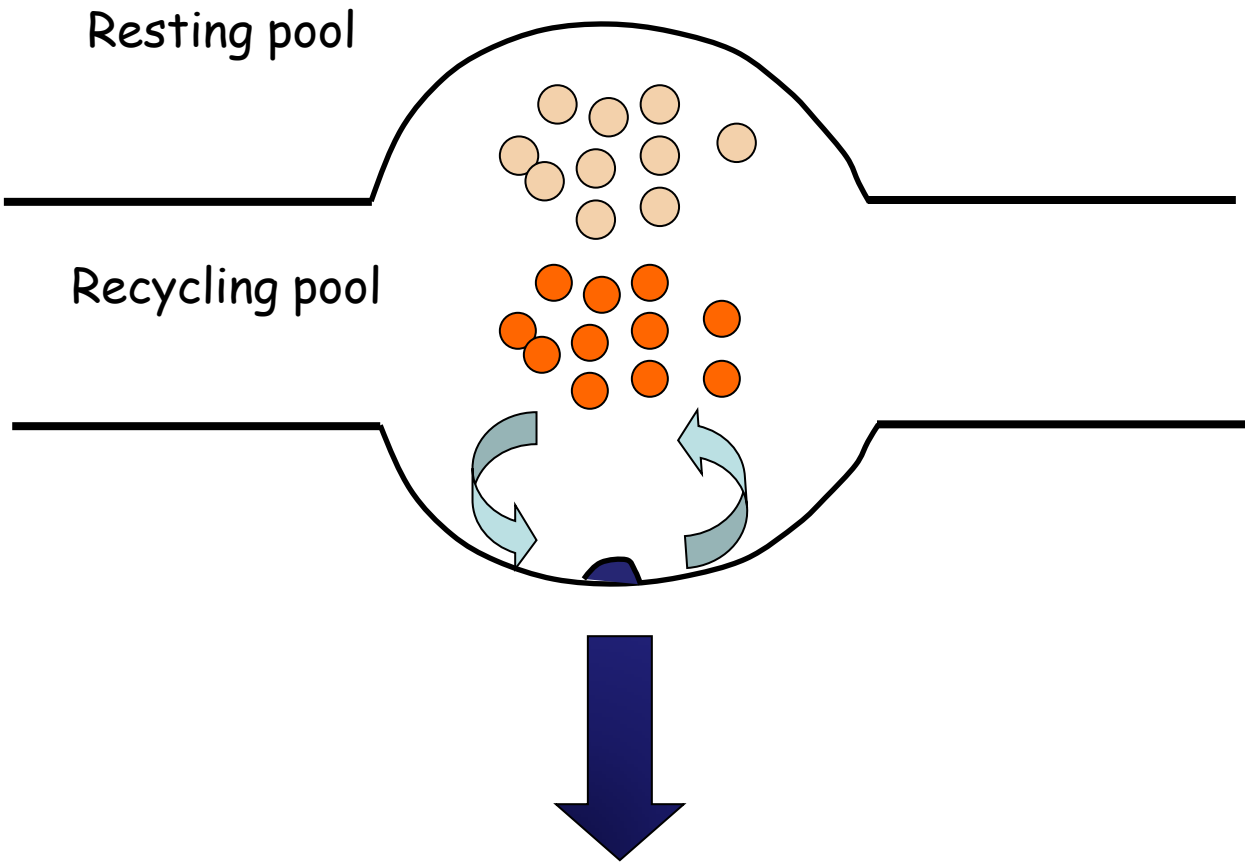
C Synaptic density of WT and transgenic boutons



Scott et al., J Neurosci, **2010** PMID: 205548

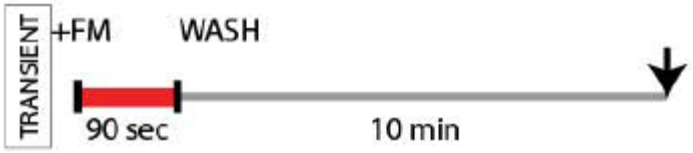


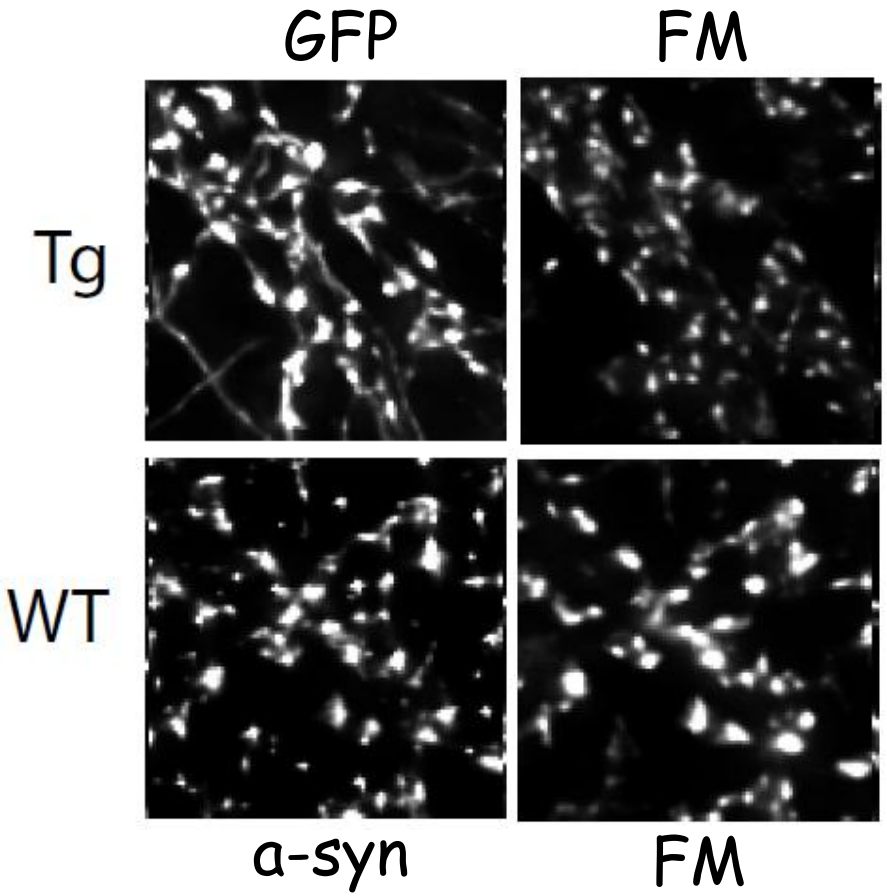
Scott and Roy, J Neurosci. July **2012** (PMID: 22836248)


Resting pool
Recycling pool

Exo *and* endocytic deficits in α-synuclein over-expressing neurons

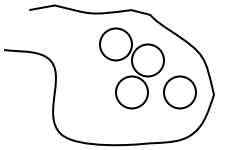
Experiment: Load WT or Tg boutons with FM4-64



