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

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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)

Time Course of Adaptation

- ~35% basal collapse rate (control not shown)
- Sema3a desensitization after 2min, resensitization after 4min; Netrin desens’s’n 1min, resens’n 4min

Adaptation adjusts sensitivity

► 5min pre-treatment (i.e. ‘fully resensitized’ at HD?)

► Dose-dependence argues for ‘recalibration’

Resens’n requires protein synthesis

- Cycloheximide/Anisomicin included in pre-treatment, blocking synthesis of new protein
- Still get desensitization, but not resensitization

Desens’n depends on endocytosis

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

Receptor localization follows sens’n

Detect only receptors on outside of membrane (non-permeabilized prep)

Same time-course as de-/re-sensitization!

Receptor localization follows sens’n: II

► 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

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

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?