

# Endocytosis-dependent desensitization and protein synthesis–dependent resensitization in retinal growth cone adaptation

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► Tom Davidson ► 9. 181 ► 29 Mar 05

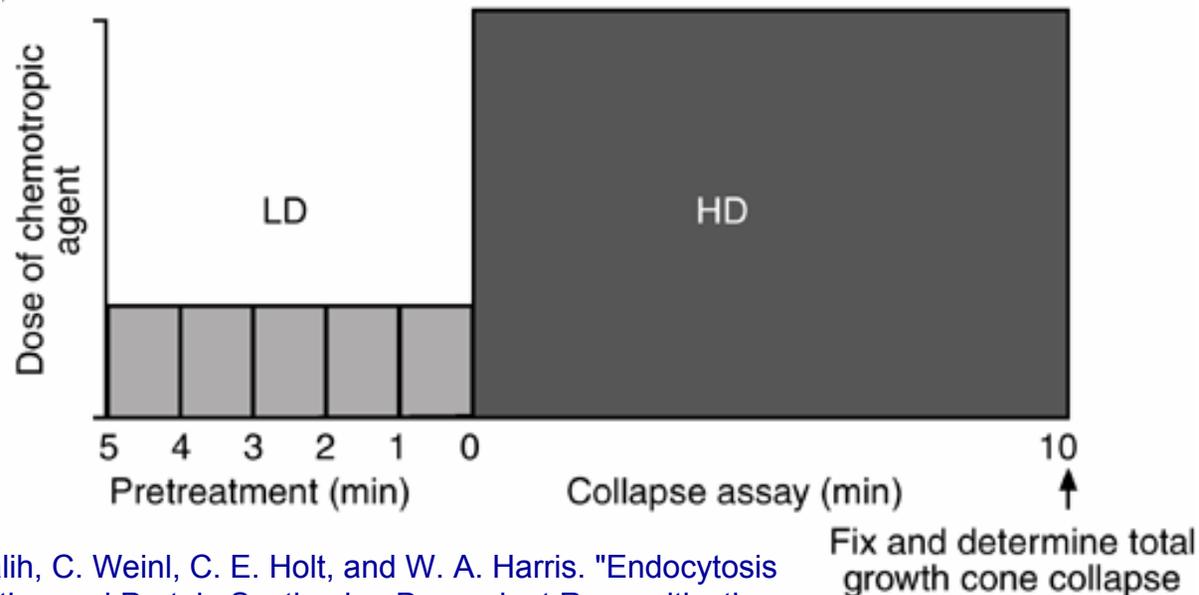
- ▶ Across biology, other receptor-mediated systems show adaptation (de-/re-sensitization...
- ▶ Does axonal pathfinding involve adaptation?
- ▶ If so, how is this adaptation accomplished?

# Uses of adaptation (proposed):

- ▶ ‘Homeostatic reset’ -- getting out of the grasp of a gradient to be able to see another gradient of the same ligand
- ▶ ‘recalibration’ -- Adjust sensitivity in a graded way to increase dynamic range, allow growth up/down long gradients

# Growth Cone Collapse Assay

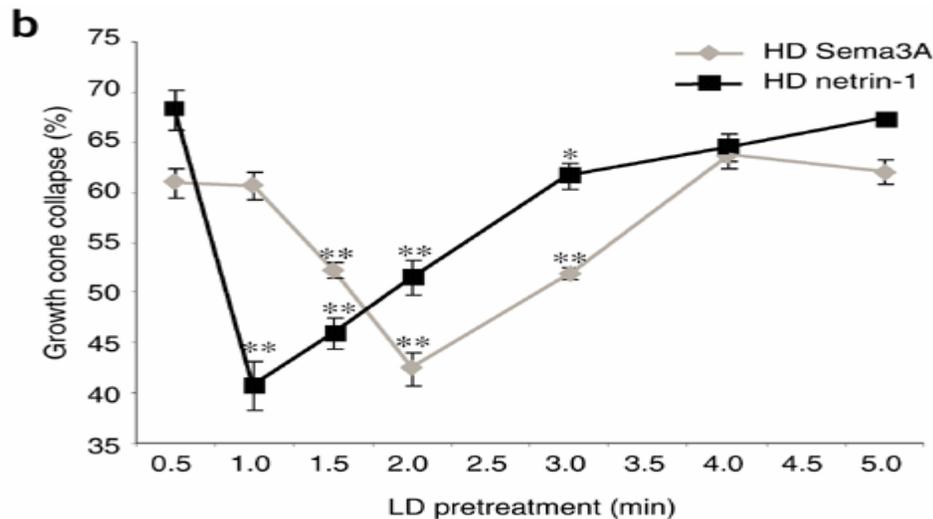
- ▶ Embryonic *Xenopus* retinal culture
- ▶ Pre-treat with low doses (LD) of chemotropic agents for varying times (+/-drugs), then treat with a high dose of same agent for 10 min
- ▶ Fix and count collapsed growth cones (movie)