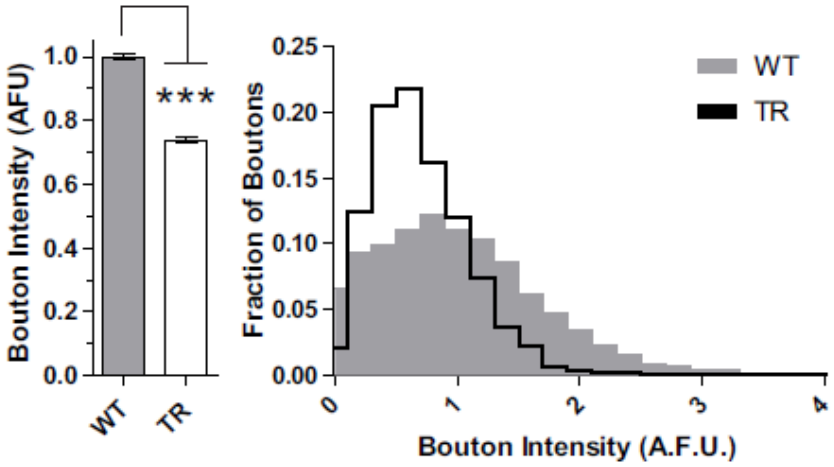


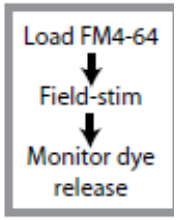
K^+ or field stimulation

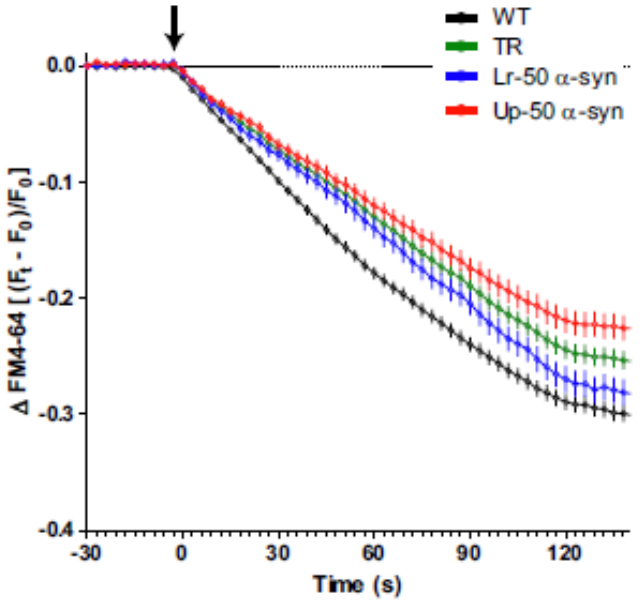
FM dye



Data from ~ 4000-6000 boutons

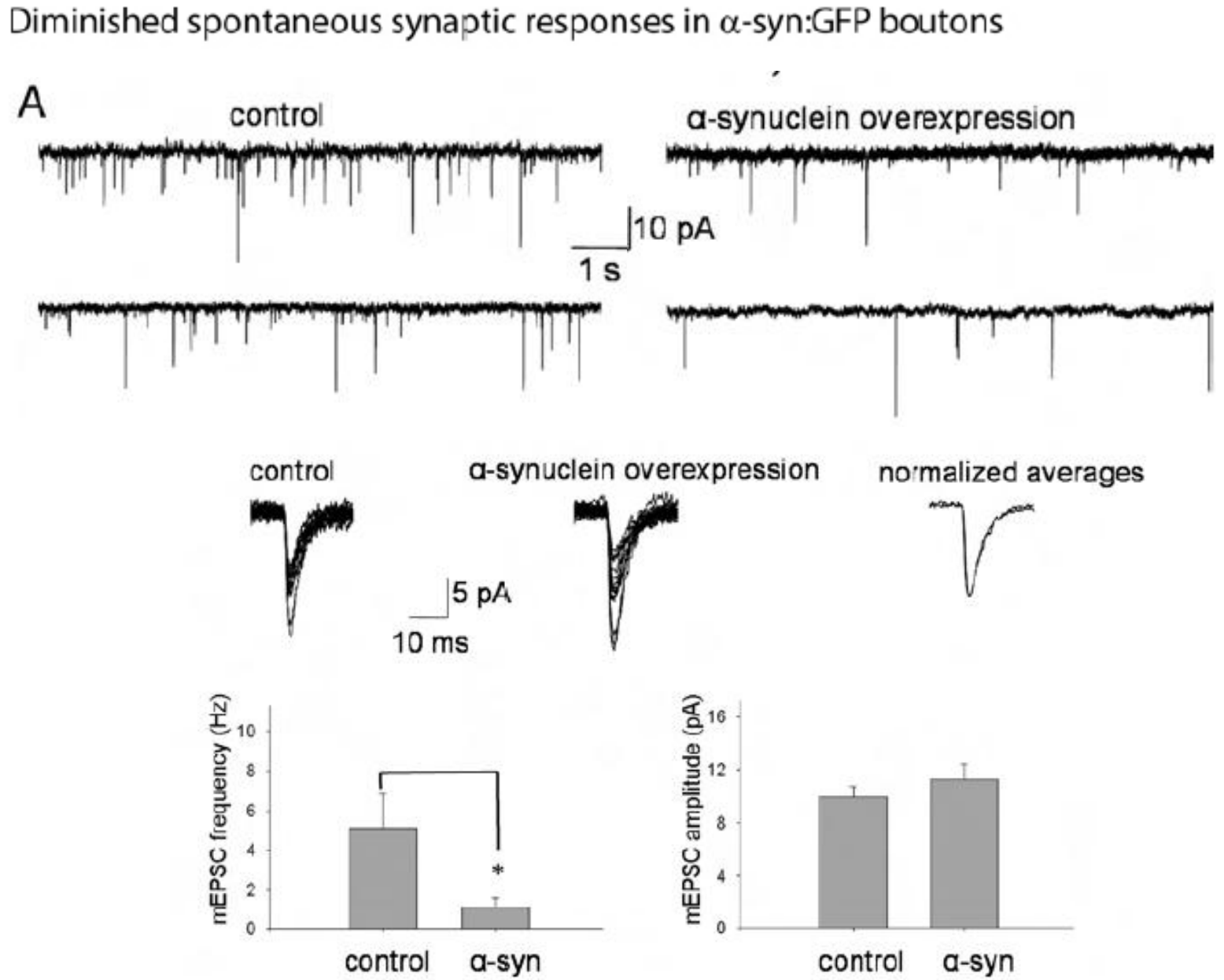






Scott et al., J Neurosci, **2010** PMID: 20554859
Scott and Roy, J Neurosci. July **2012** (PMID: 22836248)

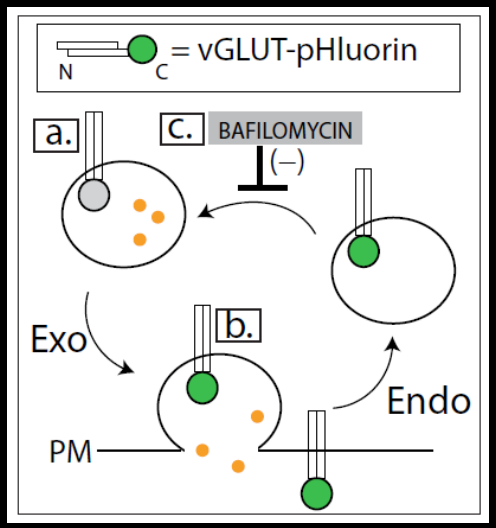
DIMINISHED SYNAPTIC RESPONSES IN α-SYN OVEREXPRESSING NEURONS

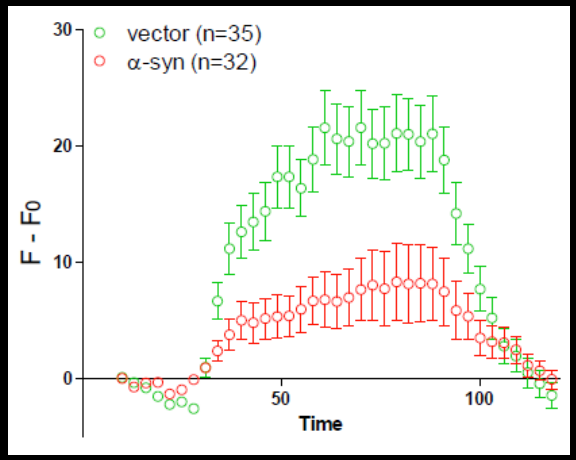


Scott et al., J Neurosci, **2010** PMID: 20554859

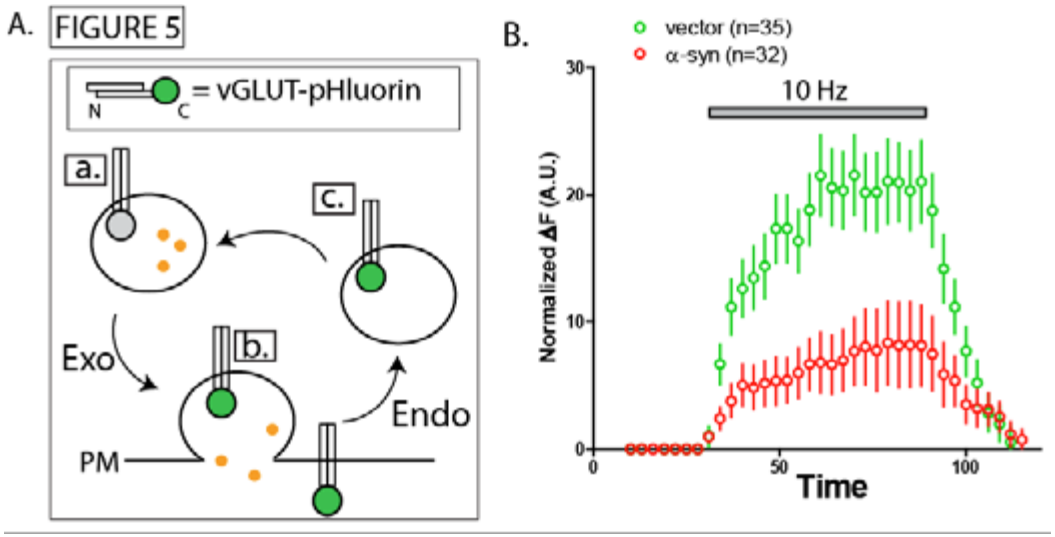
Iustin Tabarean, Scripps

pHluorin assays





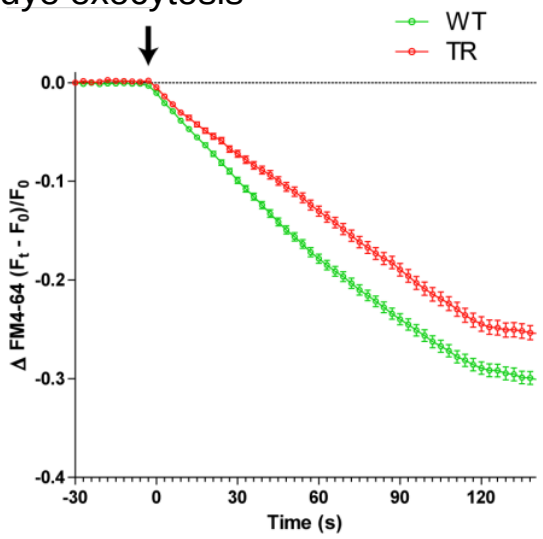
Diminished neurotransmitter release demonstrated using pHluorins



