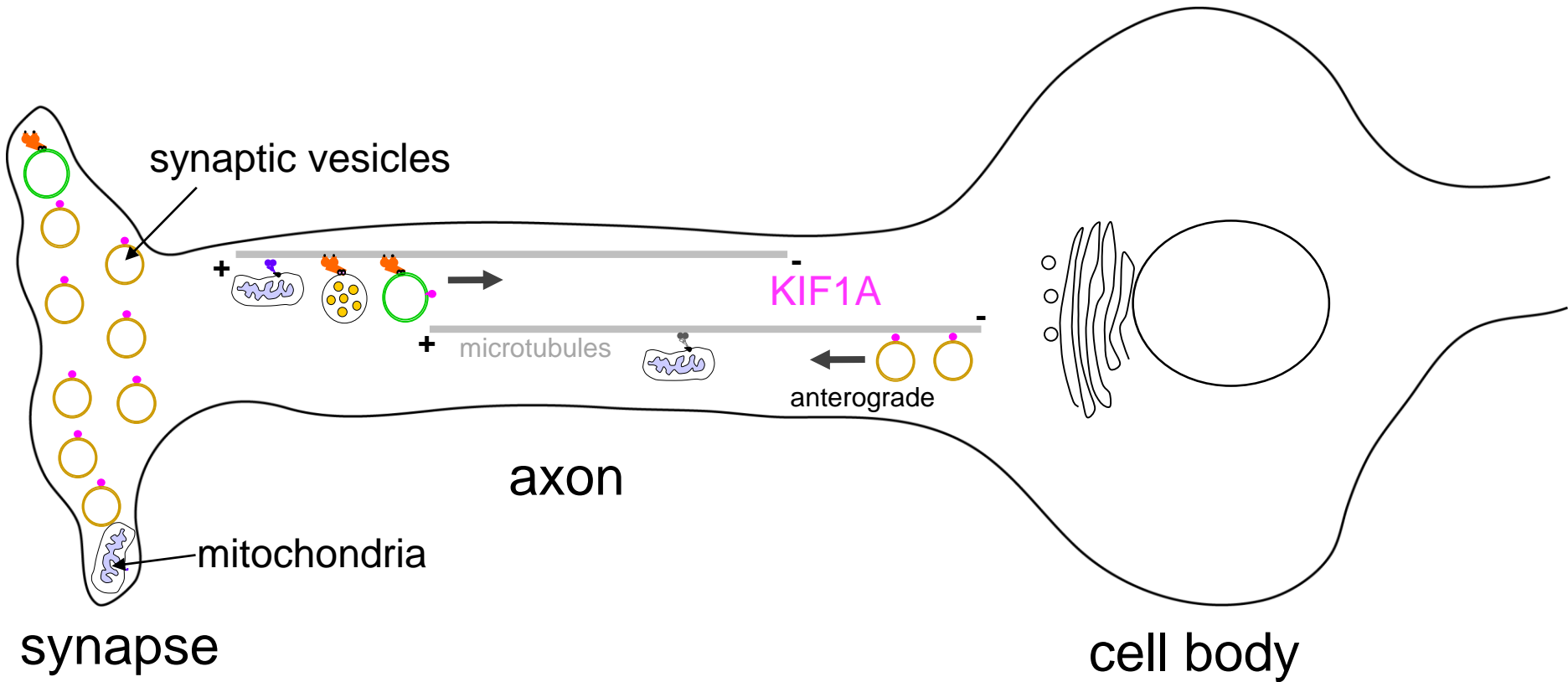# Cargo crowding at actin-rich regions causes traffic jams in neurons

Sandhya P. Koushika

Department of Biological Sciences, TIFR

Mumbai, India
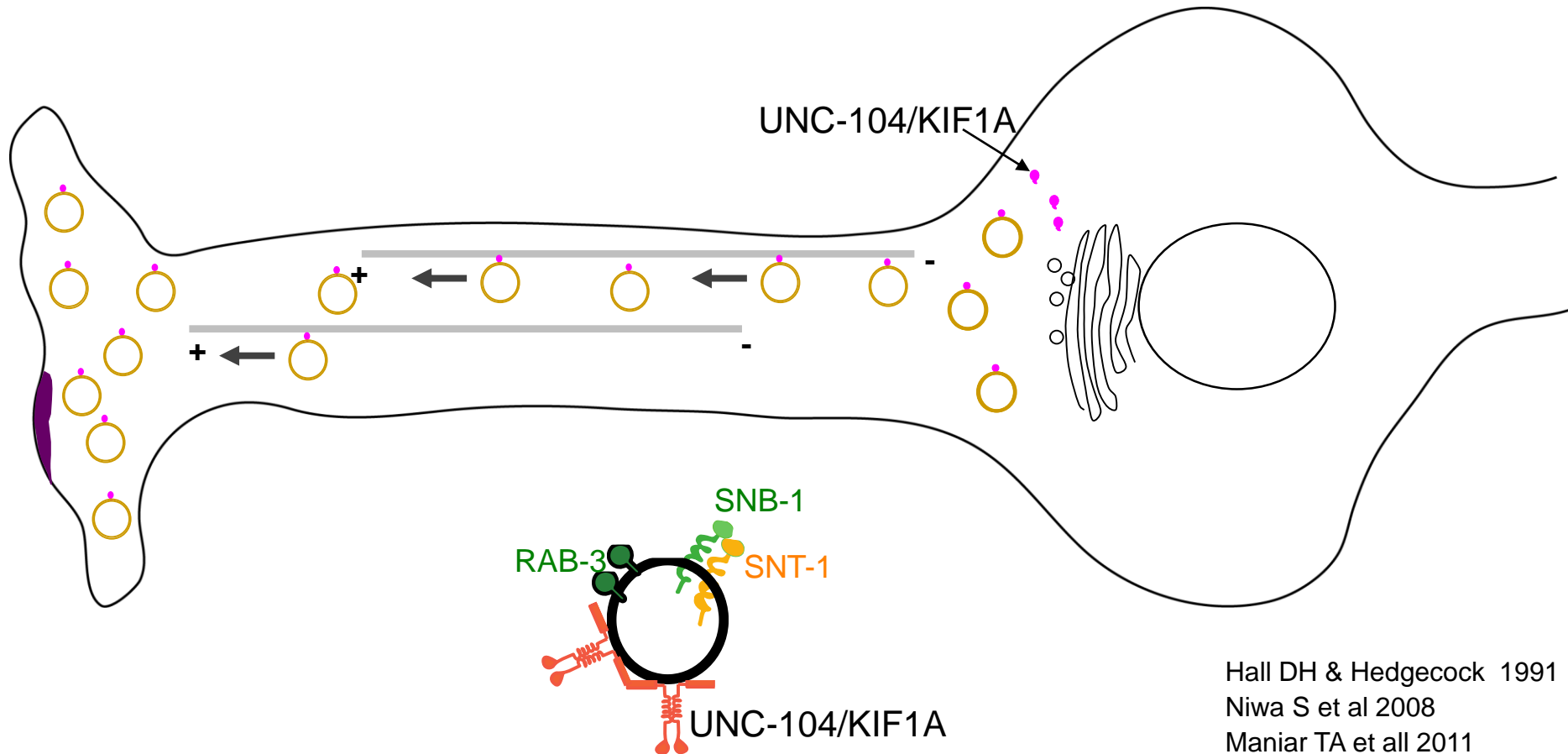
tifr

Axonal transport is an essential process

synaptic vesicles

+

KIF1A

microtubules

anterograde

axon

mitochondria

synapse

cell body

Hall D H & Hedgecock E 1991
Otsuka AJ et al 1991
Okada Y et al 1995
Zhao C et al 2001
Pack-Chung E et al 2007
Barkus RV et al 2008

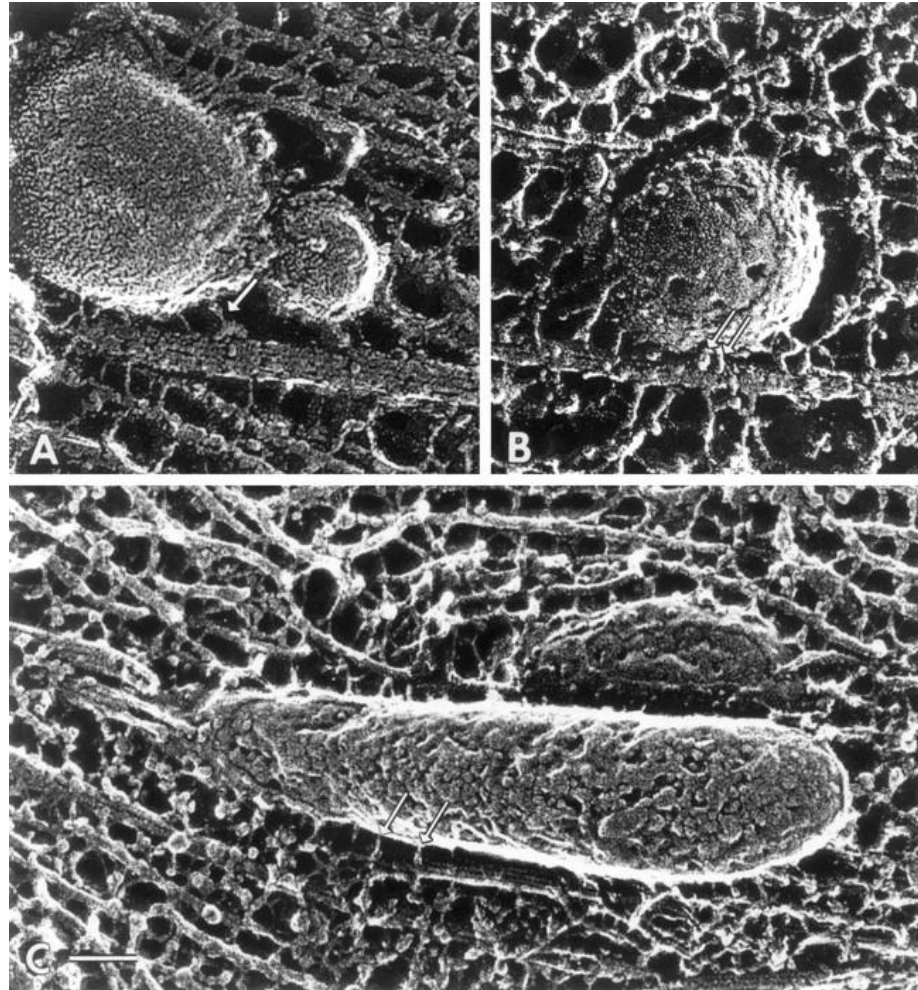# The journey of an individual cargo vesicle is a multi-step process

UNC-104/KIF1A

+ - + -

SNB-1
RAB-3
SNT-1
UNC-104/KIF1A

Hall DH & Hedgecock 1991
Niwa S et al 2008
Maniar TA et all 2011
Klassen MP et al 2010
Wagner OI et al 2009
Wu YE et al 2013
Kumar J et al 2010*