Source: Piper, M., S. Salih, C. Weinl, C. E. Holt, and W. A. Harris. "Endocytosis-Dependent Desensitization and Protein Synthesis-Dependent Resensitization in Retinal growth Cone Adaptation." *Nature Neuroscience*, 2005. Published online. Courtesy of the authors. Used with permission.

Fig. 1a

# Time Course of Adaptation



Source: Piper, M., S. Salih, C. Weinl, C. E. Holt, and W. A. Harris. "Endocytosis -Dependent Desensitization and Protein Synthesis - Dependent Resensitization in Retinal growth Cone Adaptation." *Nature Neuroscience*, 2005. Published online. Courtesy of the authors. Used with permission.

Fig. 1b

- ▶ ~35% basal collapse rate (control not shown)
- ▶ Sema3a desensitization after 2min, resensitization after 4min; Netrin desensitization after 1min, resensitization after 4min

# Adaptation adjusts sensitivity

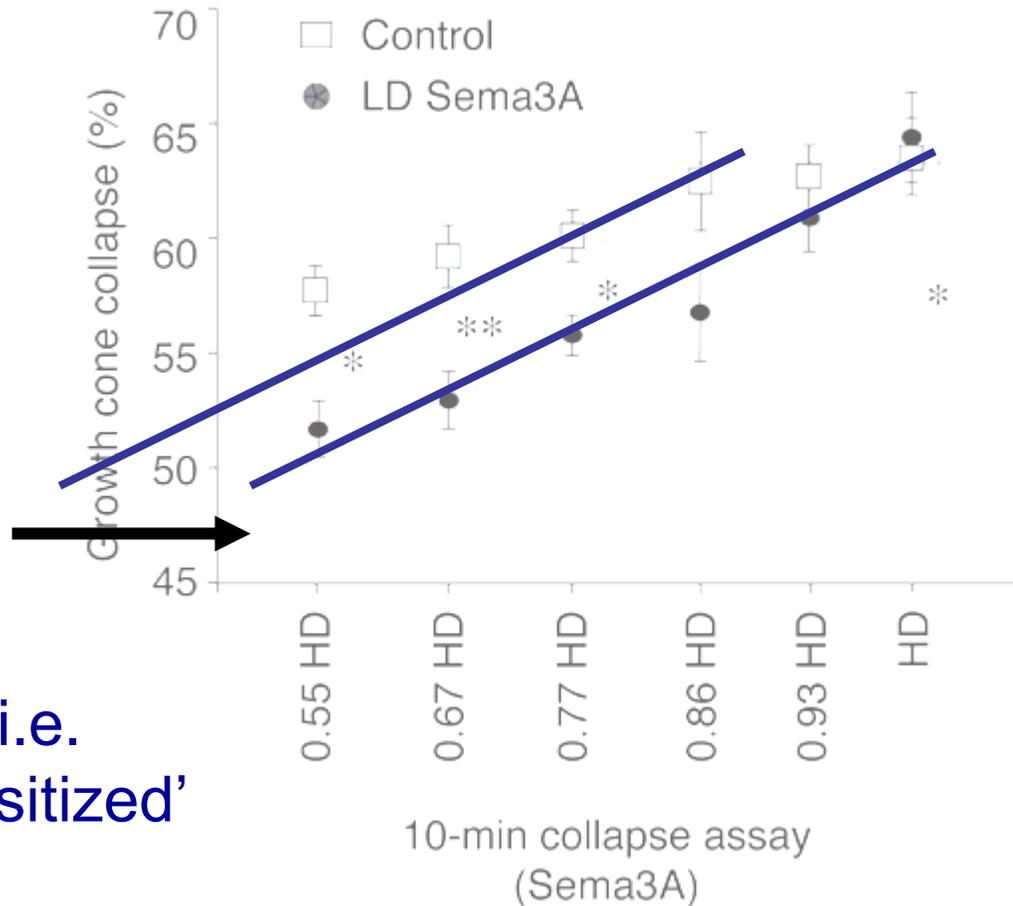


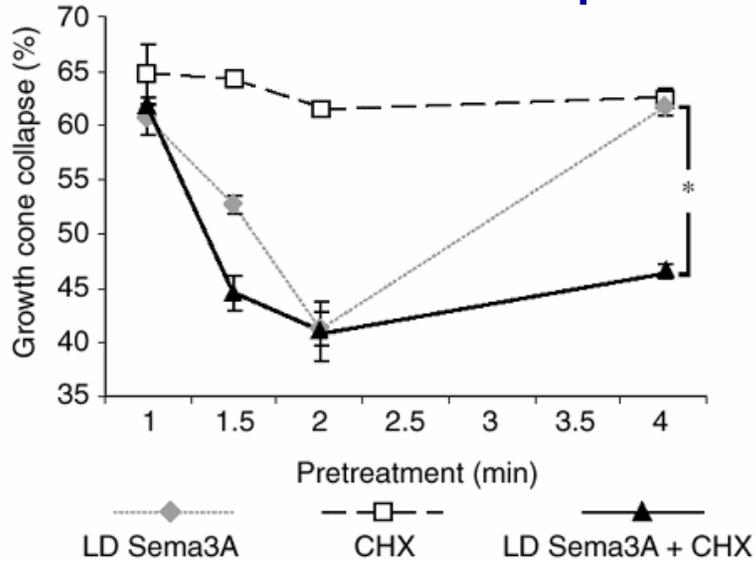
Fig. 2

- ▶ 5min pre-treatment (i.e. 'fully resensitized' at HD?)
- ▶ Dose-dependence argues for 'recalibration'

Source: Piper, M., S. Salih, C. Weigl, C. E. Holt, and W. A. Harris. "Endocytosis - Dependent Desensitization and Protein Synthesis - Dependent Resensitization in Retinal growth Cone Adaptation." *Nature Neuroscience*, 2005. Published online. Courtesy of the authors. Used with permission.