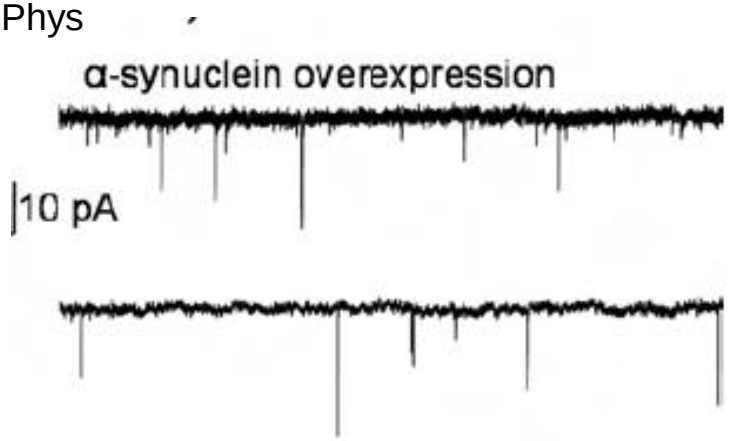
Wang et al., unpublished

Effects of modestly elevated a-synuclein on neurotransmission

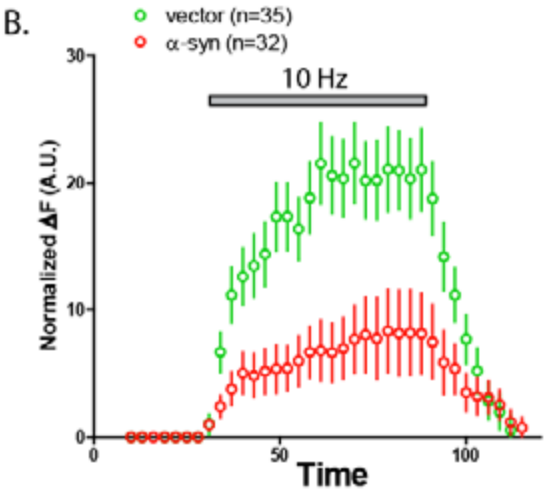
FM-dye exocytosis

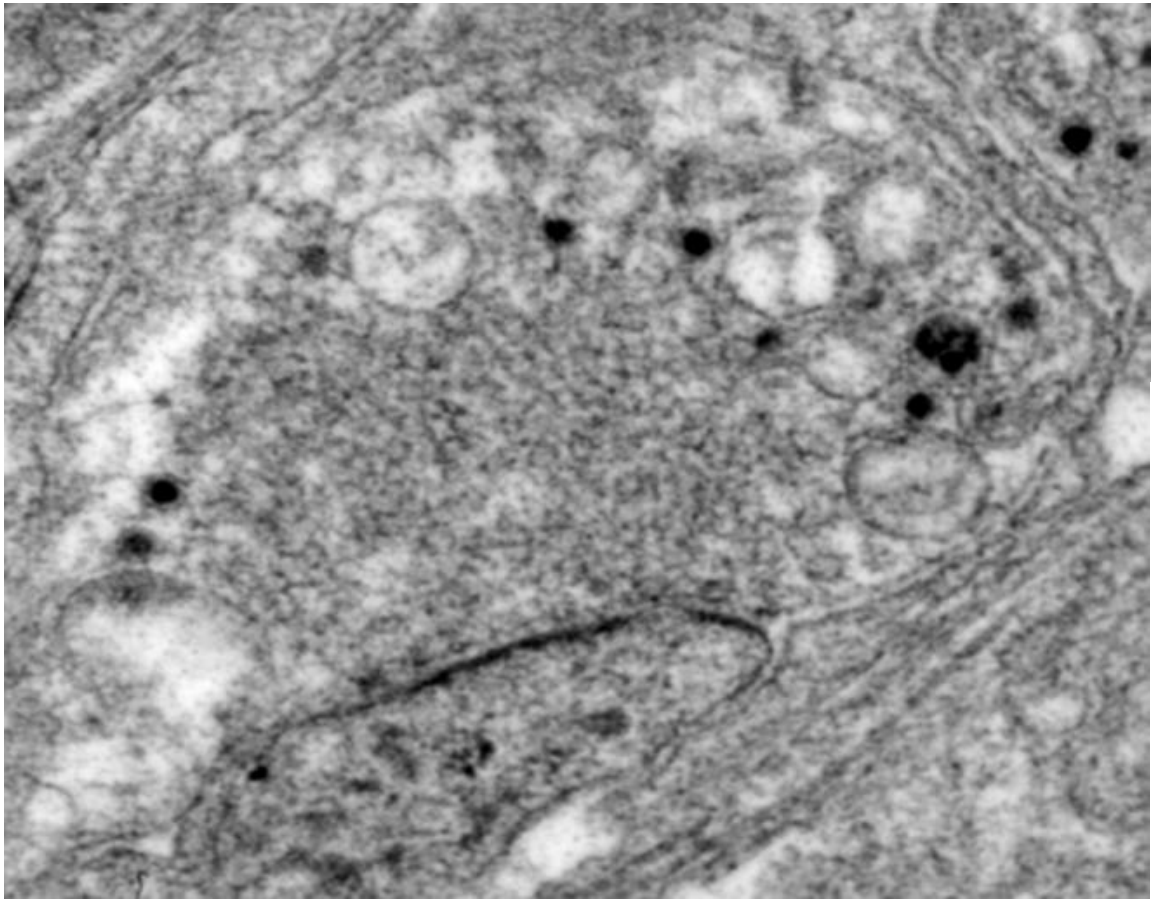


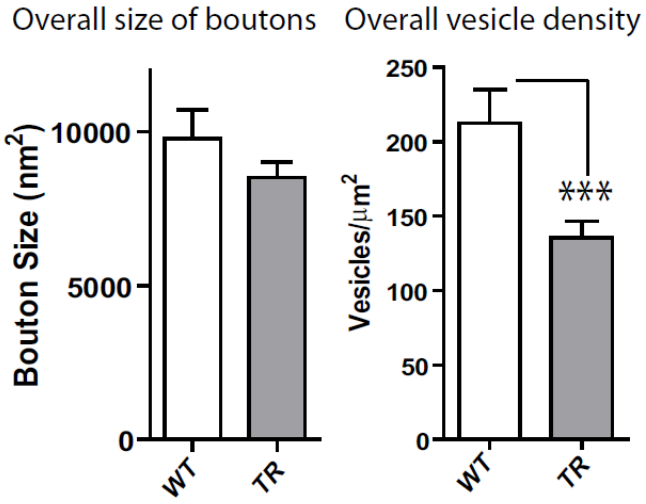
EPhys



Vglut:pHluorin assay



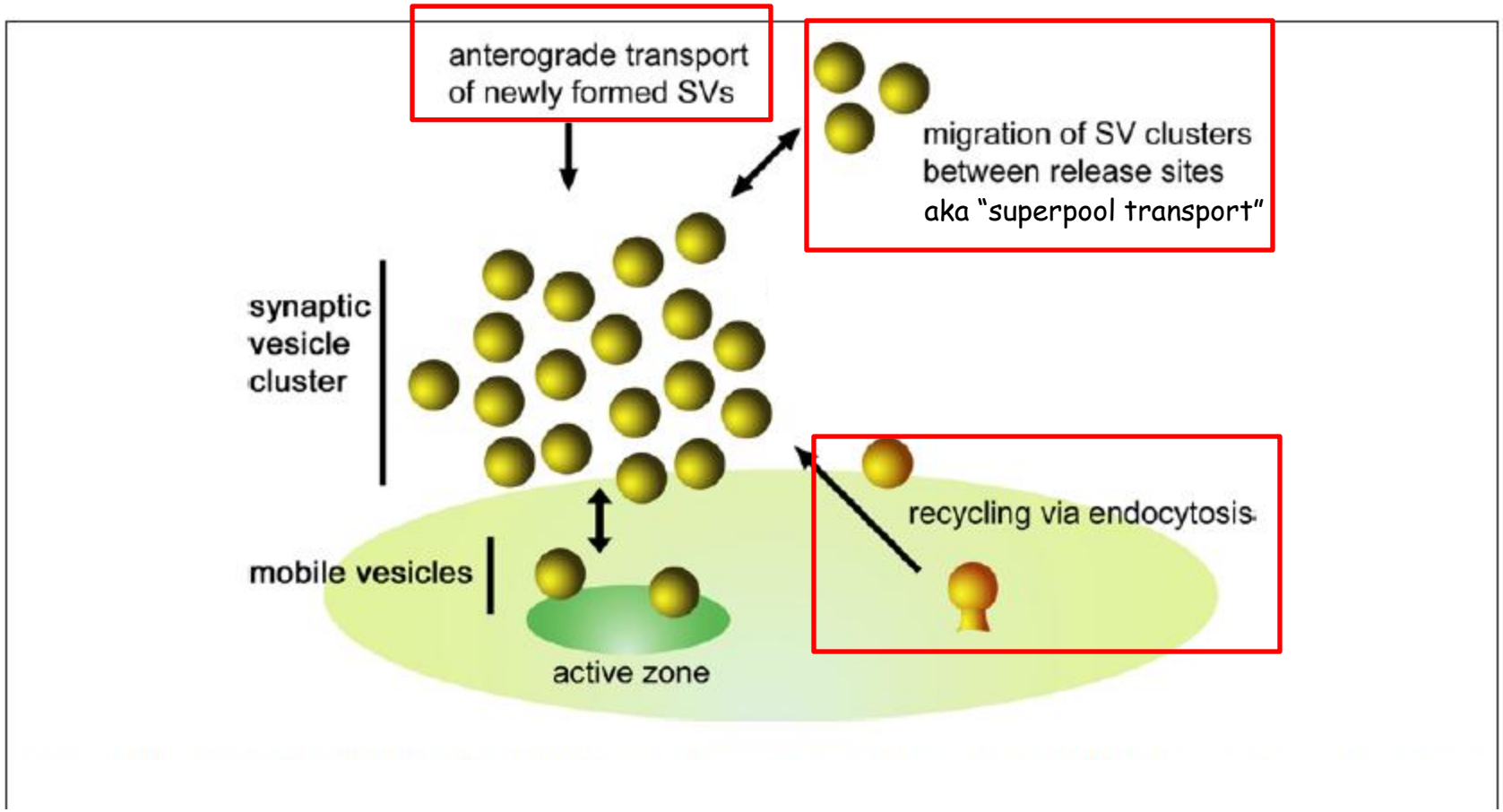



Overall size of boutons
Bouton Size (nm²)
10000
5000
0
WT
TR
Overall vesicle density
Vesicles/µm²
250
200
150
100
50
0
***
WT
TR