How do you maintain cargo flux in an axon?
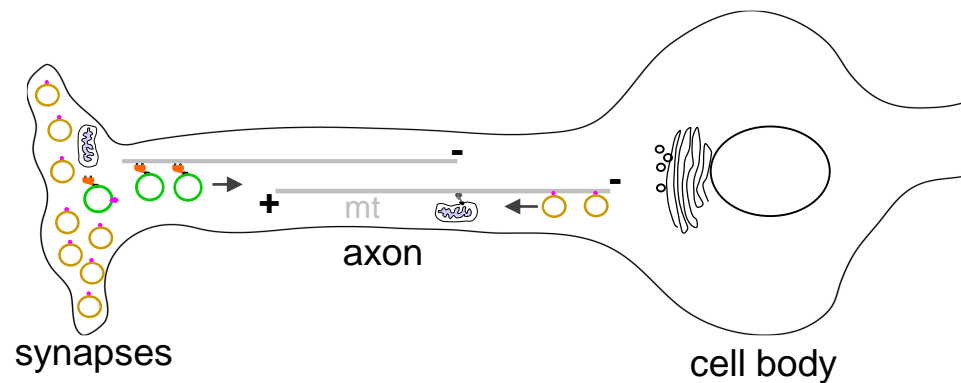
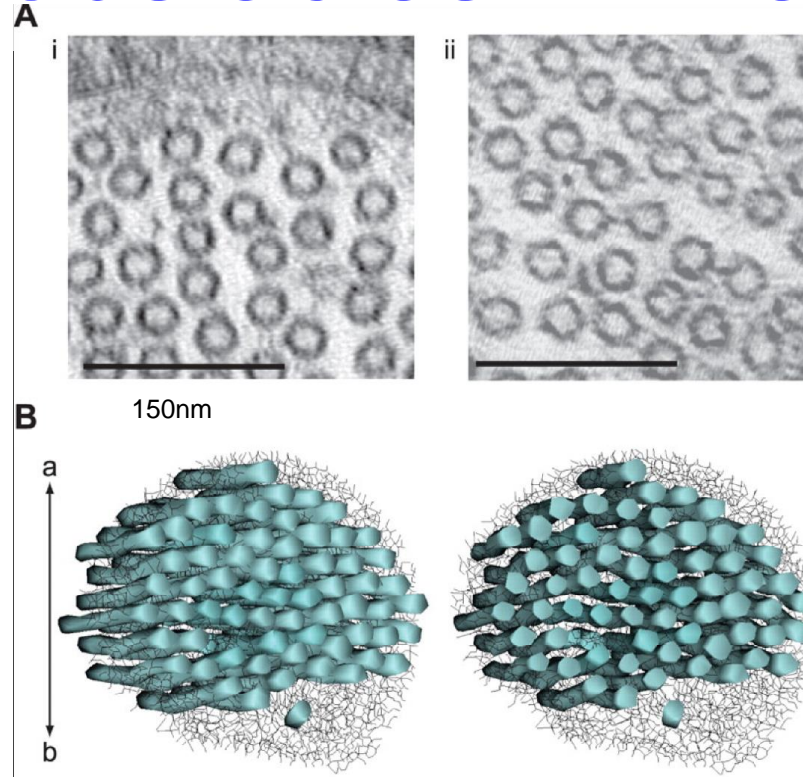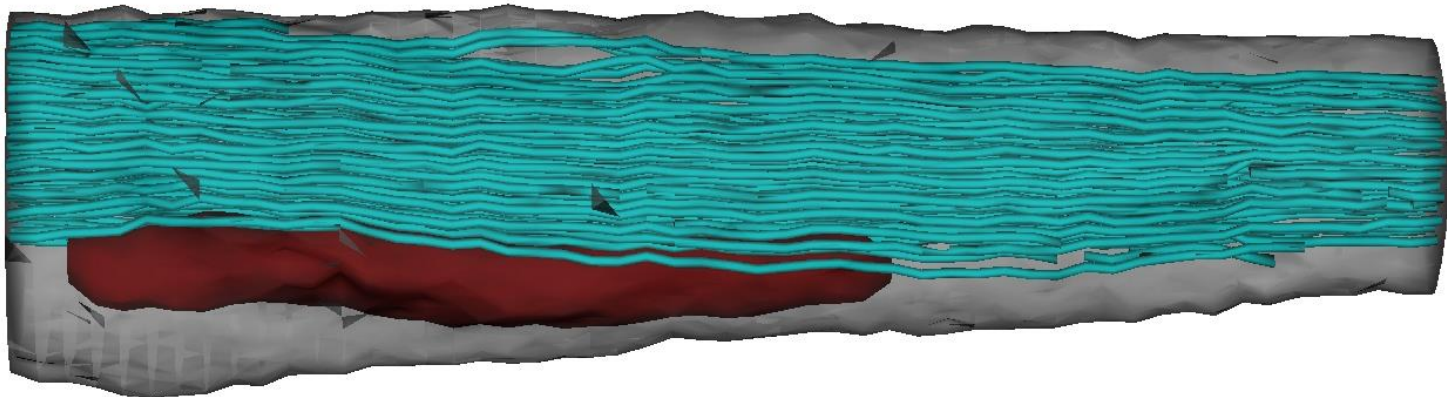# Cargo move in cytoskeletally-rich environments in axons

Hirokawa N and Noda Y 2008
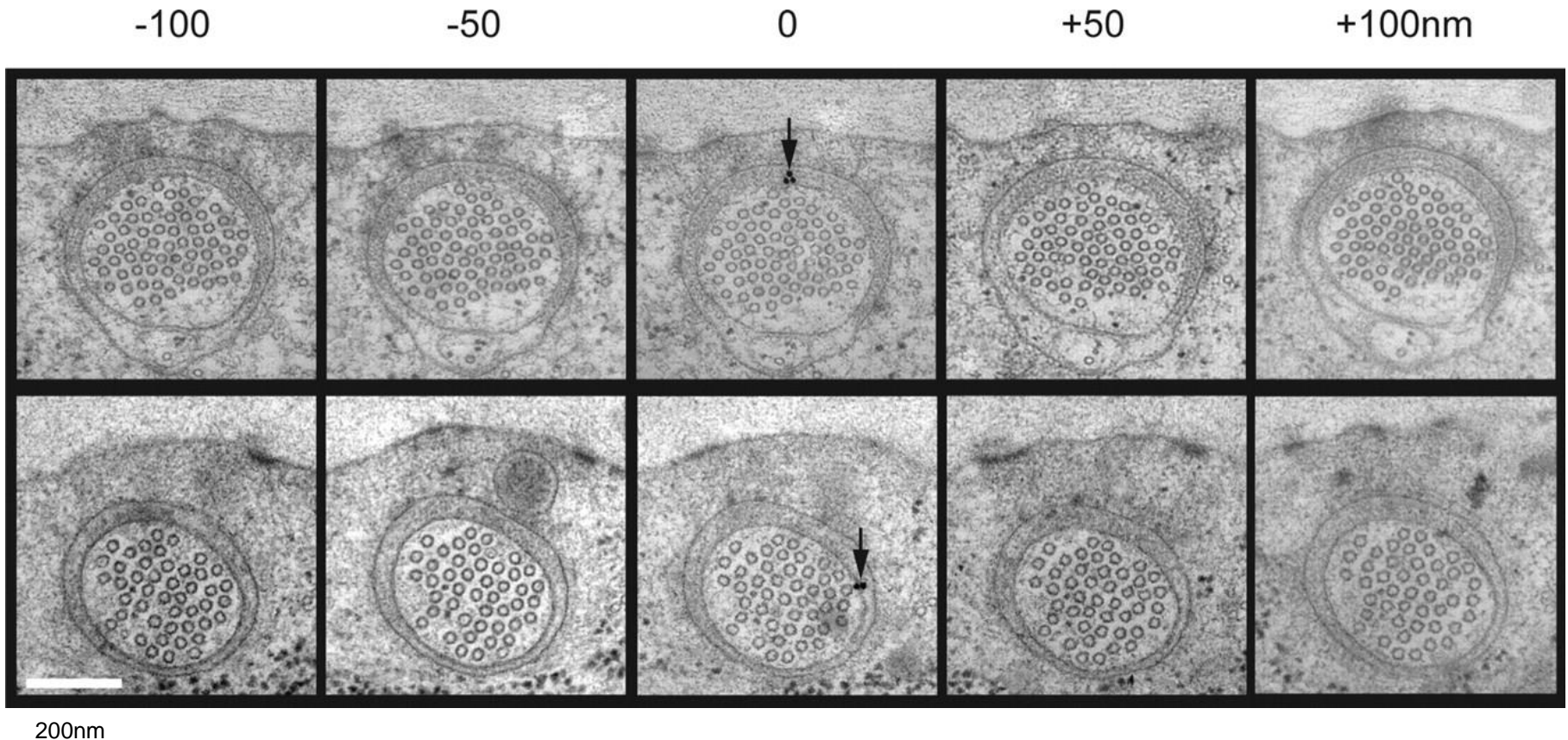Hirokawa N 1998
Hirokawa N 1982

Many obstacles in the path
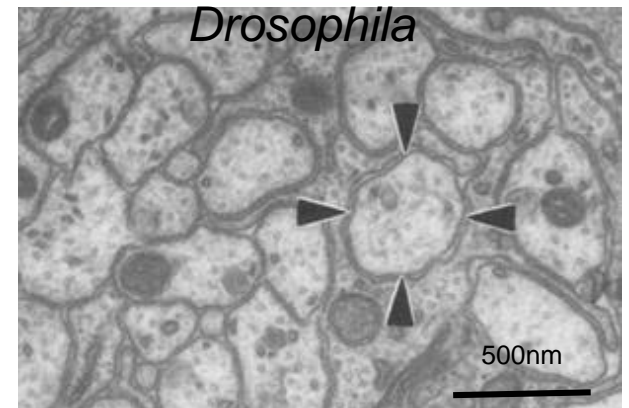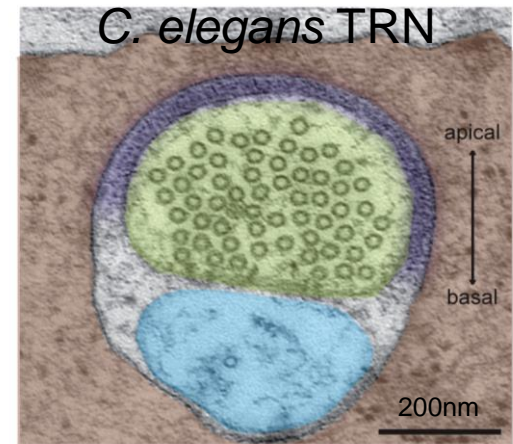