# Resens'n requires protein synthesis

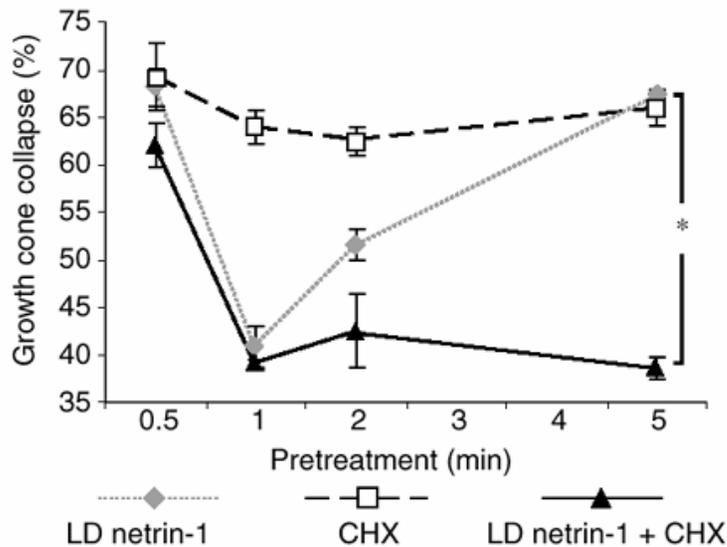
a



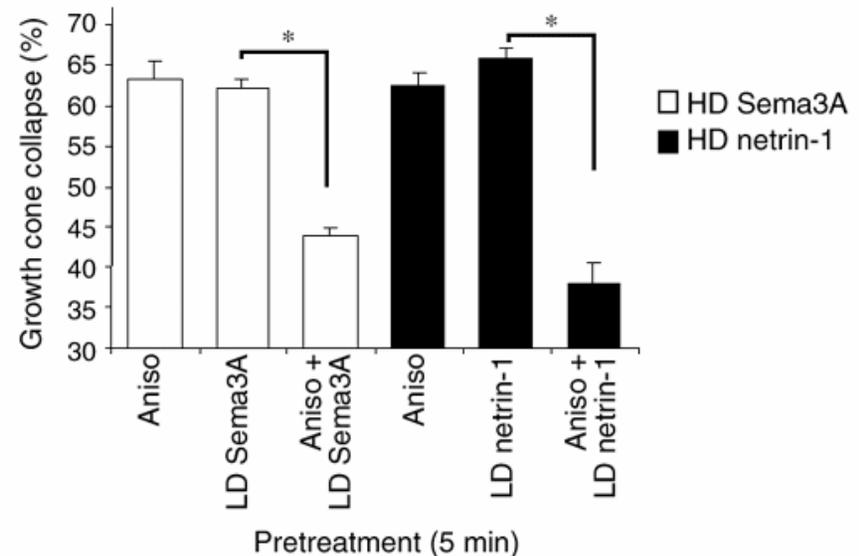
► Cycloheximide/Anisomycin included in pre-treatment, blocking synthesis of new protein