Outcomes of *modestly elevated* alpha-synuclein

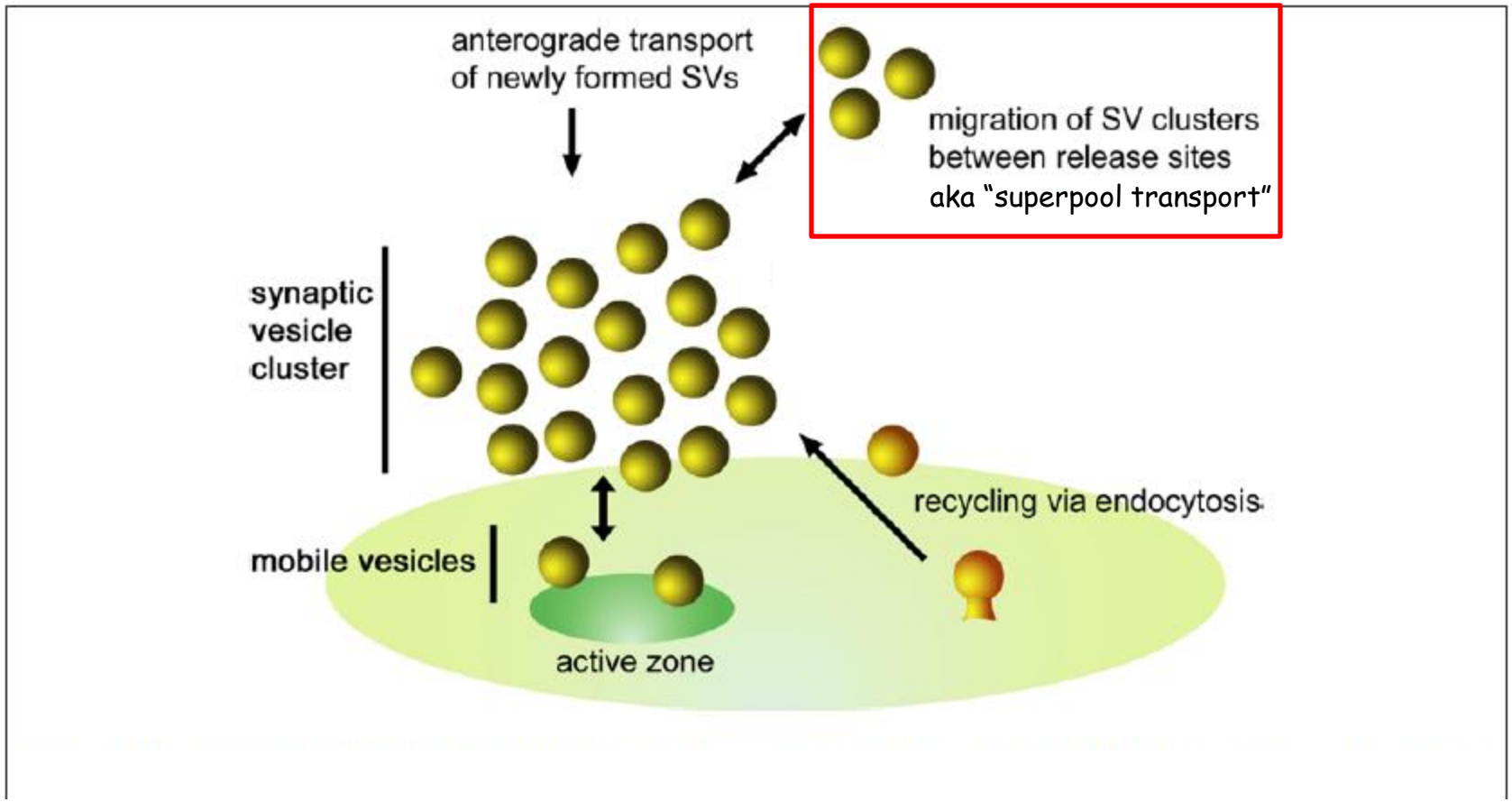
- Reduced synaptic RECYCLING POOLS
- Reduced neurotransmitter release

Replenishment of synaptic recycling pools

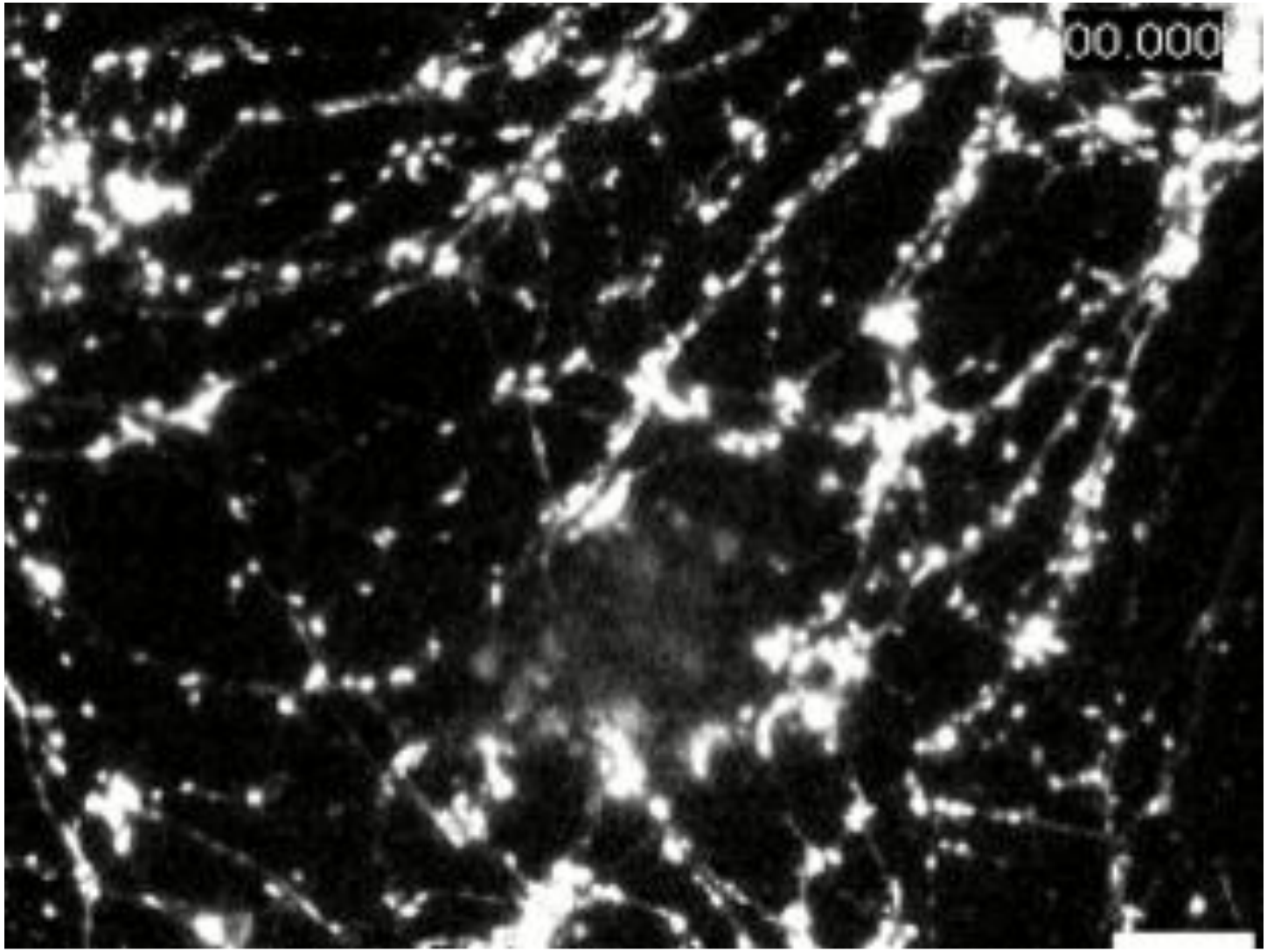


Adapted from Pechstein and Shupliakov, frontiers in synaptic neuroscience

Replenishment of synaptic recycling pools



Adapted from Pechstein and Shupliakov, frontiers in synaptic neuroscience


00.000

α-Synuclein Blocks ER-Golgi Traffic and Rab1 Rescues Neuron Loss in Parkinson's Models

