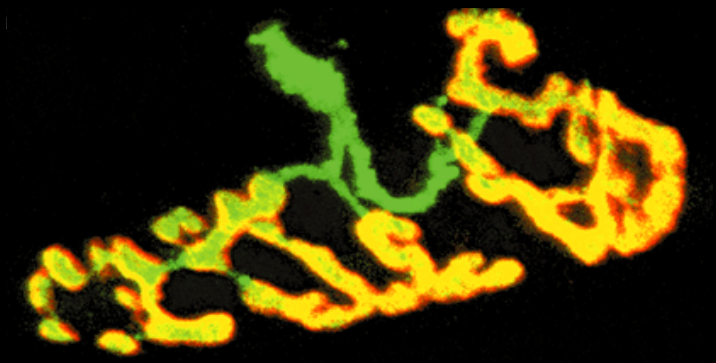
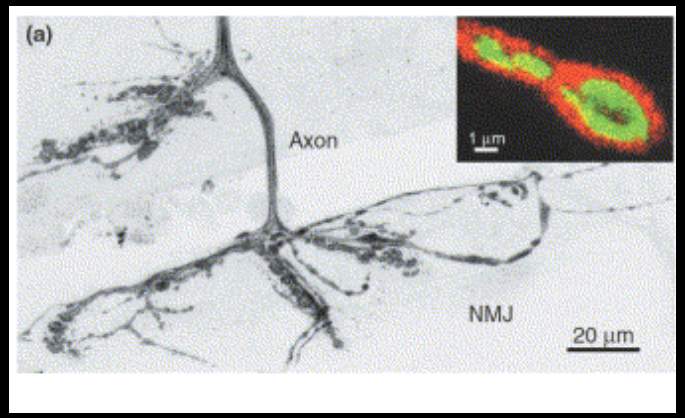
Neuronal Differentiation: Synapse Formation NMJ

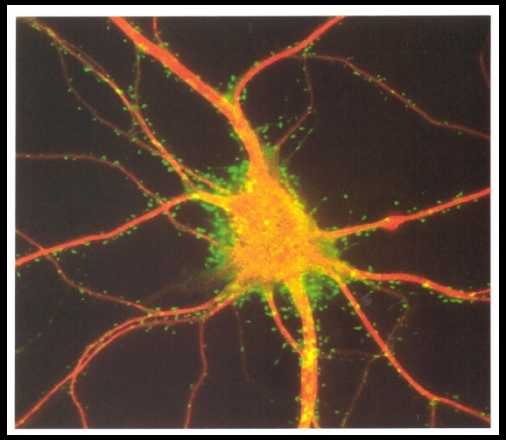
Approaches to study synapse formation



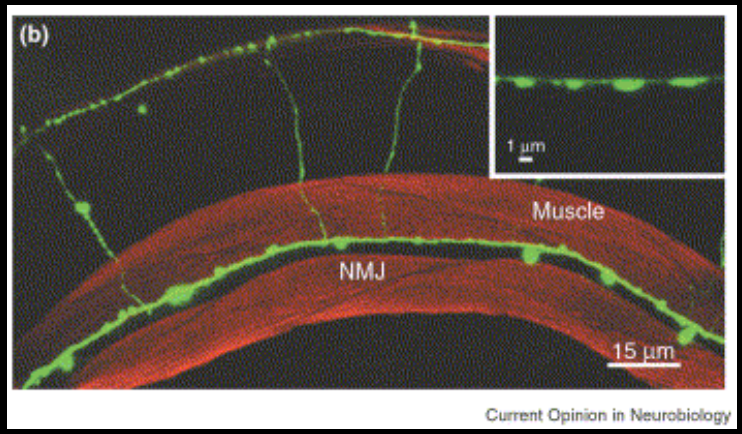
Rodent NMJ
(cholinergic)



Drosophila NMJ
(glutamatergic)



Rodent CNS synapse
(glutamatergic or GABAergic)