A

i　　　　ii

150nm

B
a

b

Cueva J.G. et al, 2007
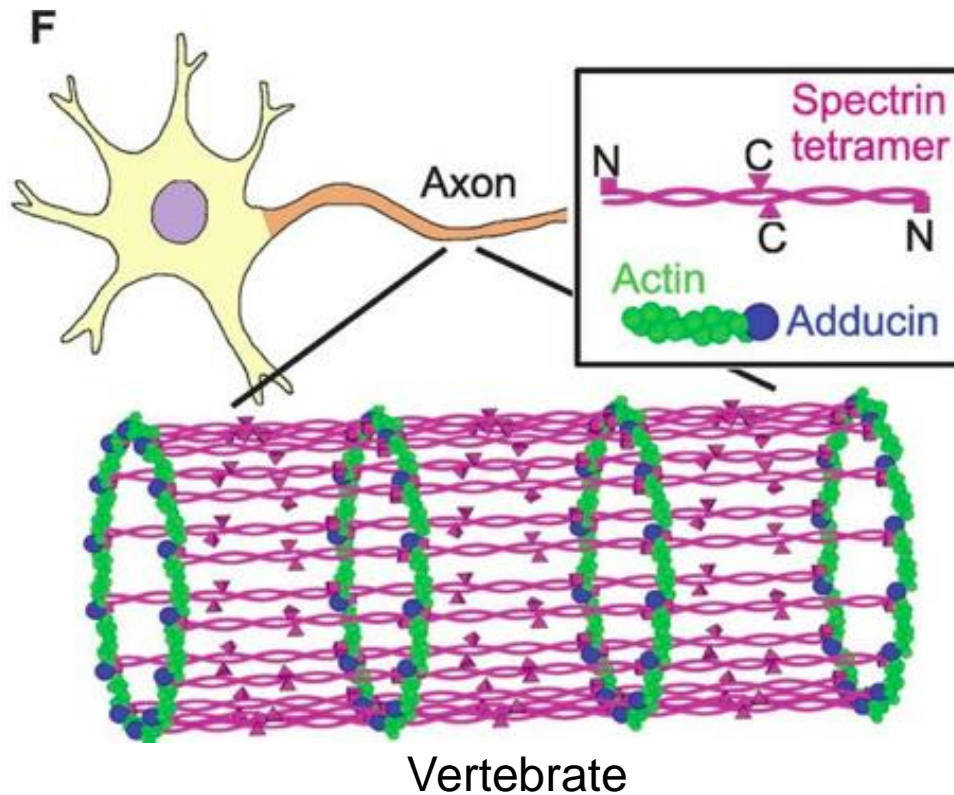
Cueva J.G. and Goodman M.B., unpublished

Transport path may be narrow
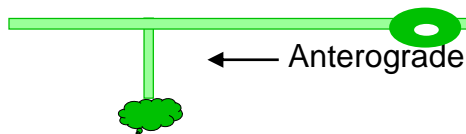

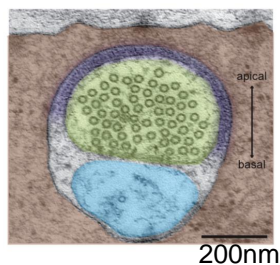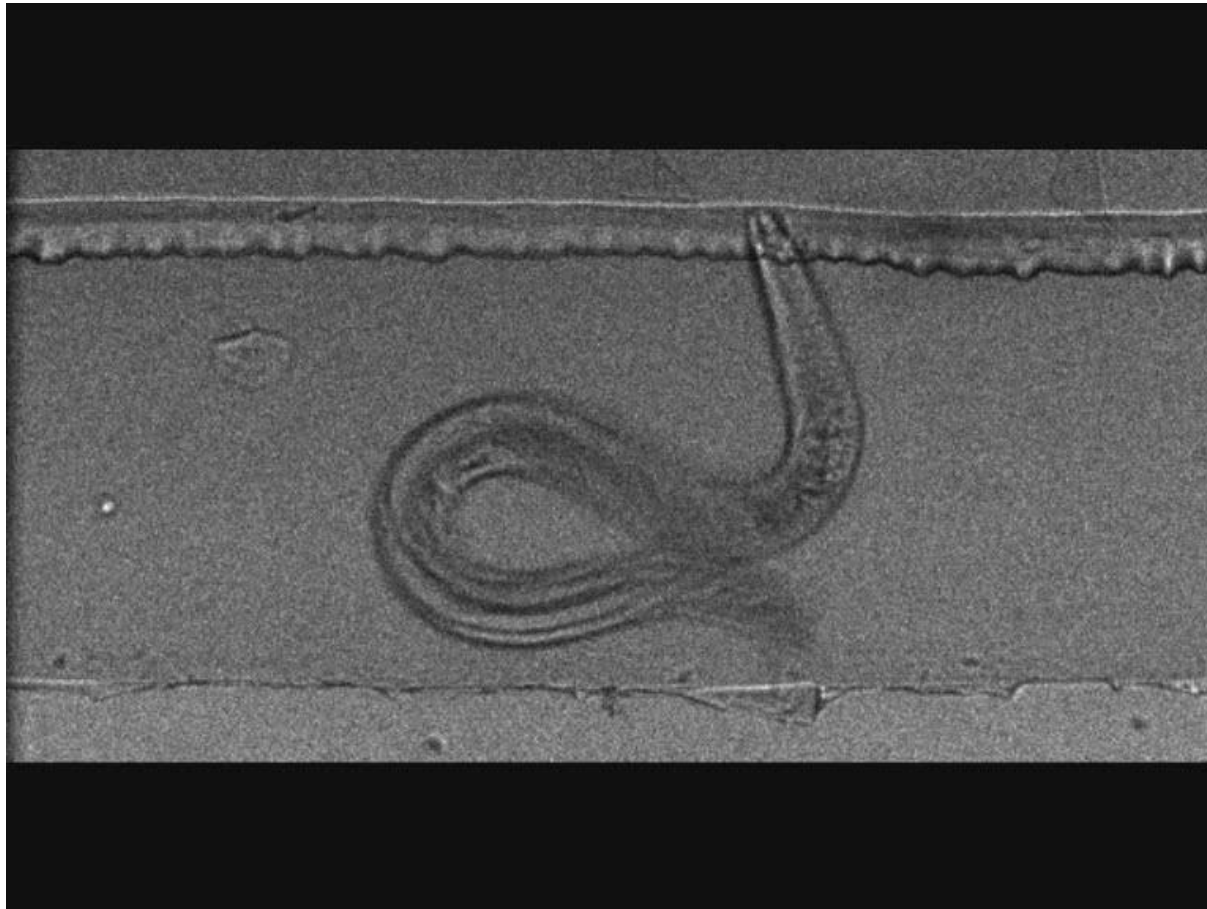-100   -50   0   +50   +100nm

200nm

Cueva J.G. et al, 2007

Neuronal highway is not homogeneous

F

Axon

Spectrin tetramer

N   C
     C   N

Actin   Adducin

Vertebrate

C. elegans TRN

apical

basal

200nm

Drosophila

500nm

Xu K et al 2013
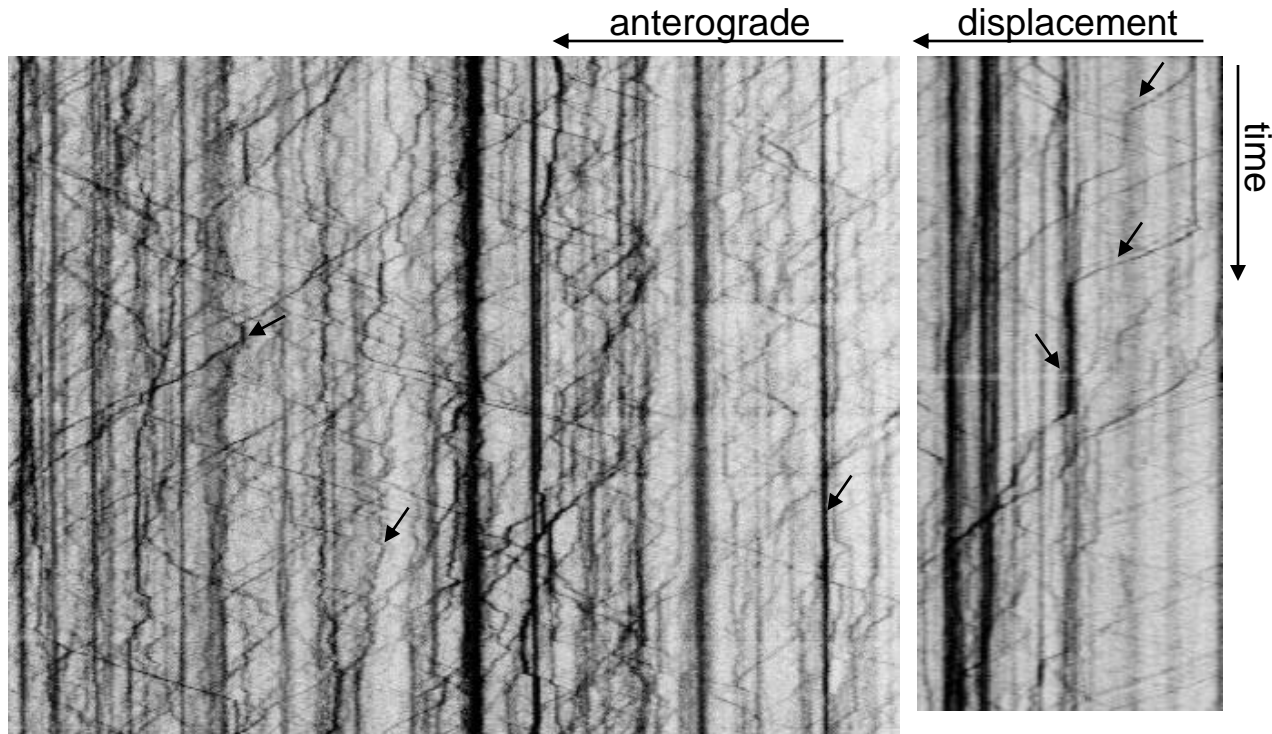Cueva J et al 2007
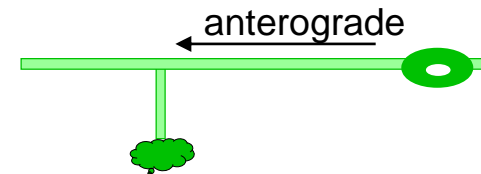Hurd DD & Saxton WM 1996

Synaptic vesicle transport *in vivo*

200nm

Anterograde

Mondal S et al 2011*
Mondal S et al 2012*
Mondal S et al 2014*
Cueva J et al 2007