Antony A. Cooper,[1*†] Aaron D. Gitler,[2*] Anil Cashikar,[2‡] Cole M. Haynes,[1§] Kathryn J. Hill,[1†] Bhupinder Bhullar,[2,3] Kangning Liu,[4,5] Kexiang Xu,[4] Katherine E. Strathearn,[6] Fang Liu,[6] Songsong Cao,[7] Kim A. Caldwell,[7] Guy A. Caldwell,[7] Gerald Marsischky,[3] Richard D. Kolodner,[8] Joshua LaBaer,[3] Jean-Christophe Rochet,[6] Nancy M. Bonini,[4,5] Susan Lindquist[2,9‖]

Alpha-synuclein (αSyn) misfolding is associated with several devastating neurodegenerative disorders, including Parkinson's disease (PD). In yeast cells and in neurons αSyn accumulation is cytotoxic, but little is known about its normal function or pathobiology. The earliest defect following αSyn expression in yeast was a block in endoplasmic reticulum (ER)–to–Golgi vesicular trafficking. In a genomewide screen, the largest class of toxicity modifiers were proteins functioning

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The Parkinson's disease protein α-synuclein disrupts cellular Rab homeostasis

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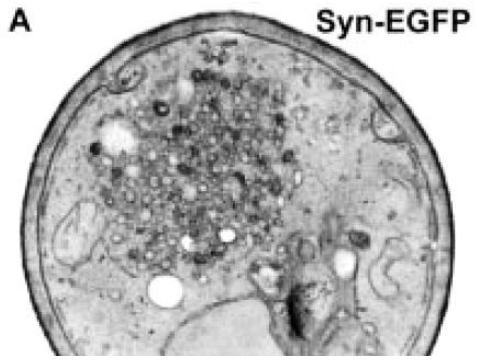
Contributed by Susan Lindquist, November 15, 2007 (sent for review August 20, 2007)

α-Synuclein (α-syn), a protein of unknown function, is the most abundant protein in Lewy bodies, the histological hallmark of

evoked neurotransmitter release owing to an increase in the pool of docked, but not yet fused, secretory vesicles (14, 15). Other



Molecular Biology of the Cell
Vol. 19, 1093–1103, March 2008

α-Synuclein–induced Aggregation of Cytoplasmic Vesicles in *Saccharomyces cerevisiae*

James H. Soper,*† Subhojit Roy,*† Anna Stieber,*† Eliza Lee,*† Robert B. Wilson,† John Q. Trojanowski,*† Christopher G. Burd,‡ and Virginia M.-Y. Lee*†

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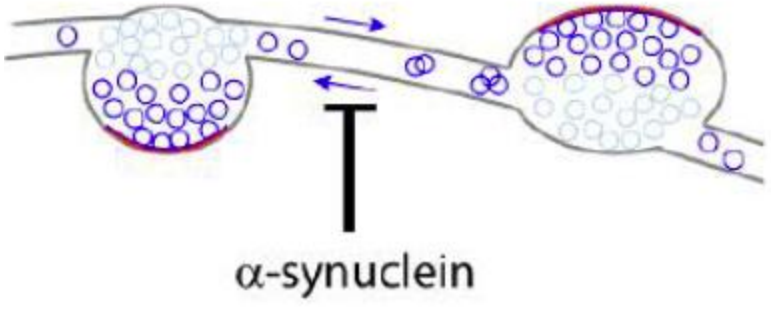
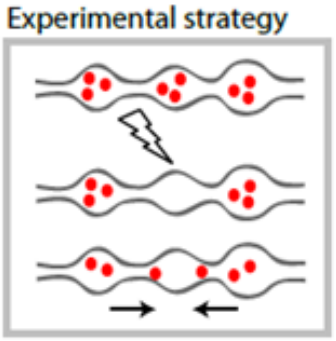
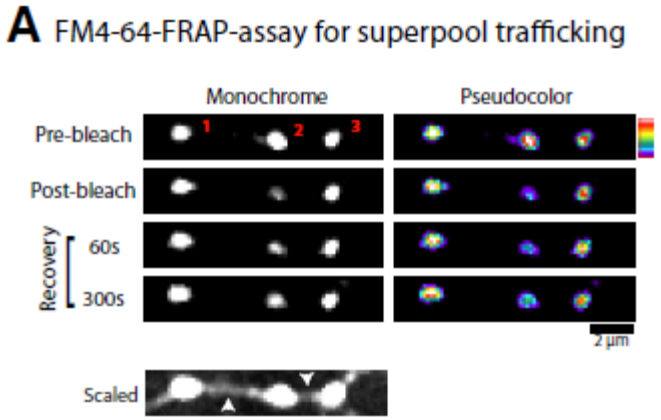
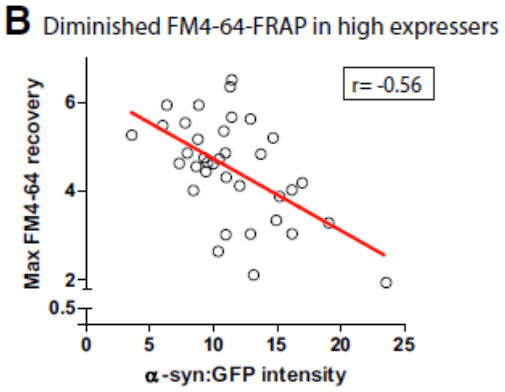
Submitted August 29, 2007; Revised December 12, 2007; Accepted December 20, 2007

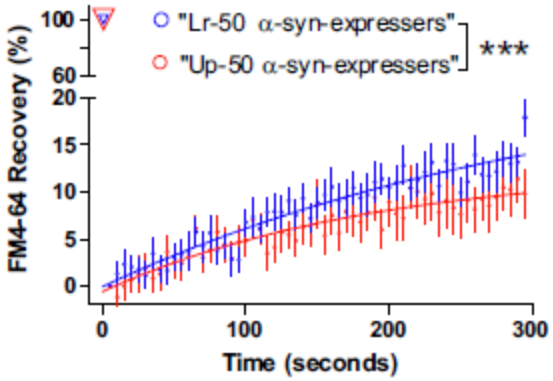


Assay for analyzing intra-synaptic exchange of recycling vesicles



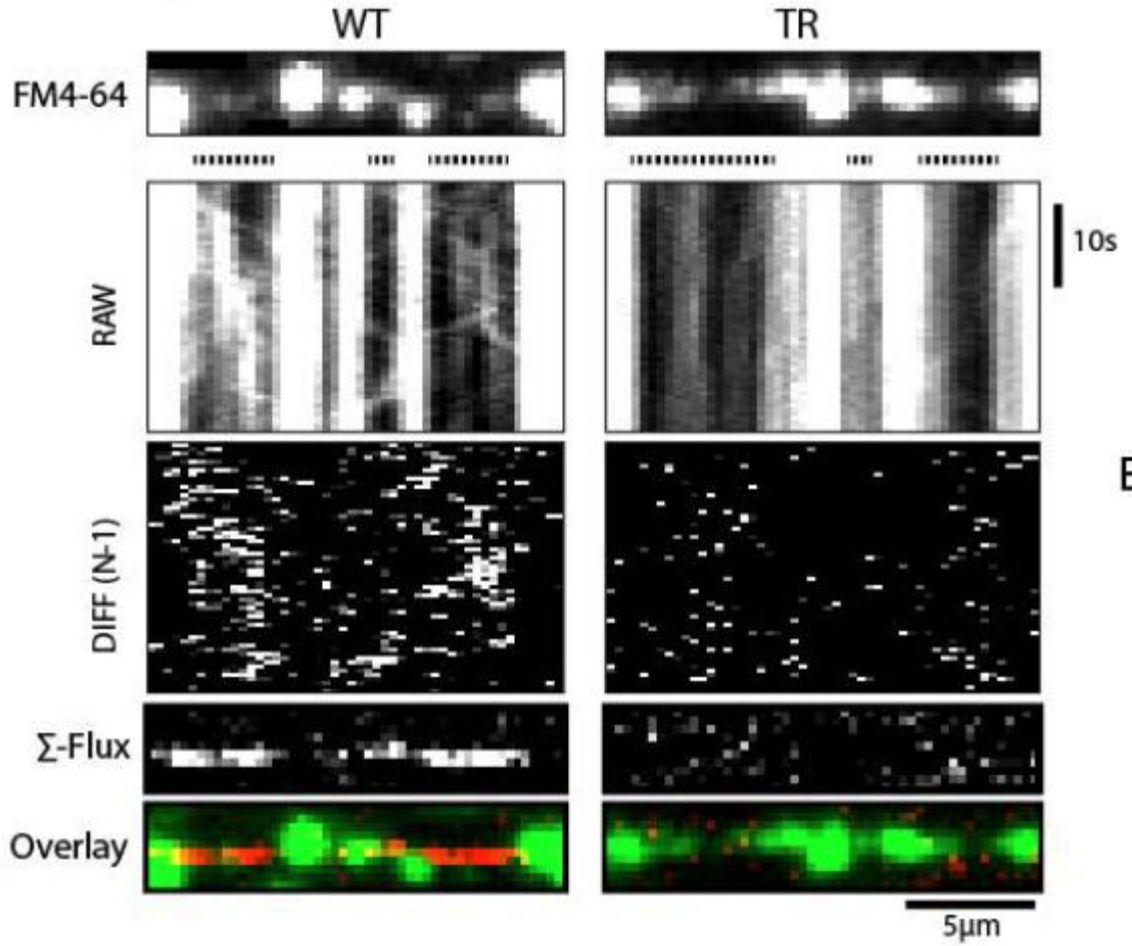




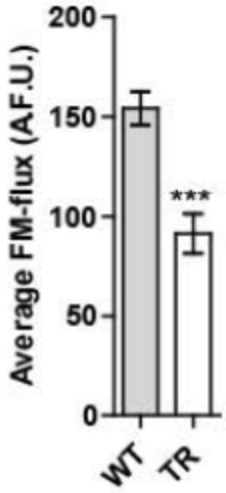


Scott and Roy, J Neurosci. July **2012** (PMID: 22836248)