C. elegans NMJ
(cholinergic and GABAergic)

Approaches to study synapse formation and elimination

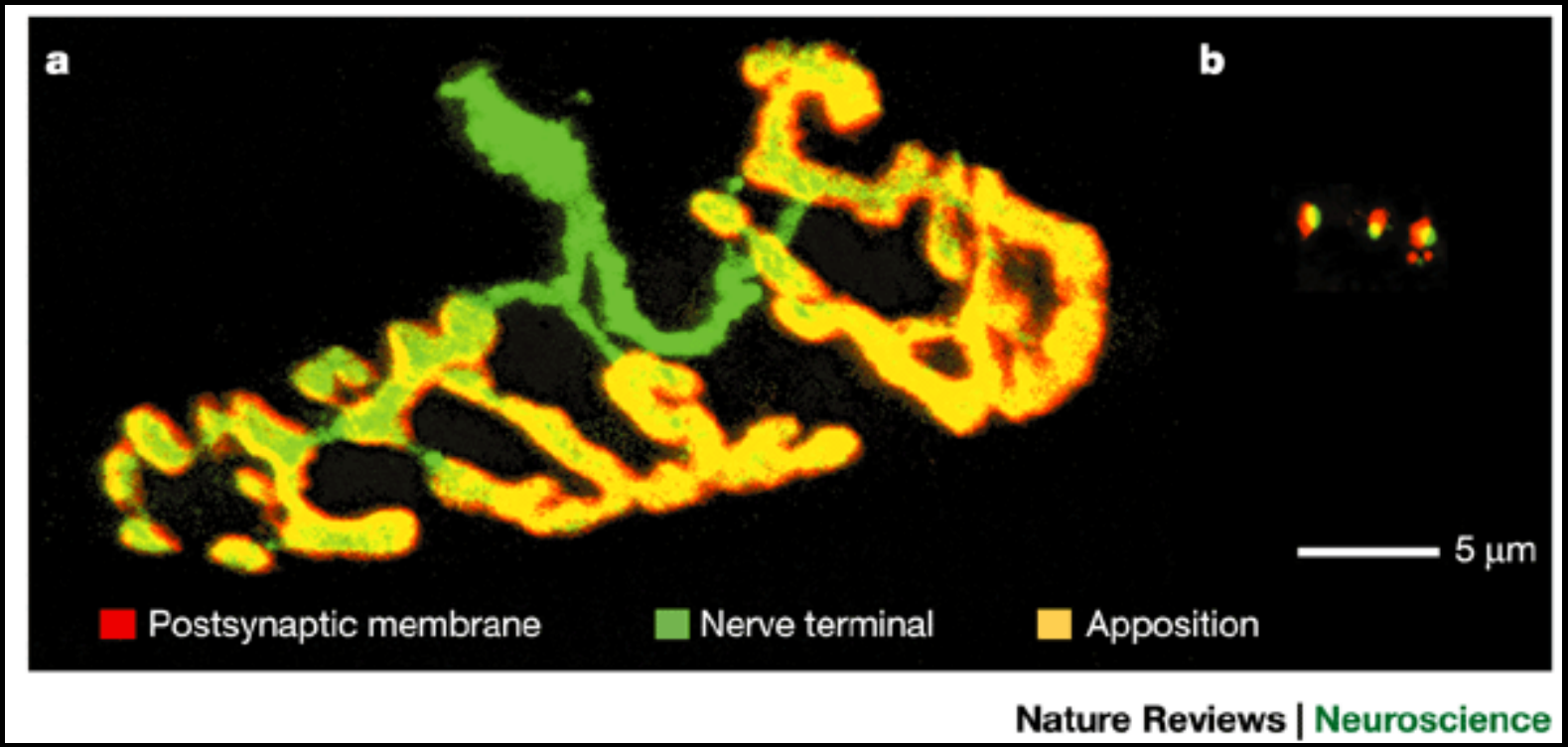
(1) Rodent NMJ (cholinergic)
-accessibility in vivo in transgenic mice allows for elegant imaging experiments: field used to be dominated by Jeff Lichtman and Josh Sanes

(2) Genetic screens:
-Drosophila NMJ (glutamatergic)…Broadie and Davis
-C. Elegans…Jin, Bargmann, and Nonet
** early screens had only limited success perhaps due to late onset of synaptogenesis
** recent development of GFP fusion reporter genes has changed this by allowing visualization of synapses in living animals

(3) Rodent CNS synapses in vitro & in vivo
-recent advances in cell culture, GFP fusions, and live imaging techniques

(4) Rodent (and frog and fish) CNS synapses in vivo
-imaging of spines, recently imaging of axons