► Still get desensitization, but not resensitization

b



c

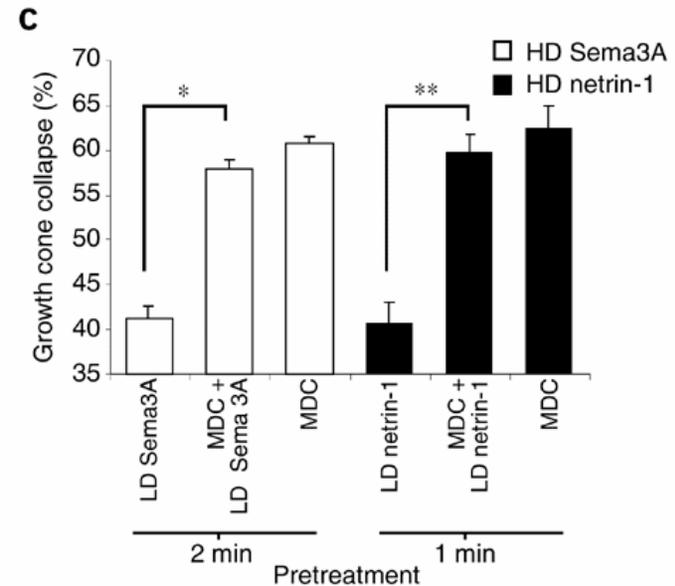
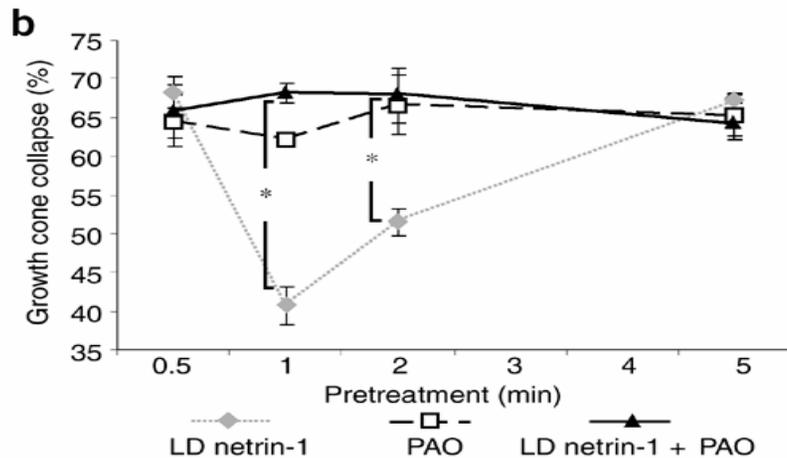
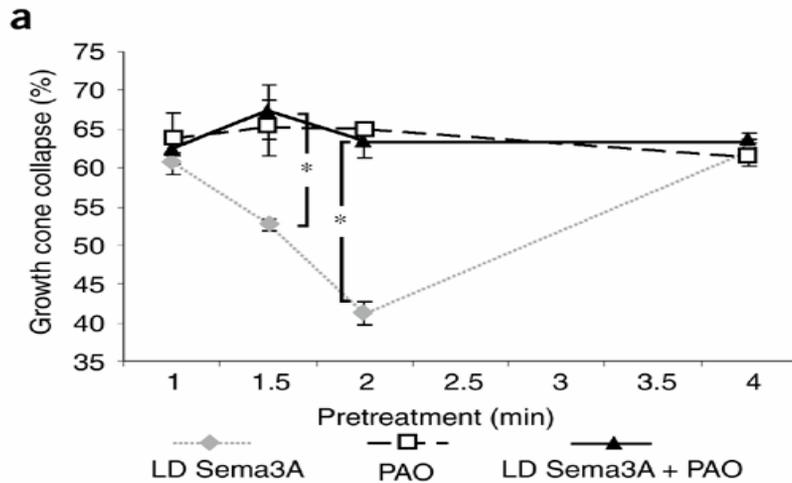