Typical movements and stationary cargo
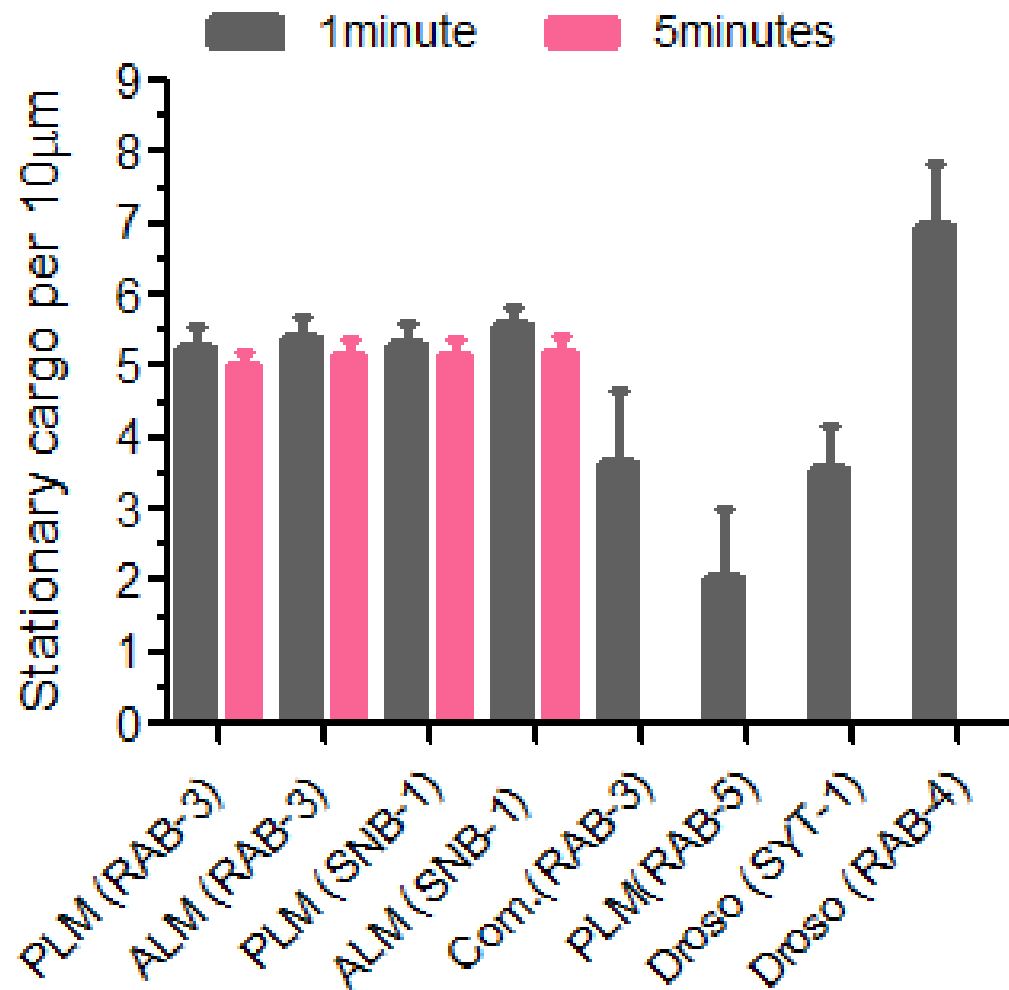

anterograde

displacement

time

Touch neuron

Motor neuron

CARGO-GFP

anterograde

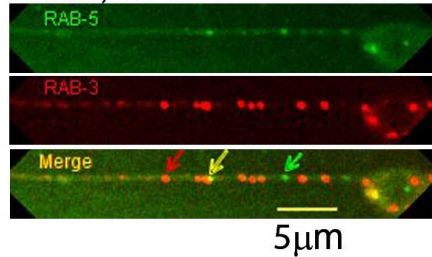# All cargo examined are stationary for long periods of time

Com.- Commissures
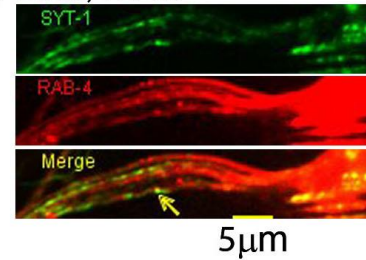*Droso- Drosophila*
N=10 animals

Do different cargo pile up together?
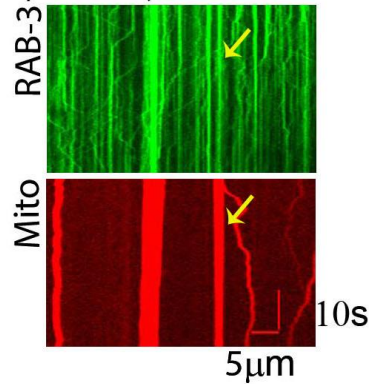
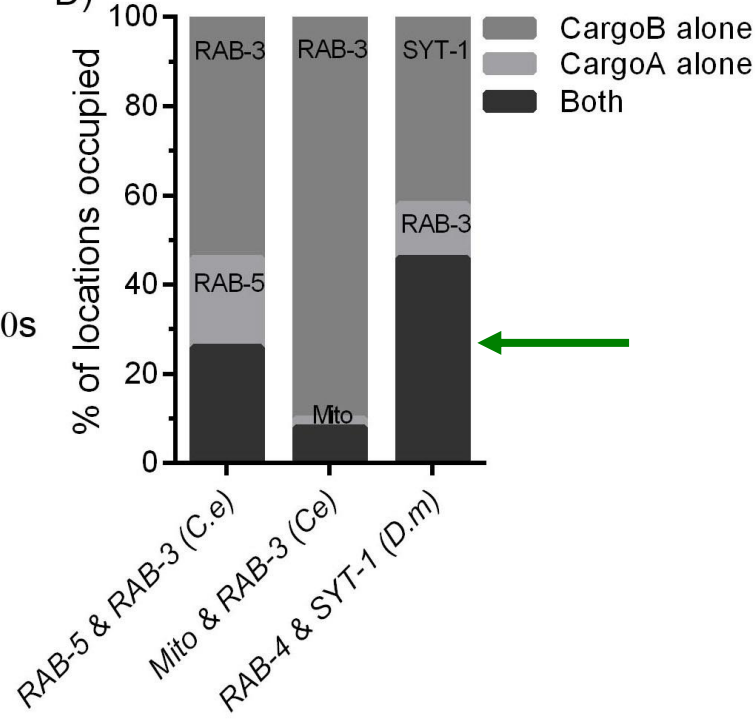# Multiple cargo types are stationary at the same locations

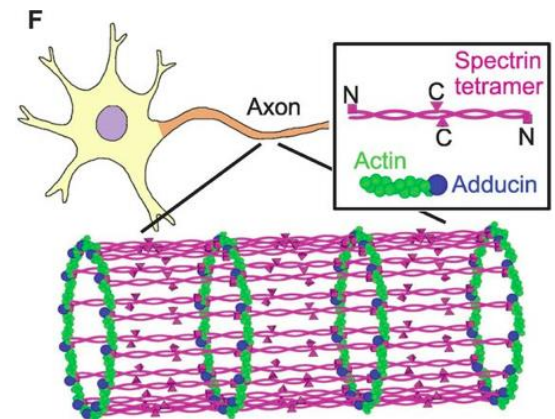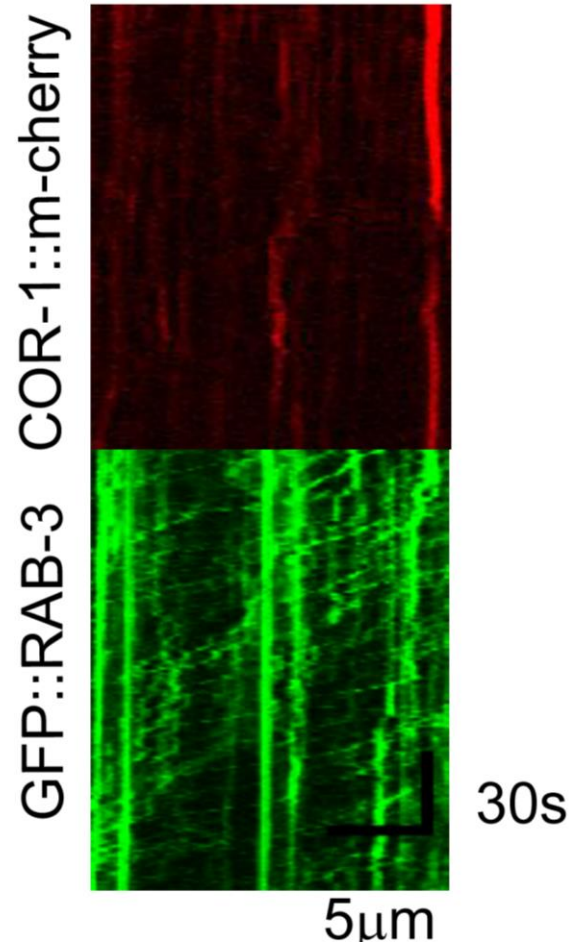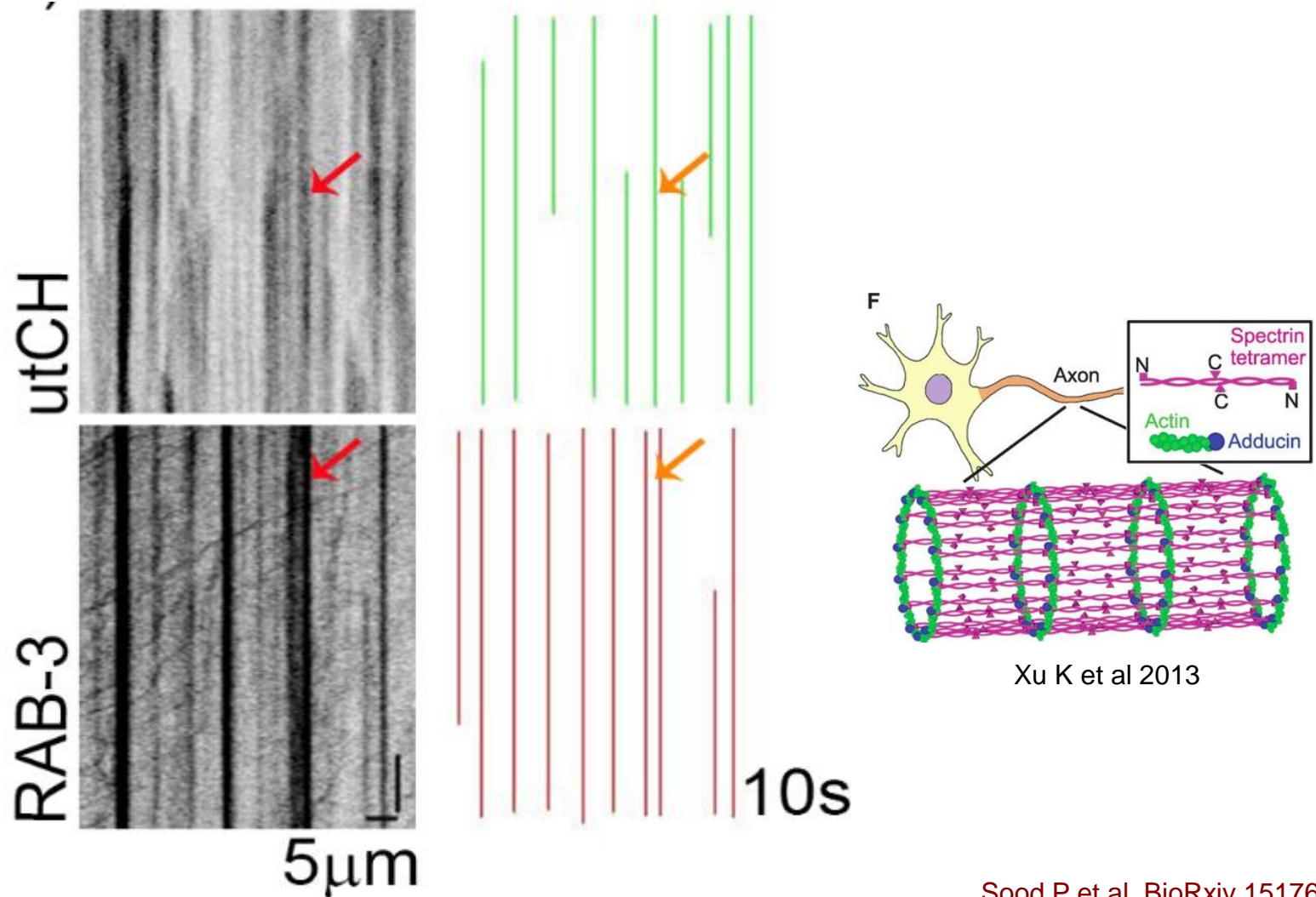A) TRNs, *C. e*

B) Ich5, *D. m*

C) TRNs, *C. e*

D)

Where do cargo pile up?