Comparison of the NMJ and Central Synapses



Advantages of using the NMJ:

(1) Large size

(2) Relative simplicity

(3) Unparalleled accessibility

**owing to these features, the concept of the postsynaptic receptor was proposed by Langley over a century ago

(4) alpha-bungarotoxin

Sanes and Lichtman 2001

The Adult NMJ

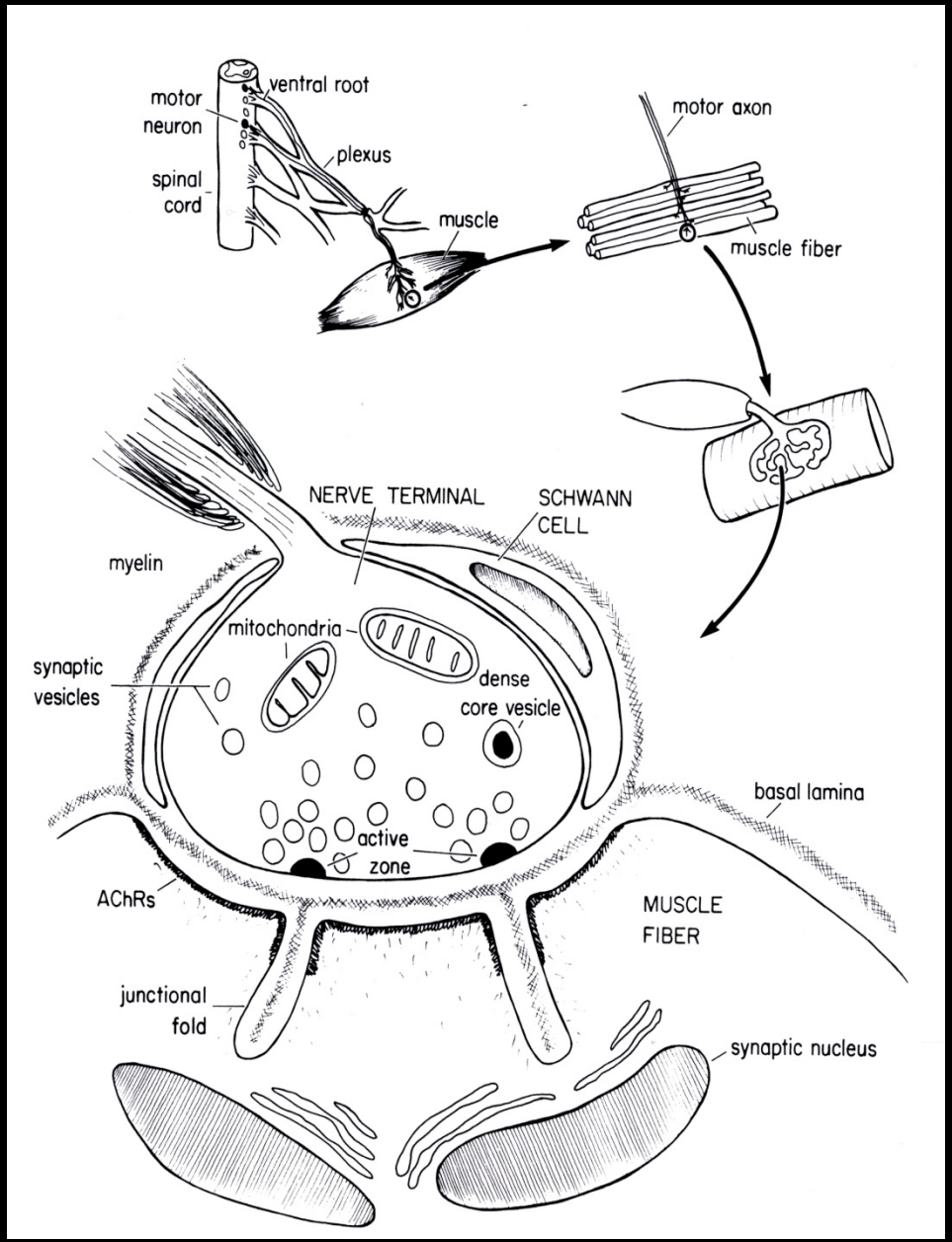
•Motor neurons in the ventral horn of the spinal cord send axons through peripheral nerves to innervate muscles