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Fig. 3

# Desens'n depends on endocytosis

► PAO/MDC included in pre-treatment, blocking receptor-mediated endocytosis.

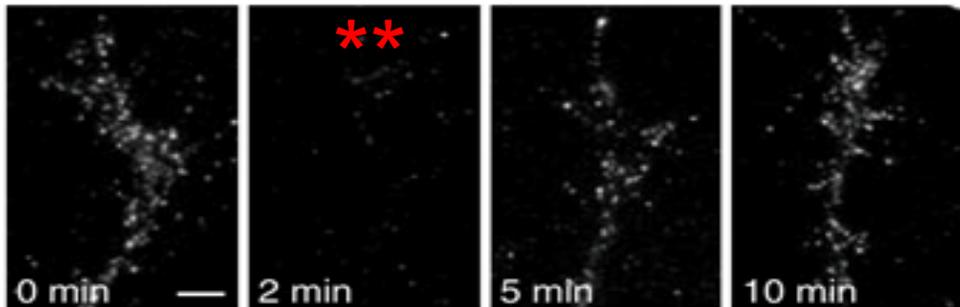


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Fig. 4

# Receptor localization follows sens'n

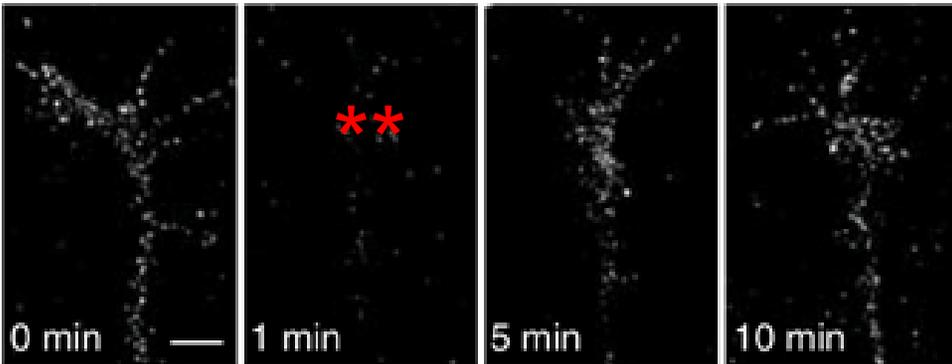
Fig. 5a-d



Neuropilin-1 immunoreactivity after LD Sema3A treatment

- ▶ Detect only receptors on outside of membrane (non-permeabilized prep)
- ▶ Same time-course as de-/re-sensitization !