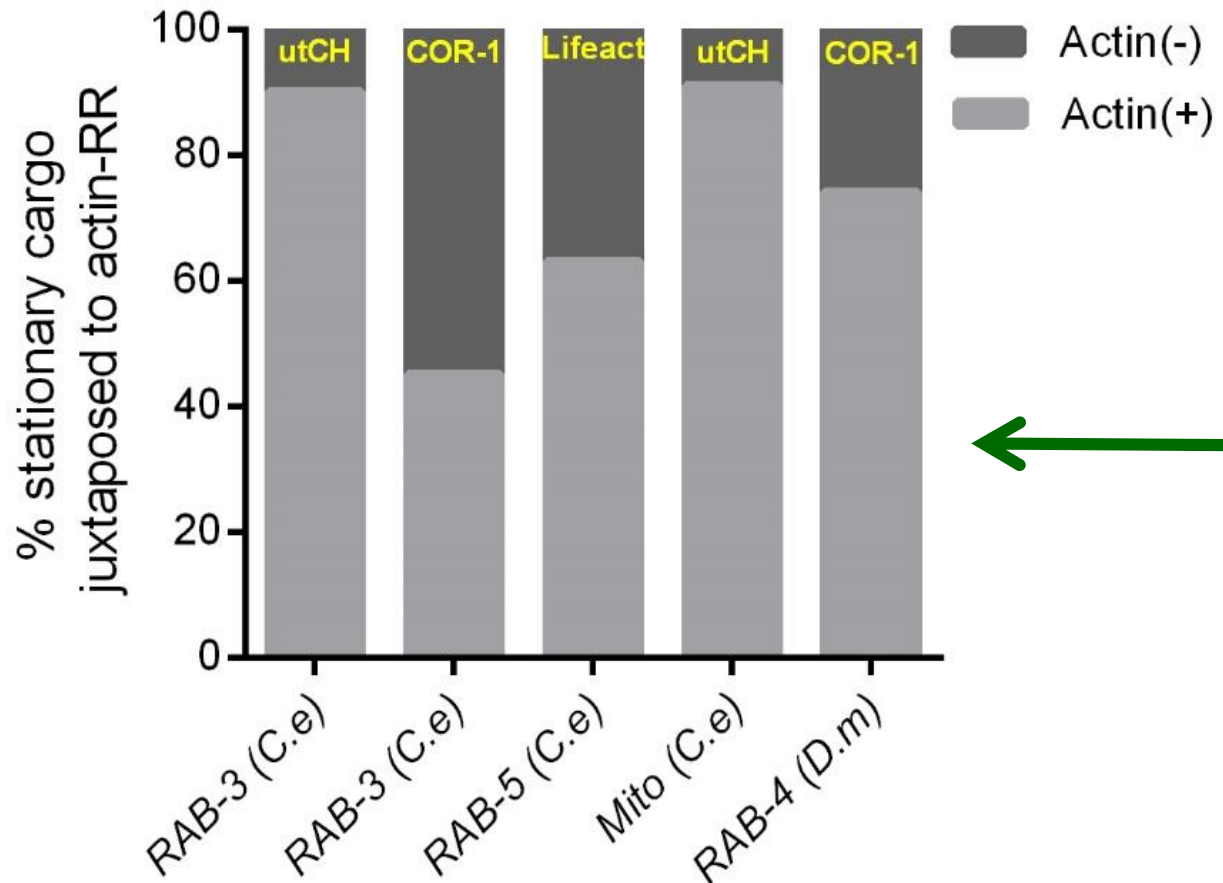# Imaging to identify locations where cargo halt

Xu K et al 2013

Imaging to identify locations where cargo halt
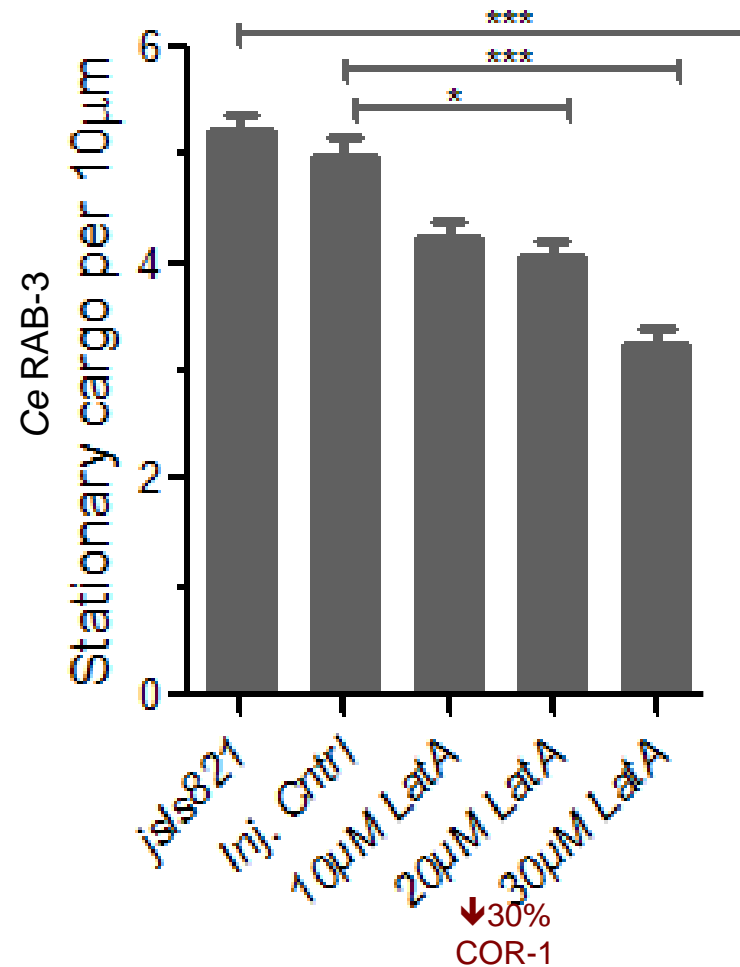

utCH

RAB-3

5μm

10s

Xu K et al 2013

Stationary cargo are at actin-rich regions

Prediction: Altering the cytoskeleton acutely should change density of stationary cargo
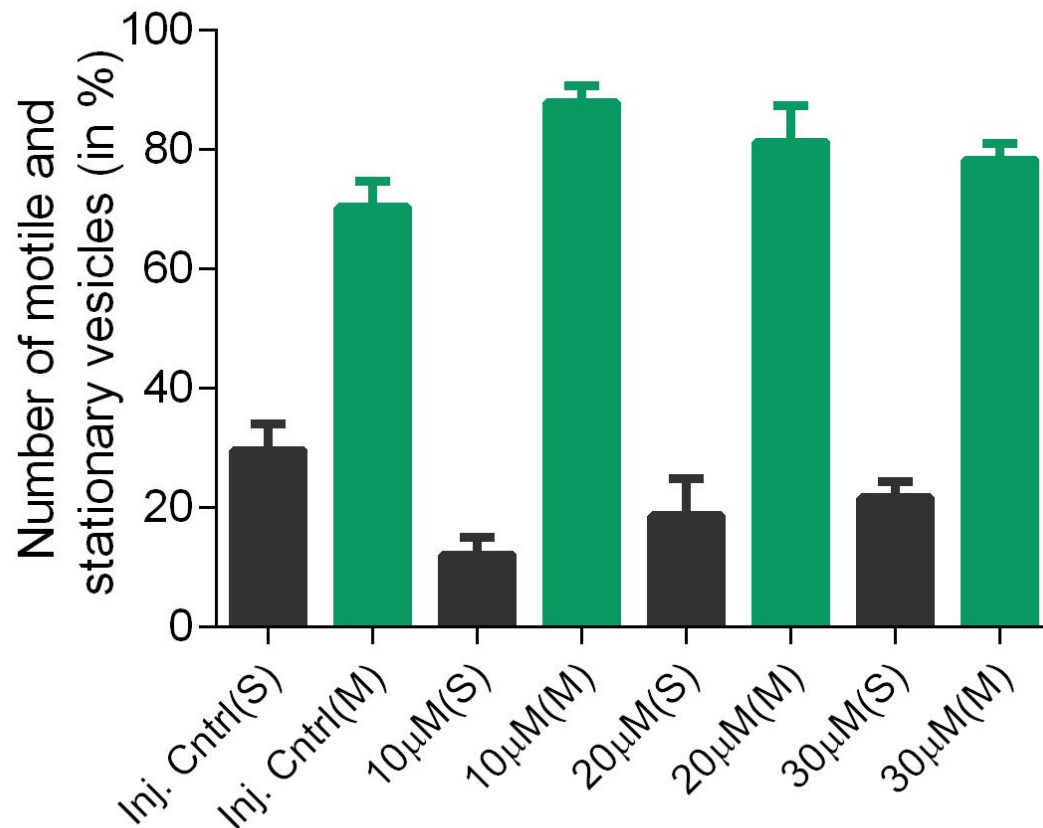
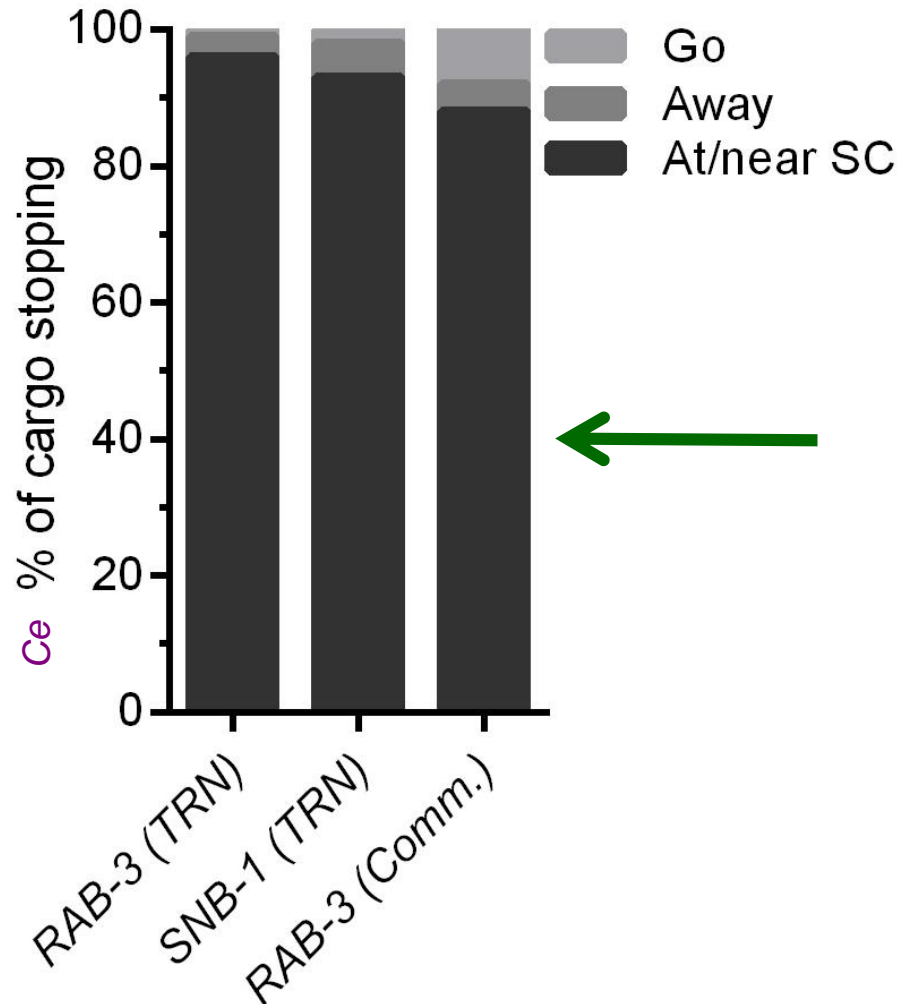# Reducing actin-rich regions alters stationary cargo density