• Axons branch intramuscularly to innervate many muscle fibers, but each fiber receives only one synapse

• The axonal branch loses its myelin sheath to terminate in a spray of boutons on the muscle fiber surface

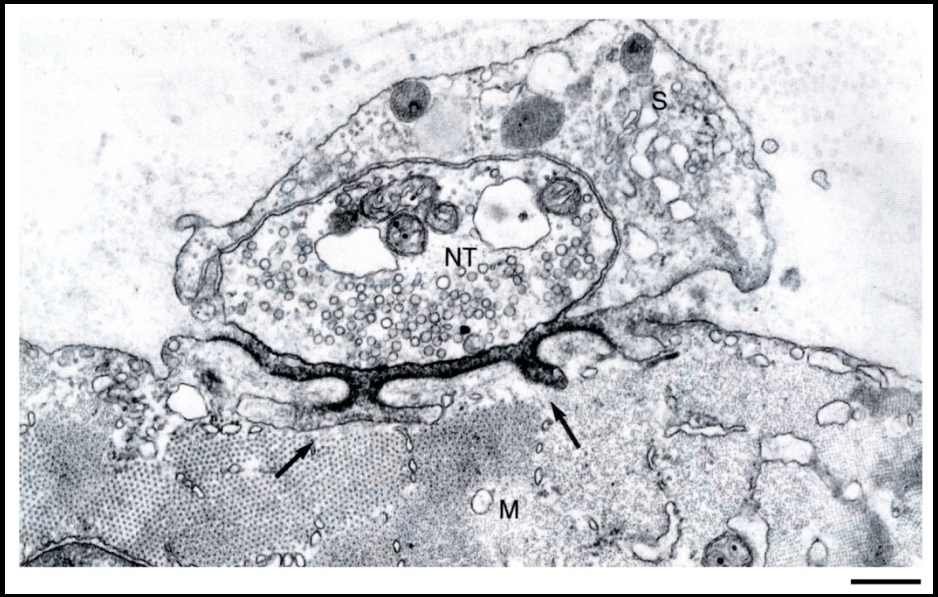
•3 cells that constitute the synapse:
(1) the motor neuron (nerve terminal)
(2) the muscle fiber
(3) the Schwann cell

**at the adult NMJ, there are > 10,000 AChRs/um^2



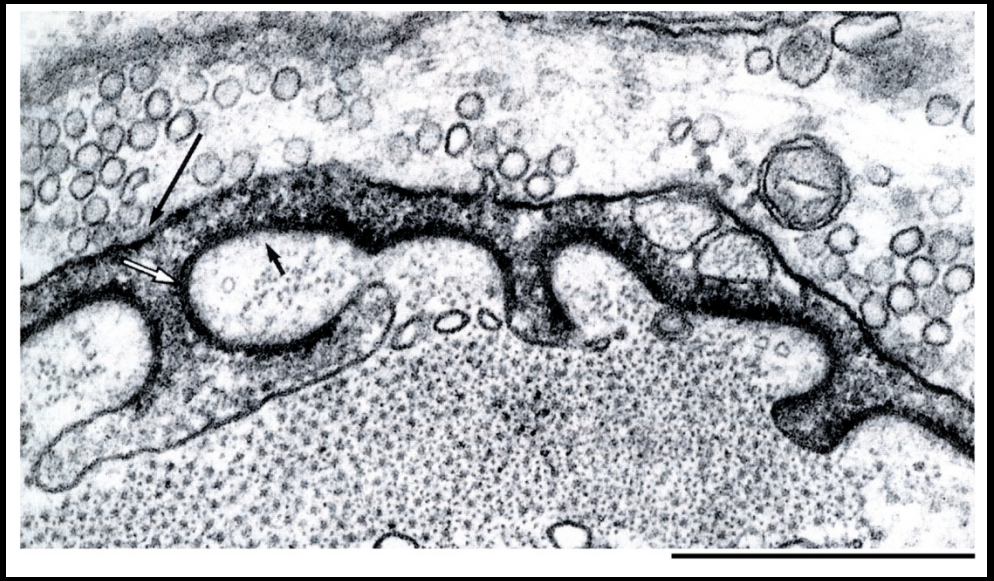
Hall and Sanes 1993

Pre- and postsynaptic membranes at the NMJ are highly specialized



- Electron micrograph of a NMJ shows the nerve terminal capped by a Schwann cell situated in a shallow depression of a muscle cell membrane, which is invaginated further into deep postjunctional folds (arrows)

**AChRs are concentrated at the synaptic site



- Higher mag view shows that AChRs are concentrated at the crests and along the sides of the postjunctional folds (white arrow).