Fig. 6a-d



DCC immunoreactivity after LD netrin-1 treatment

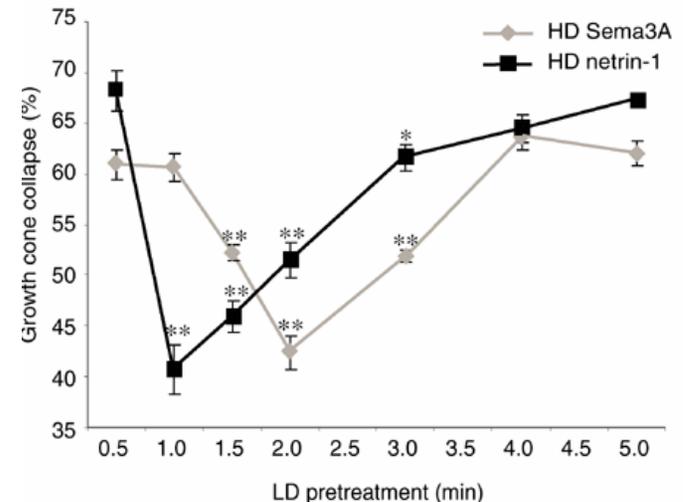
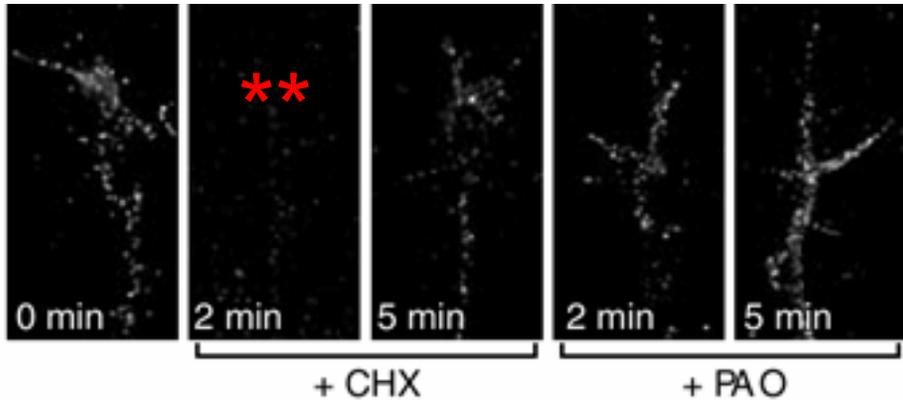


Fig. 1b

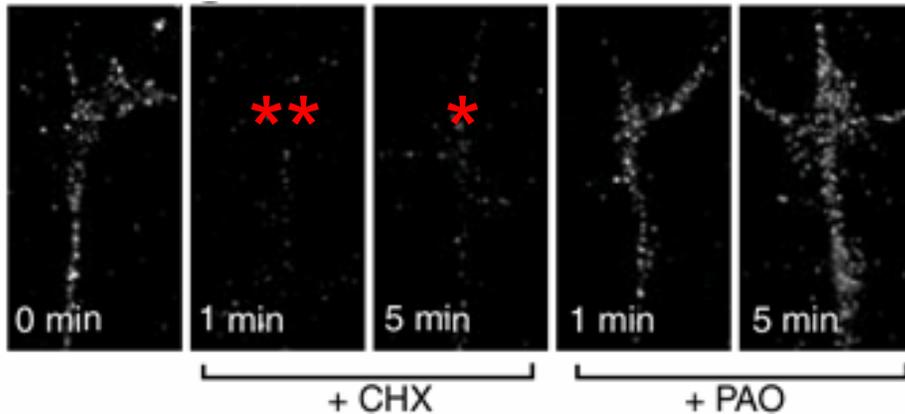
# Receptor localization follows sens'n: II