Dm RAB-4 density of LifeAct and stationary cargo reduce upon LatA treatment

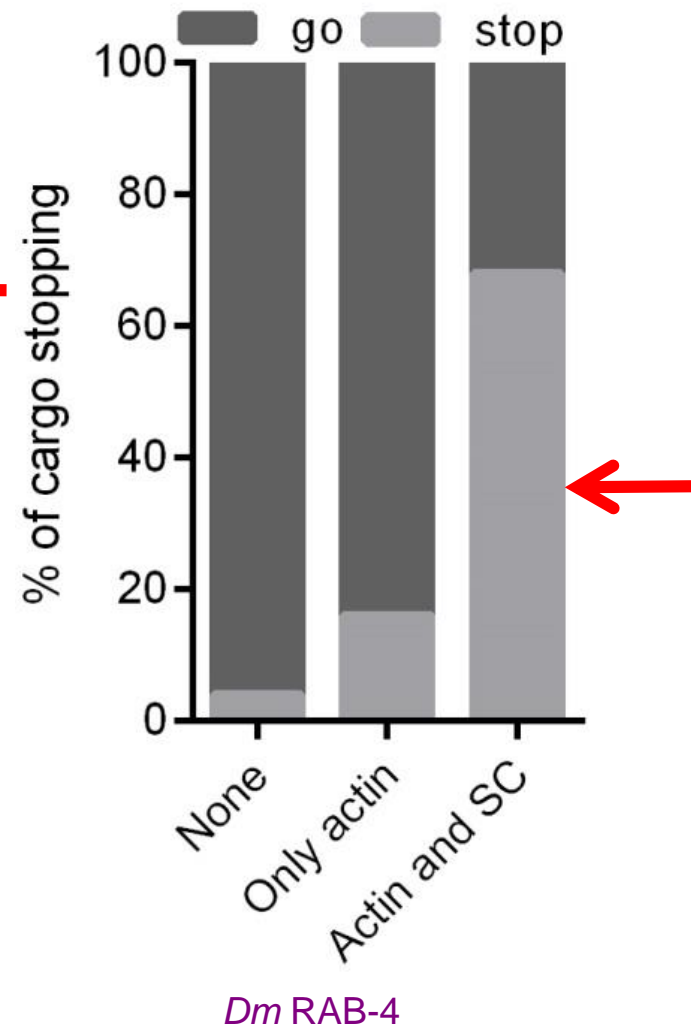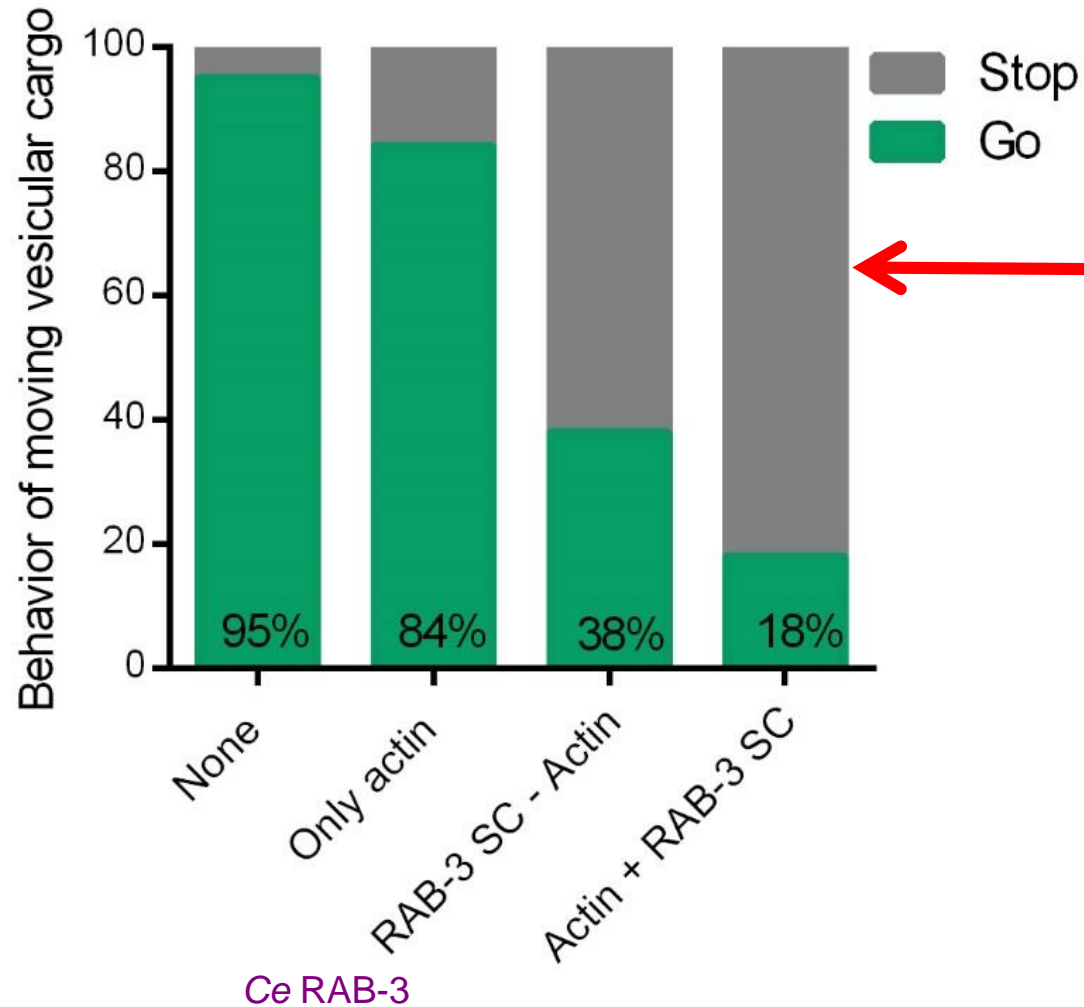# Reducing actin-rich regions increases fraction of moving cargo

What do moving cargo do when encountering a stalled cargo?

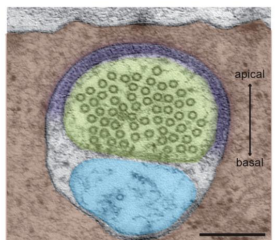# Cargo halt predominantly where other cargo are present

Cargo crowding induces traffic jams
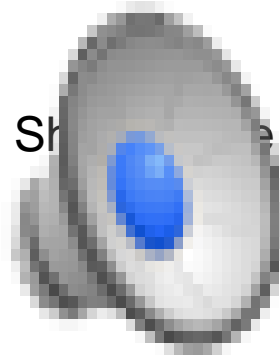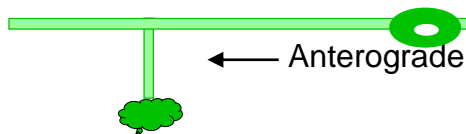

Ce RAB-3

Dm RAB-4

Sood P et al, BioRxiv 151761, 2017

Cargo stop at crowded regions

Sh[...]e

200nm

Anterograde
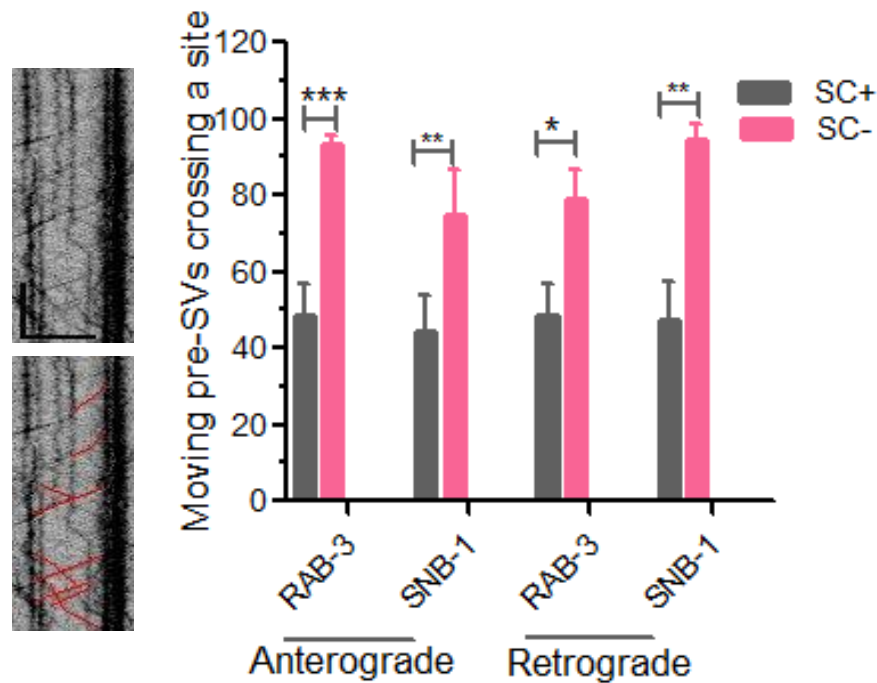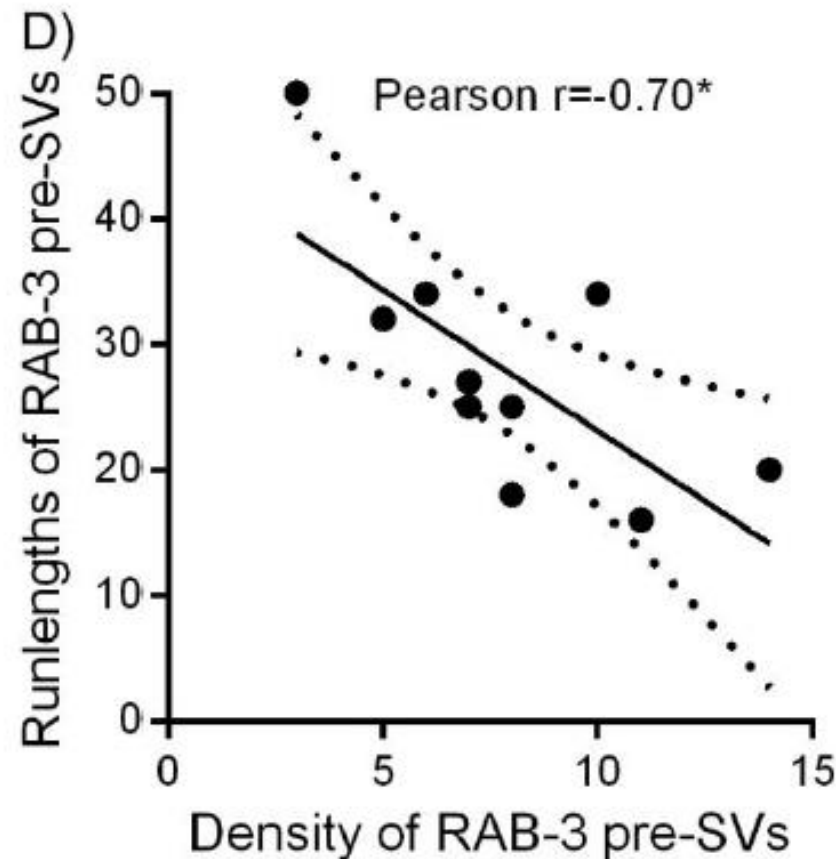
Actin rich GFP
SV cargo mCherry