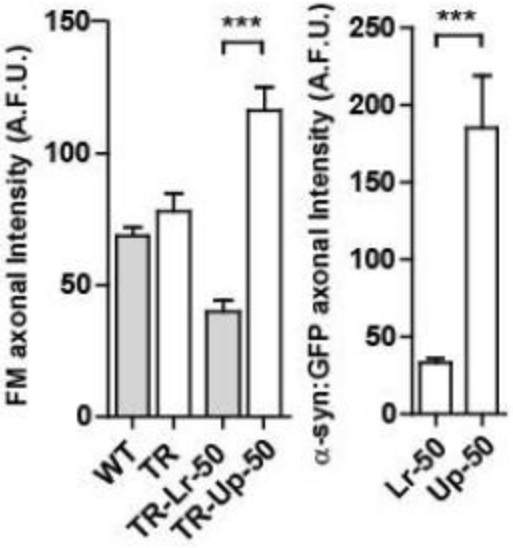
C. Diminished FM-trafficking in α-synuclein overexpressing boutons



D.

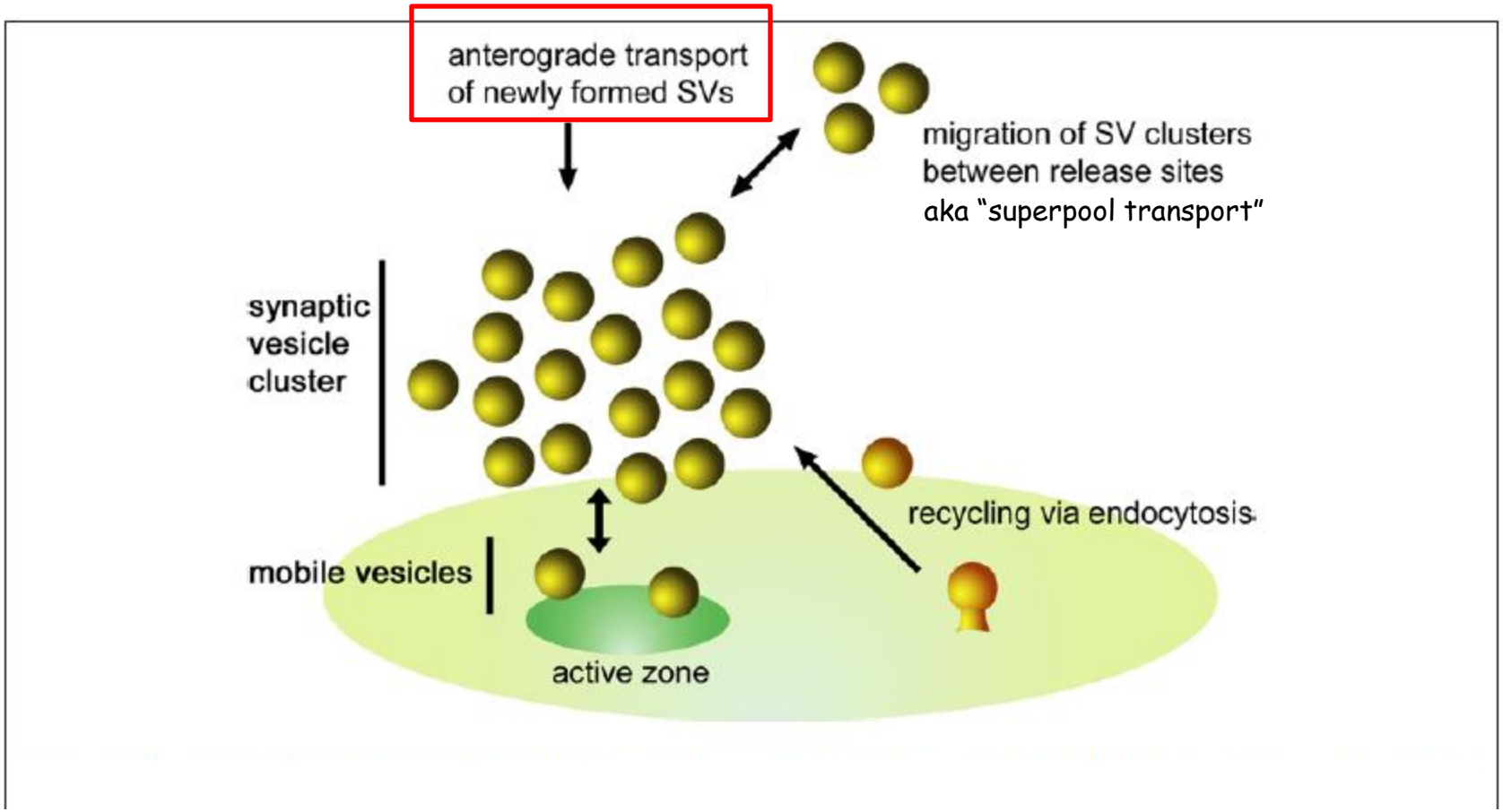


E.



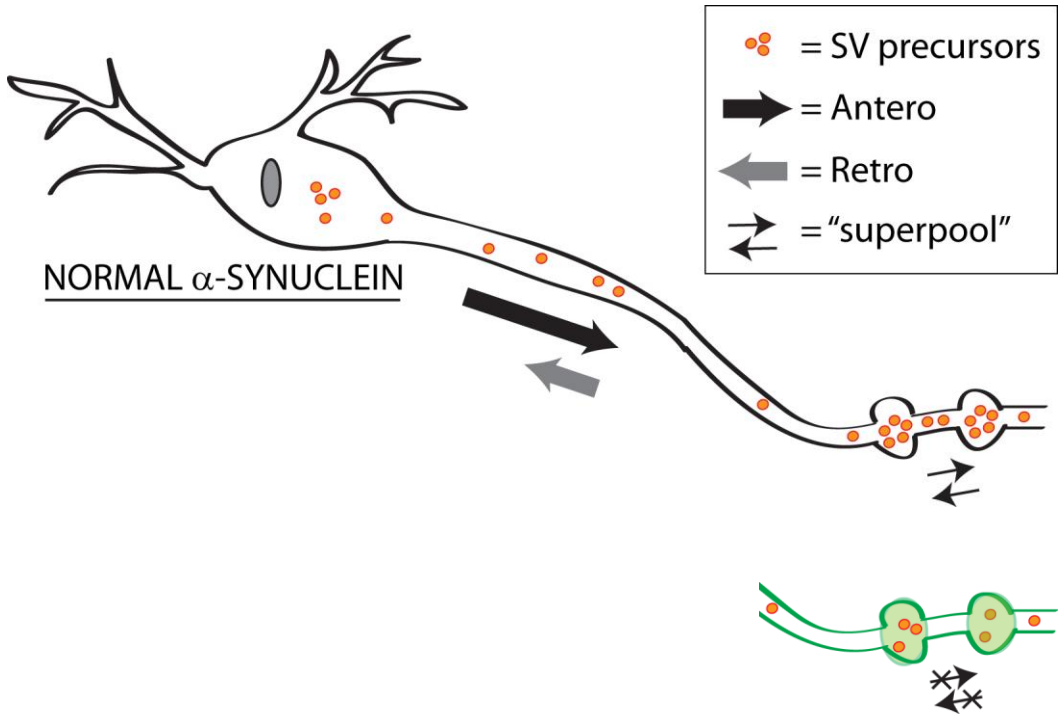
Scott and Roy, J Neurosci. July **2012** (PMID: 22836248)

Replenishment of synaptic recycling pools



Adapted from Pechstein and Shupliakov, frontiers in synaptic neuroscience

Axonal transport of synaptic vesicle (SV) precursors



ALPHA-SYNUCLEIN IS DELIVERED TO SYNAPSES BY SLOW AXONAL TRANSPORT

The slow axonal transport of alpha-synuclein – mechanistic commonalities amongst diverse cytosolic cargoes. Tang Y, Das U, Scott D and Roy S*. **Cytoskeleton** (special issue), **2012** PMID: 22309053

Mechanistic logic underlying the axonal transport of cytosolic proteins. Scott D, Das U, Tang Y and Roy S*. **Neuron** May **2011** PMID: 21555071

A simple photoactivation and image-analysis module for visualizing and analyzing axonal transport with high temporal resolution. Roy S*, Yang Ge, Tang Y and Scott D. **Nature Protocols**, **2011** Dec PMID: 22179592

Axonal transport of synaptic vesicle precursors (SVPs) in neurons transiently transfected with α-synuclein