**postjunctional folds are situated directly across from presynaptic active zones (long arrow) in which clusters of synaptic vesicles accumulate in the nerve terminal

Burden et al 1979

Molecular Components of the NMJ

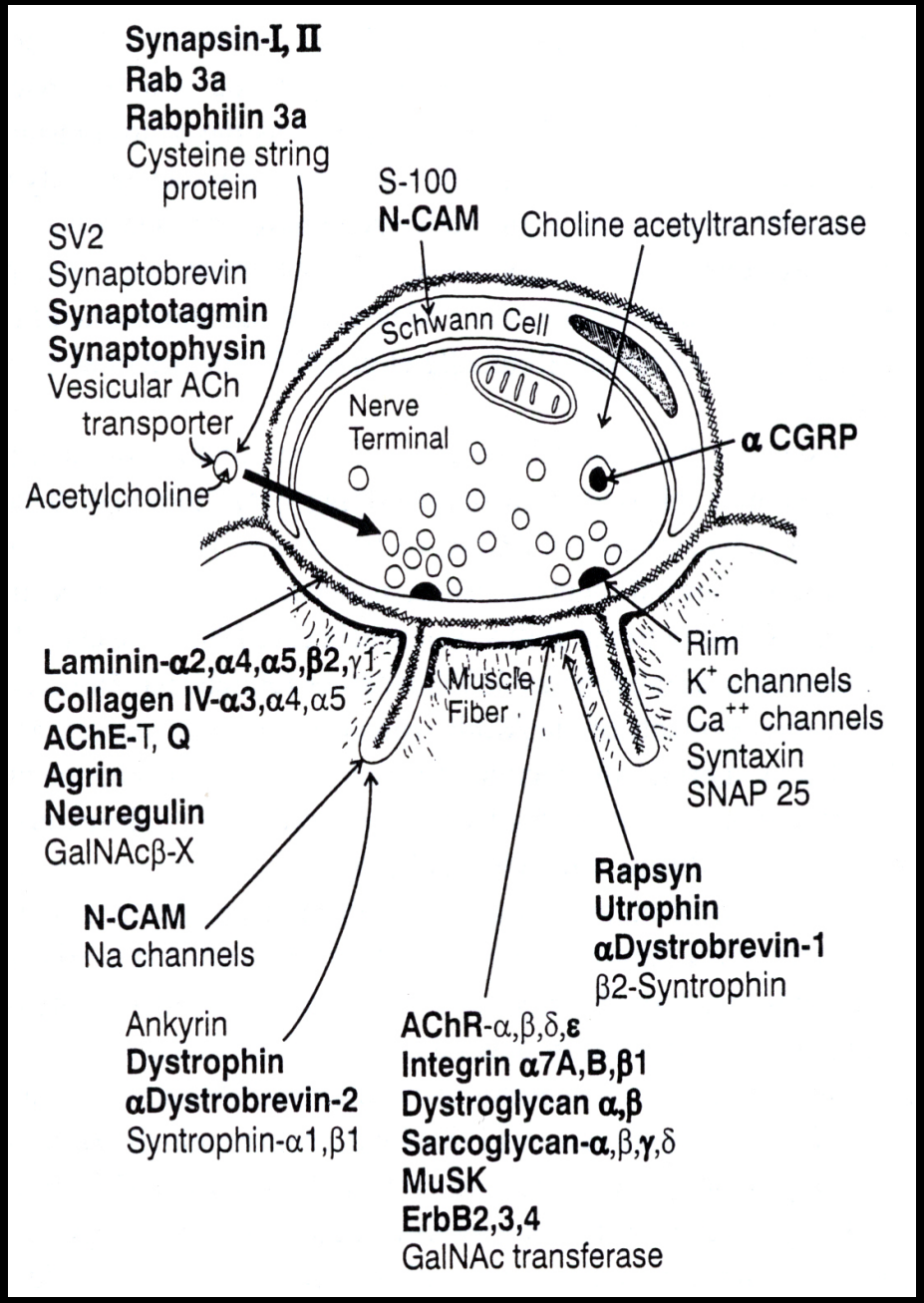
-the nerve terminal occupies a shallow gutter in the muscle fiber and is capped by processes of Schwann cells