Prediction: Cytoskeletal road blocks should control local cargo flux
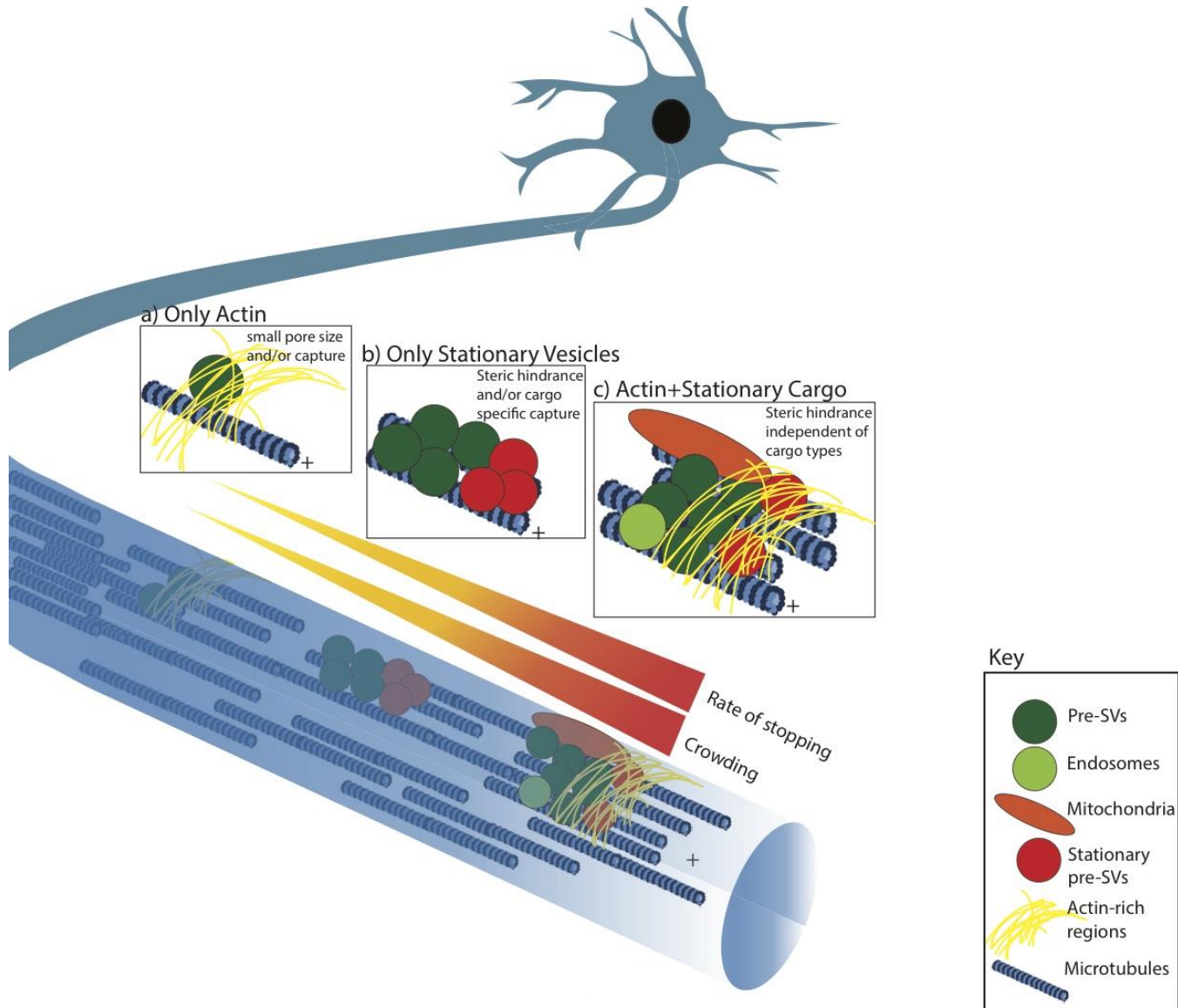
Locations where cargo become stationary regulate local flux

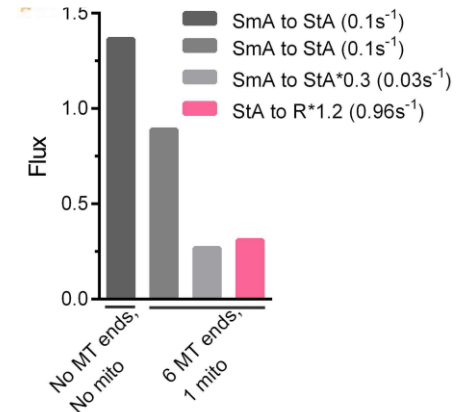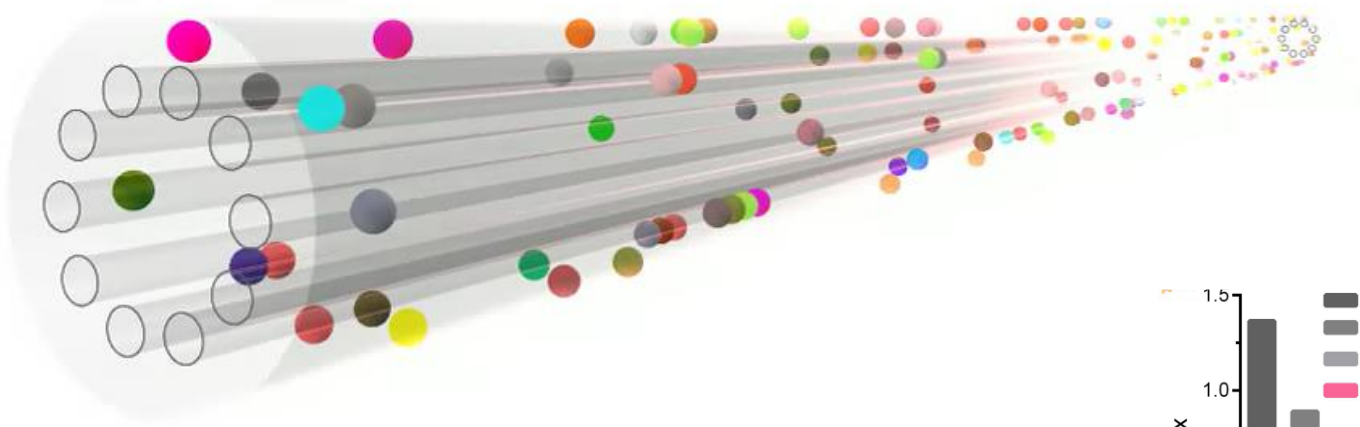# How far a vesicle moves depends on density of stationary vesicles

Physical crowding leads to stalling

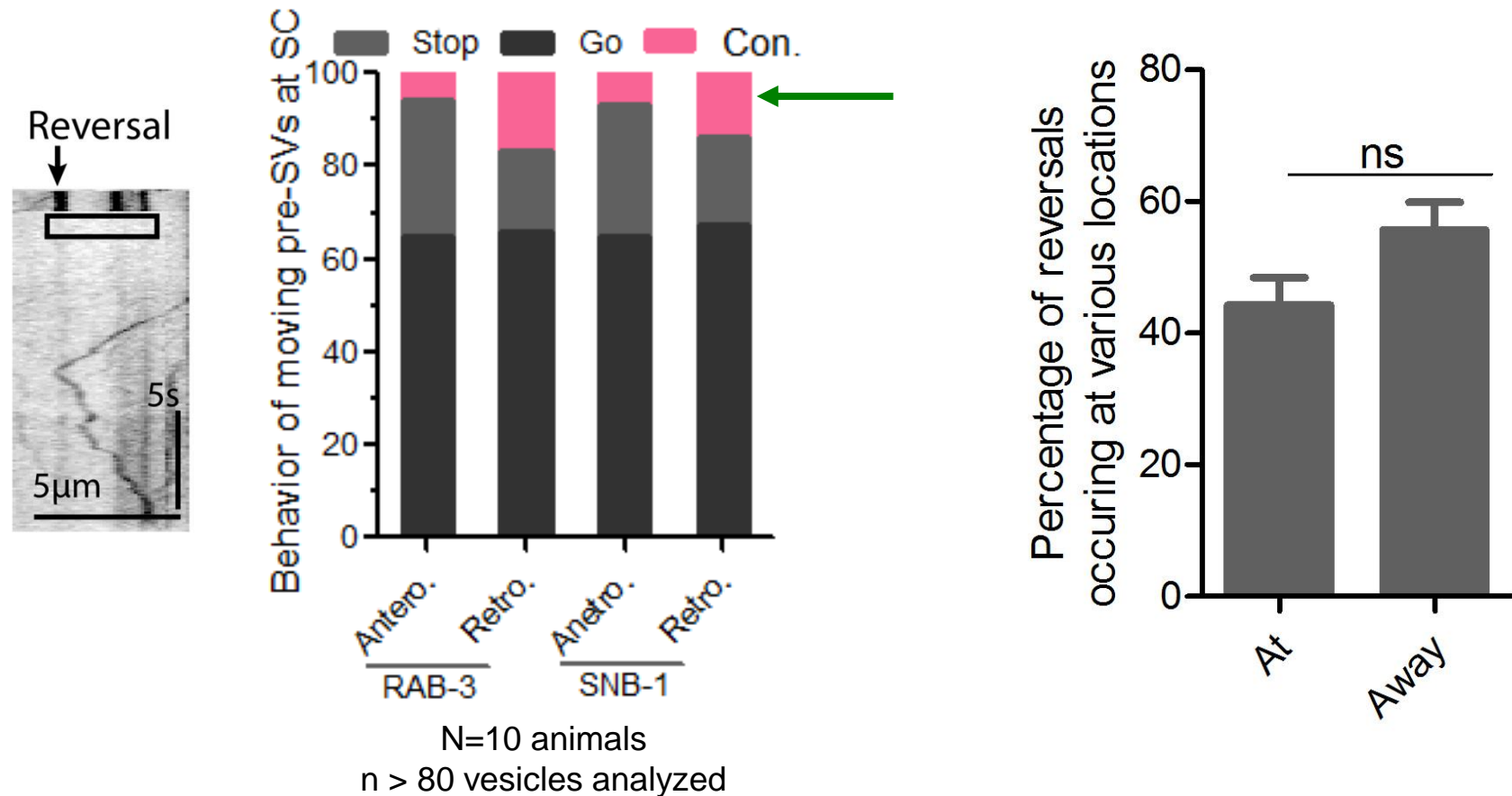# Are there ways to overcome traffic jams?