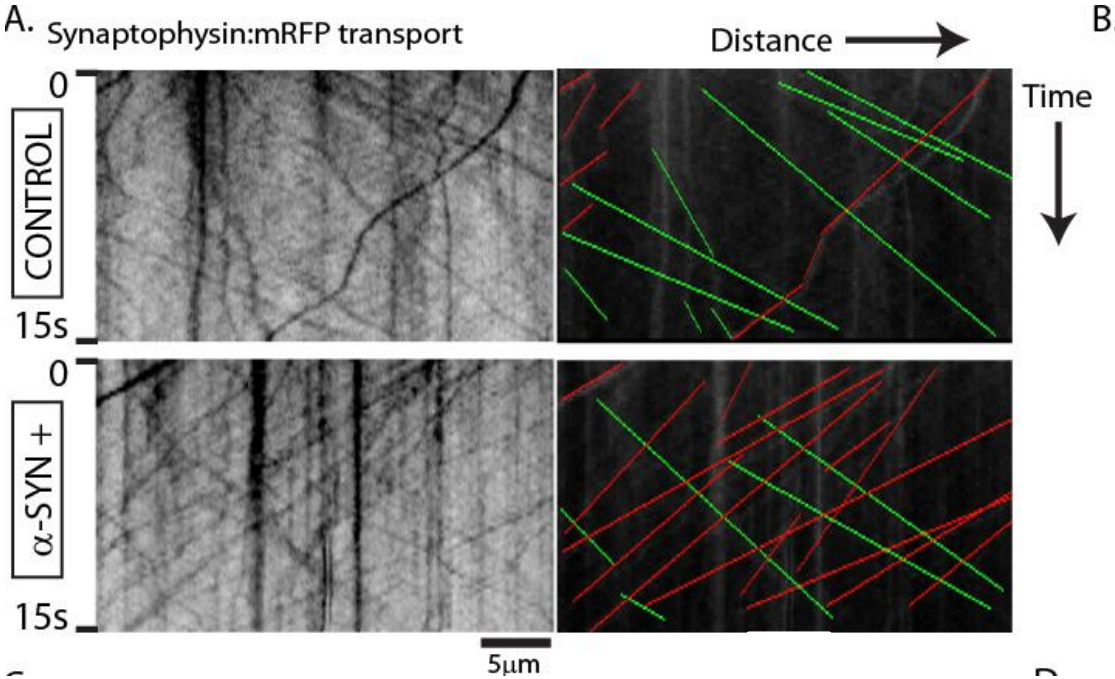
Co-transfect
GFP:α-syn + Synap:mRFP
in DIV 10 neurons

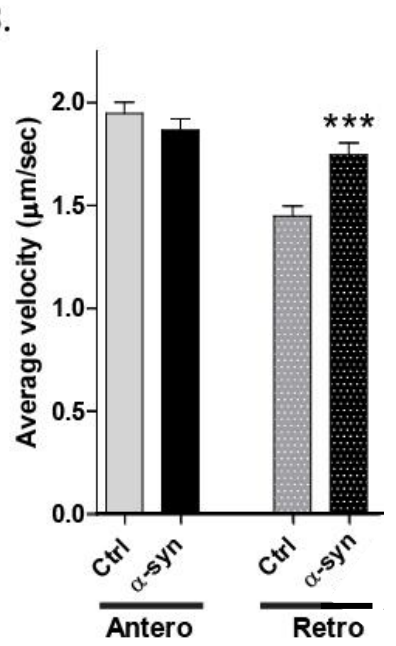
↓

Wait 24h

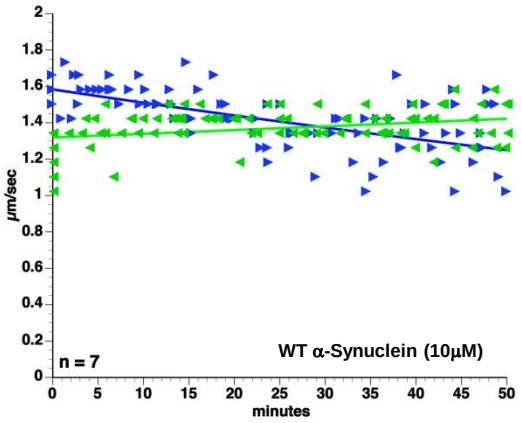
↓

Visualize Synap:mRFP
using optimized
protocols

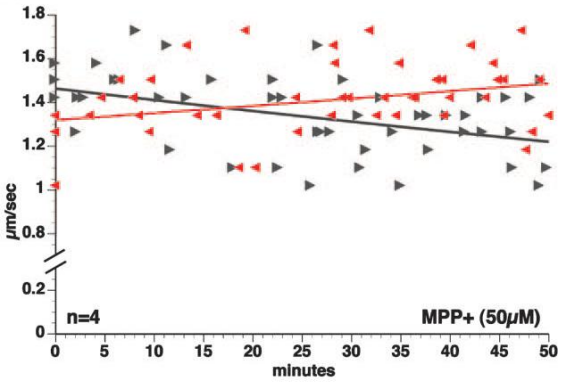




Method: Tang et al., Traffic, 2012, PMID: 22309053

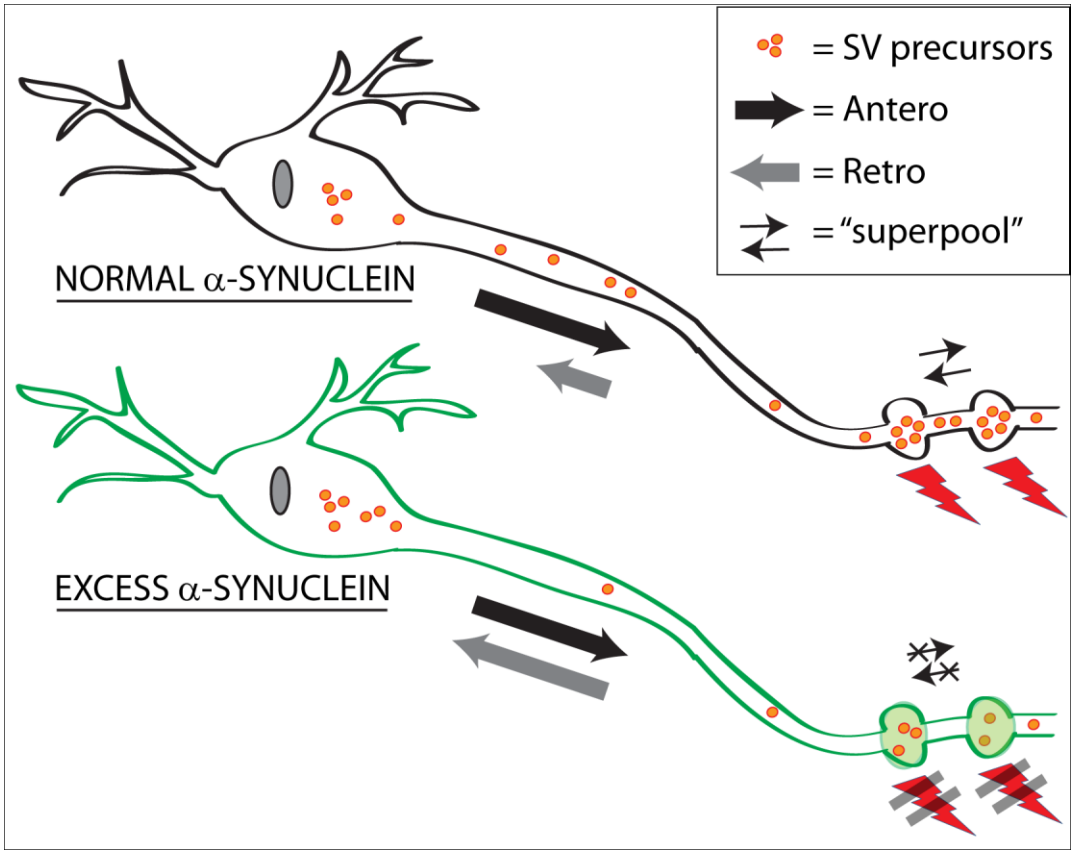


Morfini and Brady, unpublished



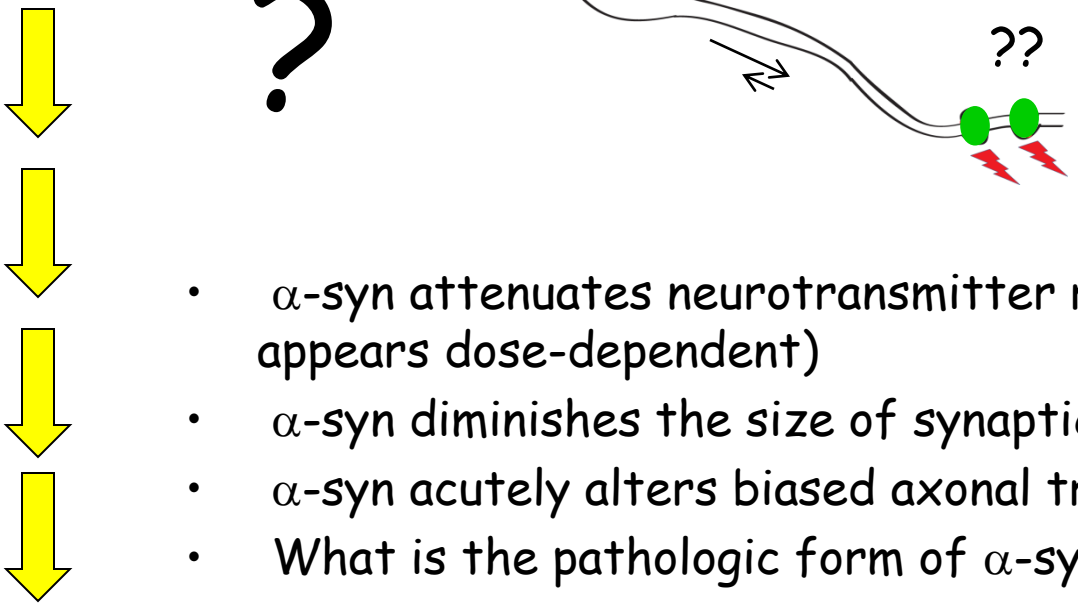
Morfini et al., PNAS 2006 PMID: PMID: 17287338