Fig. 5f-j



Neuropilin-1 immunoreactivity after LD Sema3A treatment

Fig. 6f-j

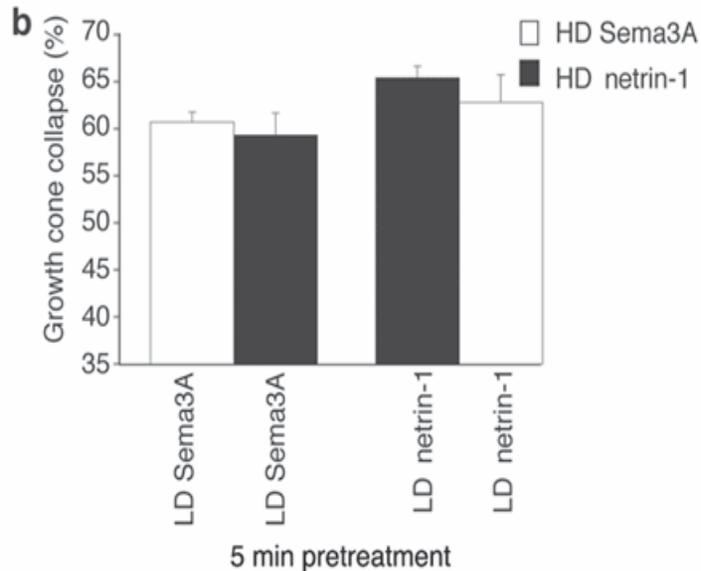
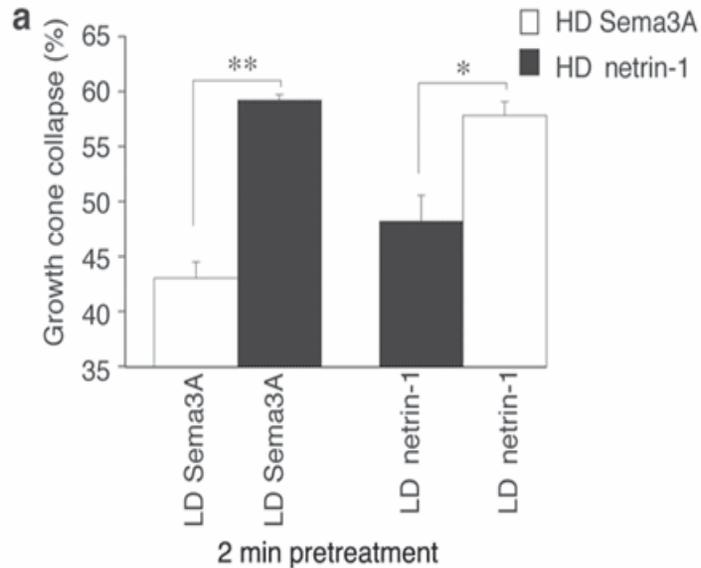


DCC immunoreactivity after LD netrin-1 treatment

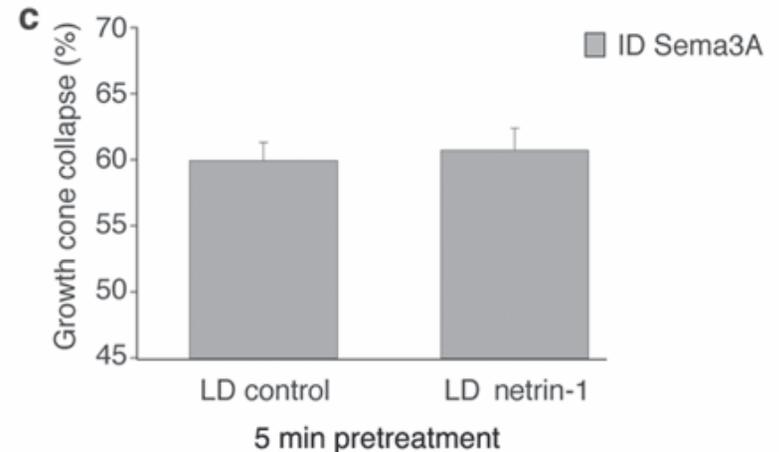
- ▶ Can block receptor removal (desensitization) with inhibitor of endocytosis (PAO)
- ▶ Can block most (but not all: fig: 5h, 6h) receptor reinsertion (resensitization) with protein synthesis inhibitor (CHX)
- ▶ Maybe endosomal recycling responsible for some reinsertion

Source: Piper, M., S. Salih, C. Weini, C. E. Holt, and W. A. Harris. "Endocytosis -Dependent Desensitization and Protein Synthesis – Dependent Resensitization in Retinal growth Cone Adaptation." *Nature Neuroscience*, 2005. Published online. Courtesy of the authors. Used with permission.

# Adaptation is 'ligand-specific'



- ▶ Pre-treat with one ligand, give high dose of other.
- ▶ No cross-desens'n (a)
- ▶ No cross-resens'n (b,c)
- ▶ Consistent with receptor trafficking hypothesis



# Summary

- ▶ Response of growth cones to chemorepellents can adapt very quickly
- ▶ Desensitization requires endocytosis
- ▶ Resensitization requires protein synthesis
- ▶ Receptor trafficking has same time course, and is blocked by same pharm. agents
- ▶ Adaptation is ligand-specific

# Questions

- ▶ All receptor trafficking, or downstream effects, too? (endocytosis as signalling step)
- ▶ Why adapt to a repellent?
- ▶ Homeostatic reset argument 'straw man'--how would you do reset except with a large rightward shift? (perhaps above physiological levels)  
Maybe there is reset after high doses, rather than LD?