Change in motion state is essential to go bypass traffic jams

SmA to StA (0.1s^{-1})
SmA to StA (0.1s^{-1})
SmA to StA*0.3 (0.03s^{-1})
StA to R*1.2 (0.96s^{-1})

Flux

1.5
1.0
0.5
0.0

No MT ends, No mito
6 MT ends, 1 mito

Vinod Kumar
Gautam Menon

Reversals occur through out the neuron

N=10 animals
n > 80 vesicles analyzed

Cargo reverse through out the axon
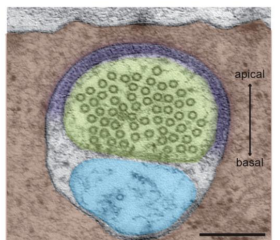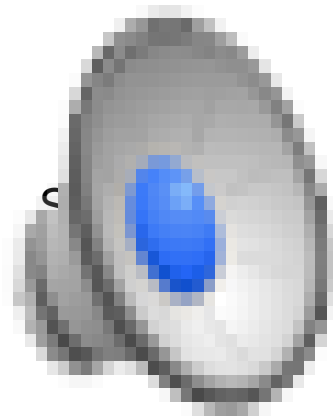
200nm
Cueva J et al 2007

← Anterograde

Actin rich GFP
SV cargo mCherry

Are naturally occurring pools of vesicles physiologically relevant?

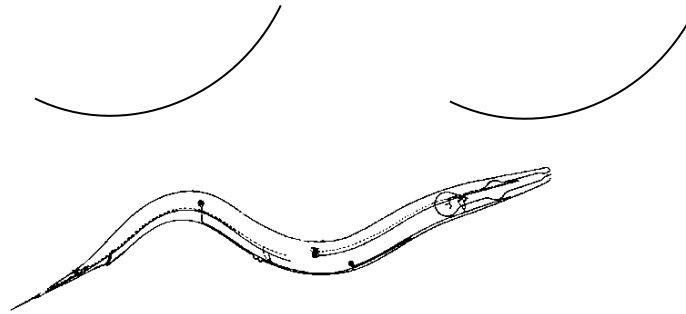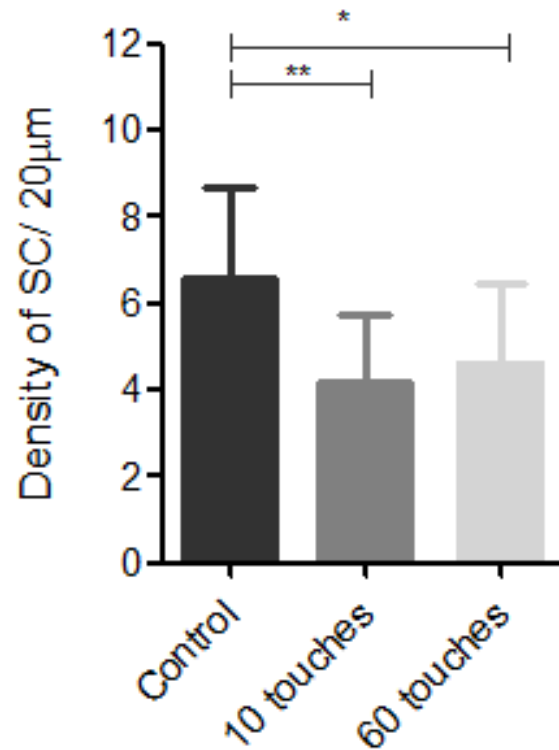# The gentle touch assay

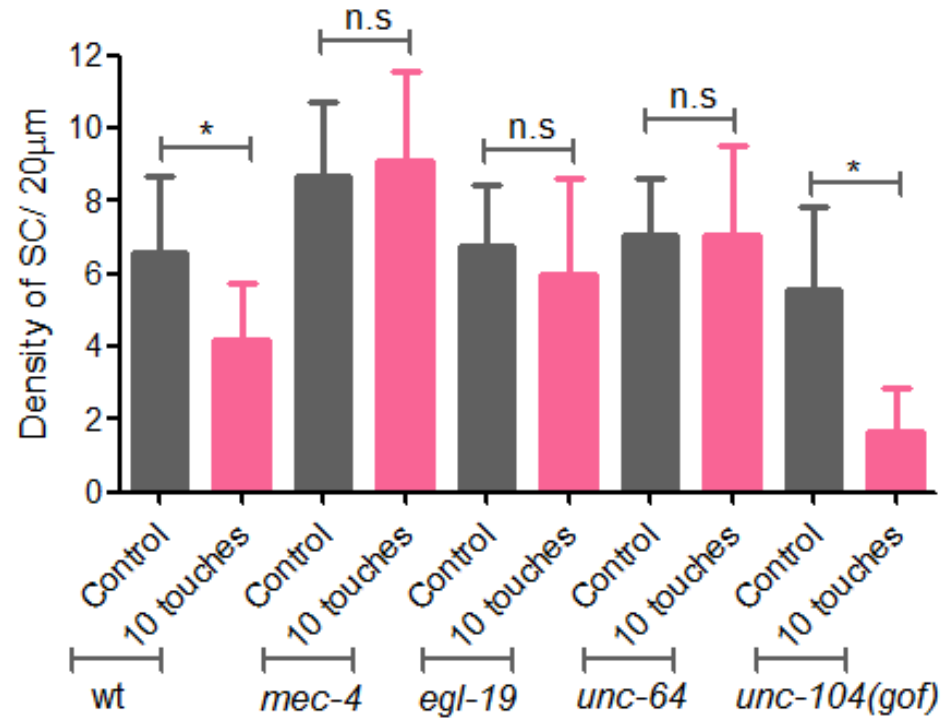

No pillar PDMS device

Pillar PDMS device

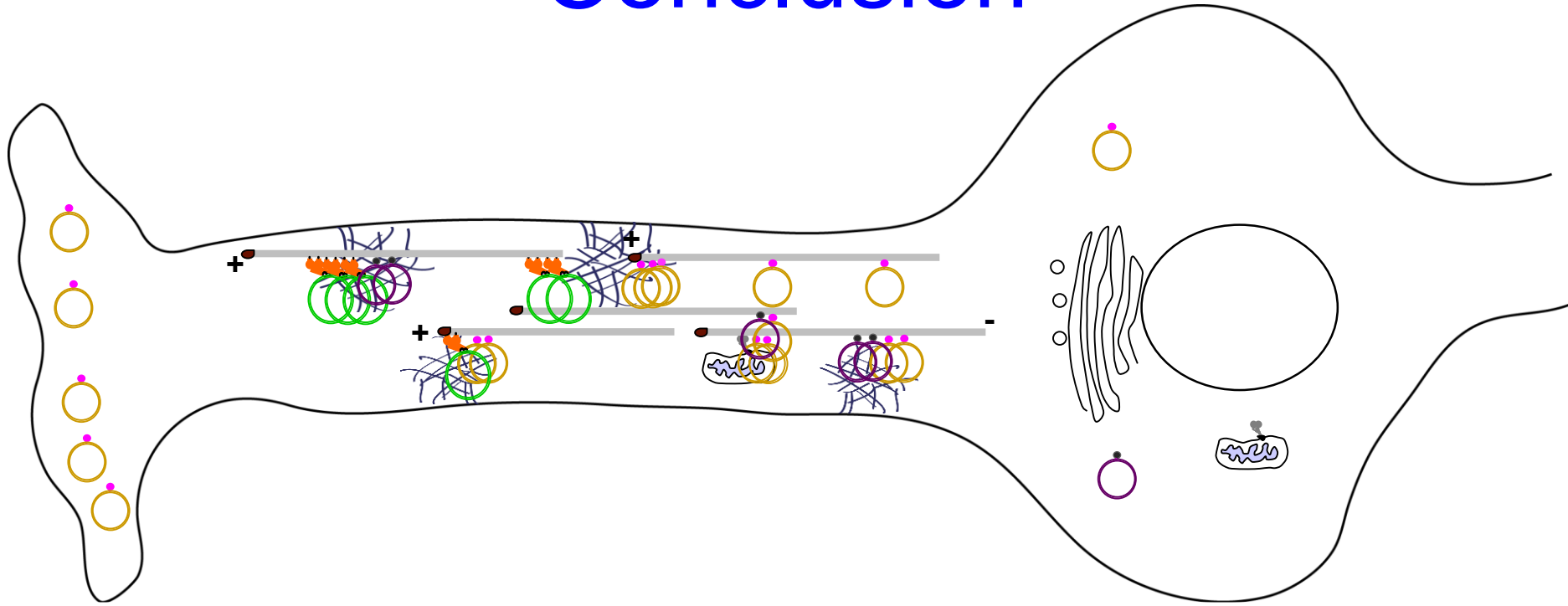# Density of stationary pre-SVs reduce after stimulation

Density of stationary pre-SVs after stimulation

- ability to sense touch
- ability of the neuron to allow calcium influx
- presence of motor
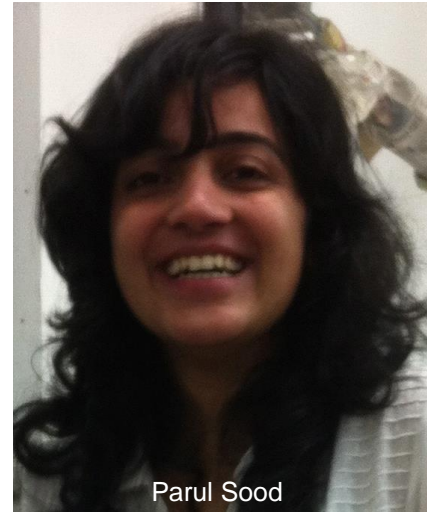- ability to release vesicles at the synapse

Conclusion

Credits-students/post-docs

- Kausalya Murthy
- Parul Sood

Dr. Kausalya Murthy
Former post-doc

Parul Sood
Graduating student

Collaborators and reagents

- Menon Gautam, IMSc, India- modeling cargo motion (Vinod Kumar)

- Michael Nonet, Wash U, USA- *elegans* unpublished strain
- Chung-Lian Pan, Taiwan, Kang Shen, Stanford, Massimo Hilliard, Australia- *elegans* strains

Funding

hhmi | Howard Hughes Medical Institute

TIFR, DST, DBT, CSIR, Prism (with GM)