An 'extra-synaptic' mechanism to explain a 'synaptic' deficit: a possible scenario…



What are the cell-biologic events following a-synuclein elevation? *A hypothesis*

- Modestly elevated a-synuclein in a neuron

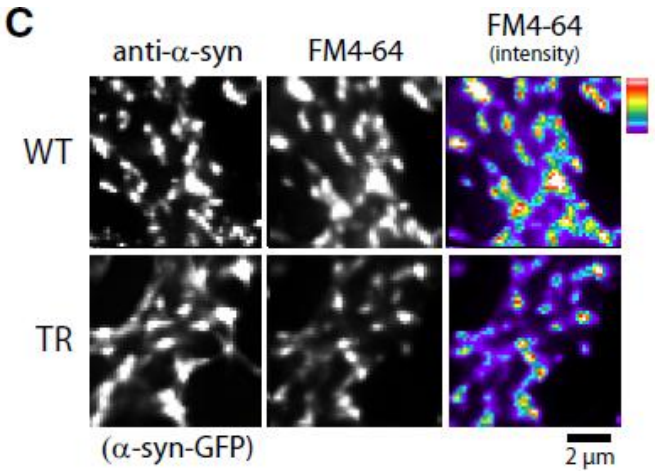


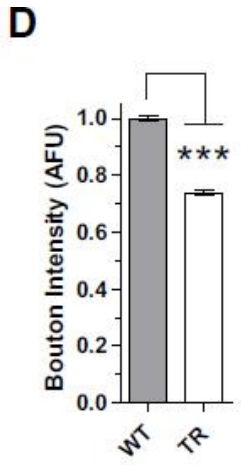
- α-syn attenuates neurotransmitter release (the effect appears dose-dependent)
- α-syn diminishes the size of synaptic recycling pools
- α-syn acutely alters biased axonal transport of SVPs
- What is the pathologic form of α-syn?

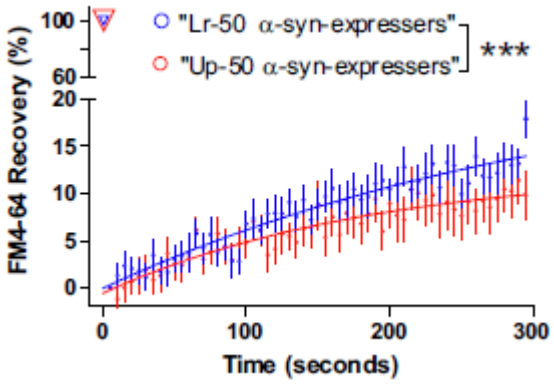
- Synaptic physiology/function

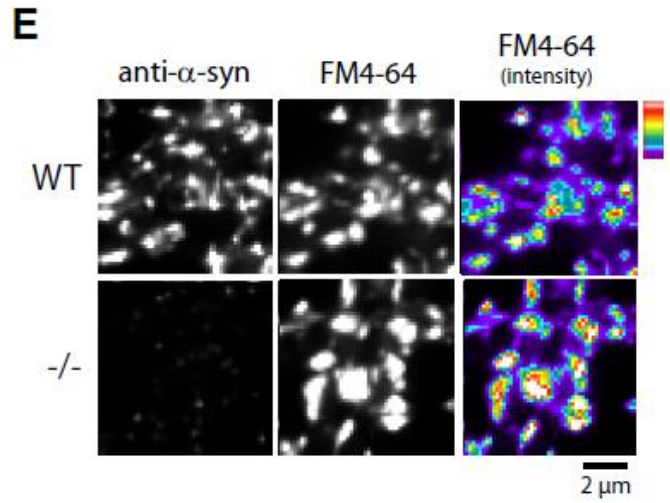
Could the α-syn induced synaptic phenotypes – decreased recycling pools/diminished superpool trafficking – reflect the normal role for α-syn ?

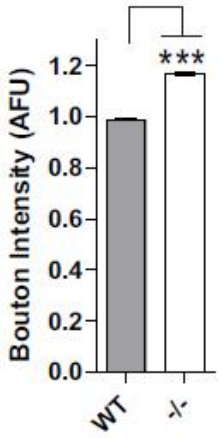
Physiologic effects of α-syn on recycling pools

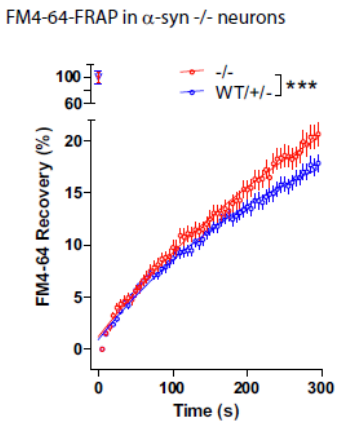


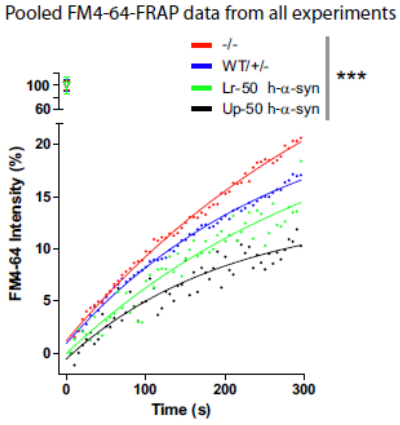








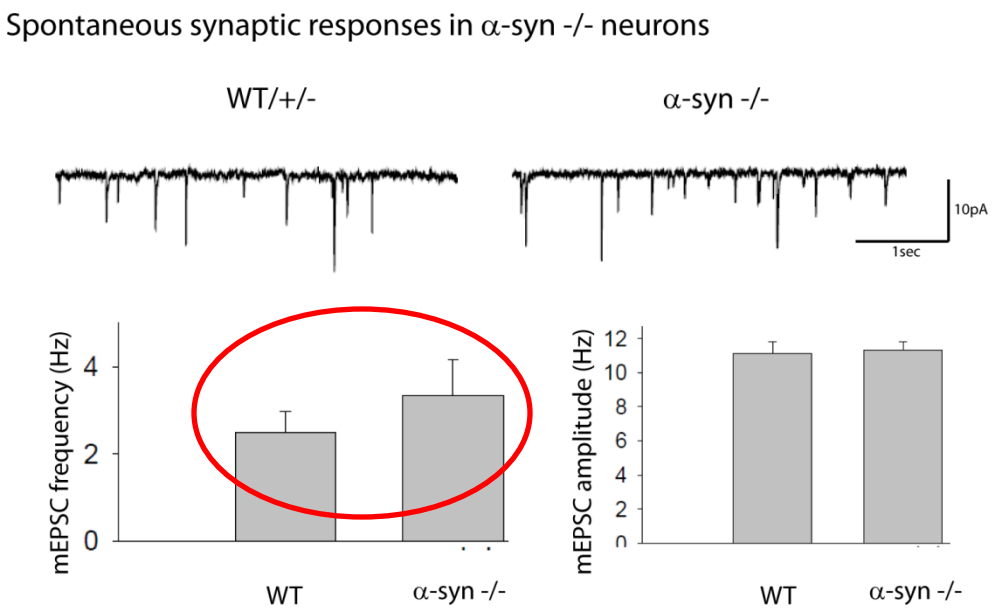




N ~ 25,000 – 50,000 boutons/condition

Scott and Roy, J Neurosci. July **2012** (PMID: 22836248)

Physiologic effects of α-syn on recycling pools



Iustin Tabarean, Scripps

α-syn may have a normal role in regulating inter-synaptic trafficking and maintaining recycling pool levels

α-synuclein…..

David Scott

Utpal Das

Yong Tang

Subhojit Roy

Early and selective impairments in axonal transport kinetics of synaptic cargoes induced by soluble amyloid-beta protein oligomers. Tang Y, Scott D, Das U, Edland S, Radomski K, Koo E and Roy S. **Traffic**, May 2012;13(5):681-93..

A pathologic cascade leading to synaptic dysfunction in a-synuclein-induced neurodegeneration (2010). Scott D, Tabarean I, Tang Y, Cartier A, Masliah E, Roy S. **Journal of Neuroscience** Jun **2010** 16;30(24):8083-95.

Alpha-synuclein inhibits inter-synaptic vesicle trafficking and regulates recycling-pool homeostasis. Scott D and Roy S. **Journal of Neuroscience**, **2012** July 25; 32(30):10129-35.

Eliezer Masliah, UCSD

Iustin Tabarean, Scripps

march of dimes march for babies

NATIONAL INSTITUTES OF HEALTH

ARDA TO EASE THE BURDEN TO FIND THE CURE American Parkinson Disease Association

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LARRY L. HILLBLOM Foundation