-active zones in the nerve terminal directly appose junctional folds in the postsynaptic membrane

-some of the proteins in the synapse are shown, with their subcellular localization indicated by arrows

-those for which knockout mice have been generated are indicated in BOLD

**Postsynaptic membrane = highly specialized to respond rapidly to NT released from the overlying nerve terminal with an extremely high conc. of AChRs: >10,000/um^2 vs. <10/um^2 extrasynaptically



Sanes and Lichtman 1999

Differentiation of the NMJ

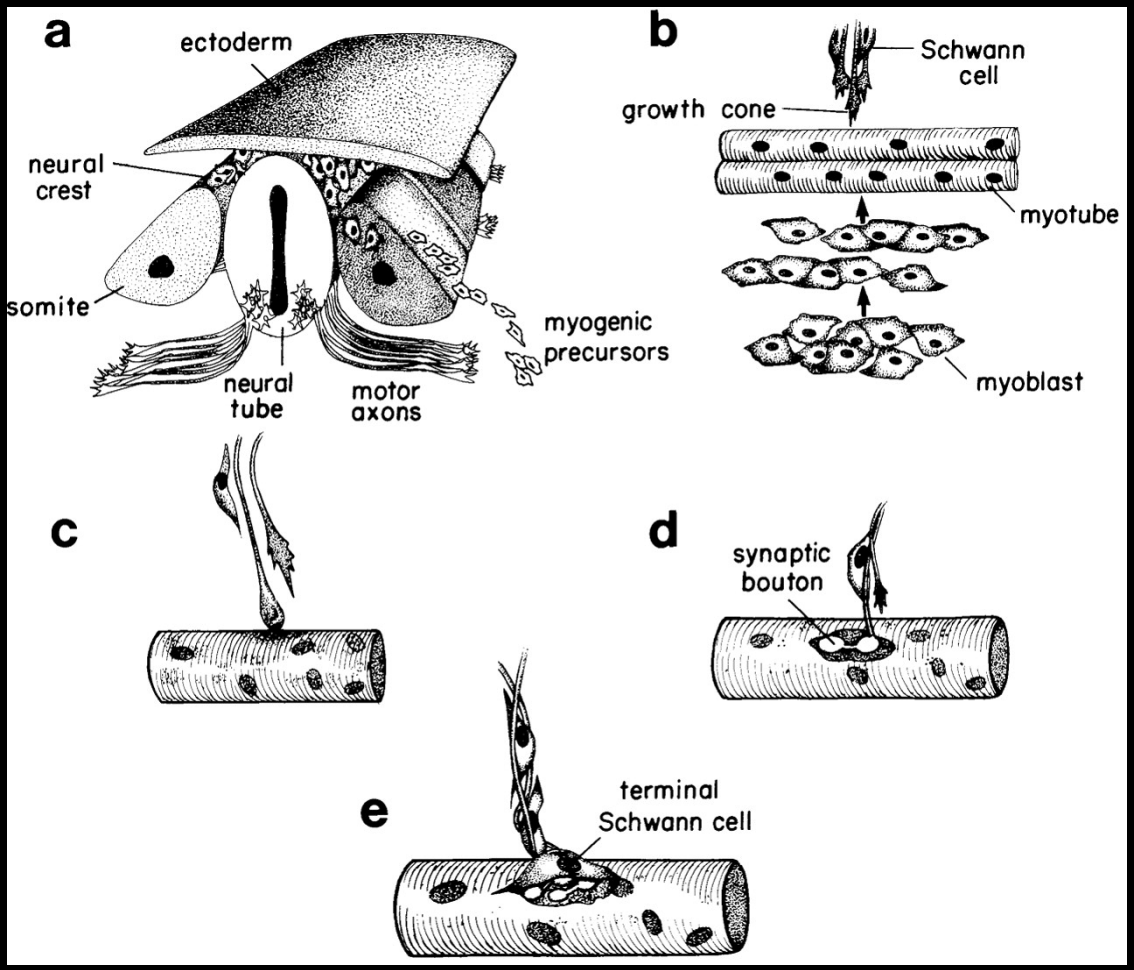
3 Steps:

(1) Formation of selective connections between the developing axon and its target

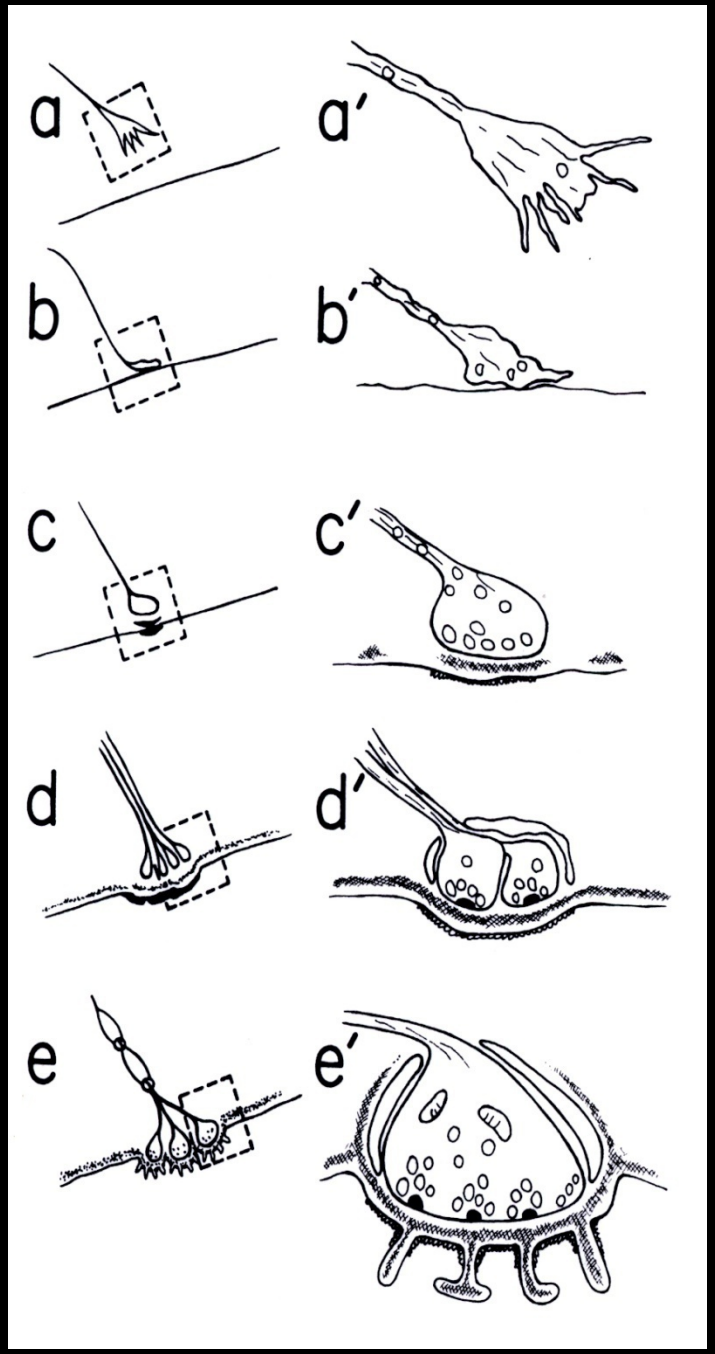
(2) Differentiation of the axon's growth cone into a nerve terminal

(3) Elaboration of a postsynaptic apparatus in the target cell

Early steps in the formation of the neuromuscular junction



Sanes and Lichtman, 1999



Differentiation of the NMJ

- A growth cone approaches a myotube (a) and forms an unspecialized but functional contact on its surface (b).

- The terminal then differentiates and basal lamina appears in a widened cleft (c).

- As basal lamina appears extrasynaptically, multiple axons converge on the synaptic site (d).

- Finally, all axons are eliminated except one. The survivor expands, each terminal bouton is ensheathed by a Schwann cell process, the preterminal axon acquires a myelin sheath, and folds form in the subterminal postsynaptic membrane (e).

Hall and Sanes 1993

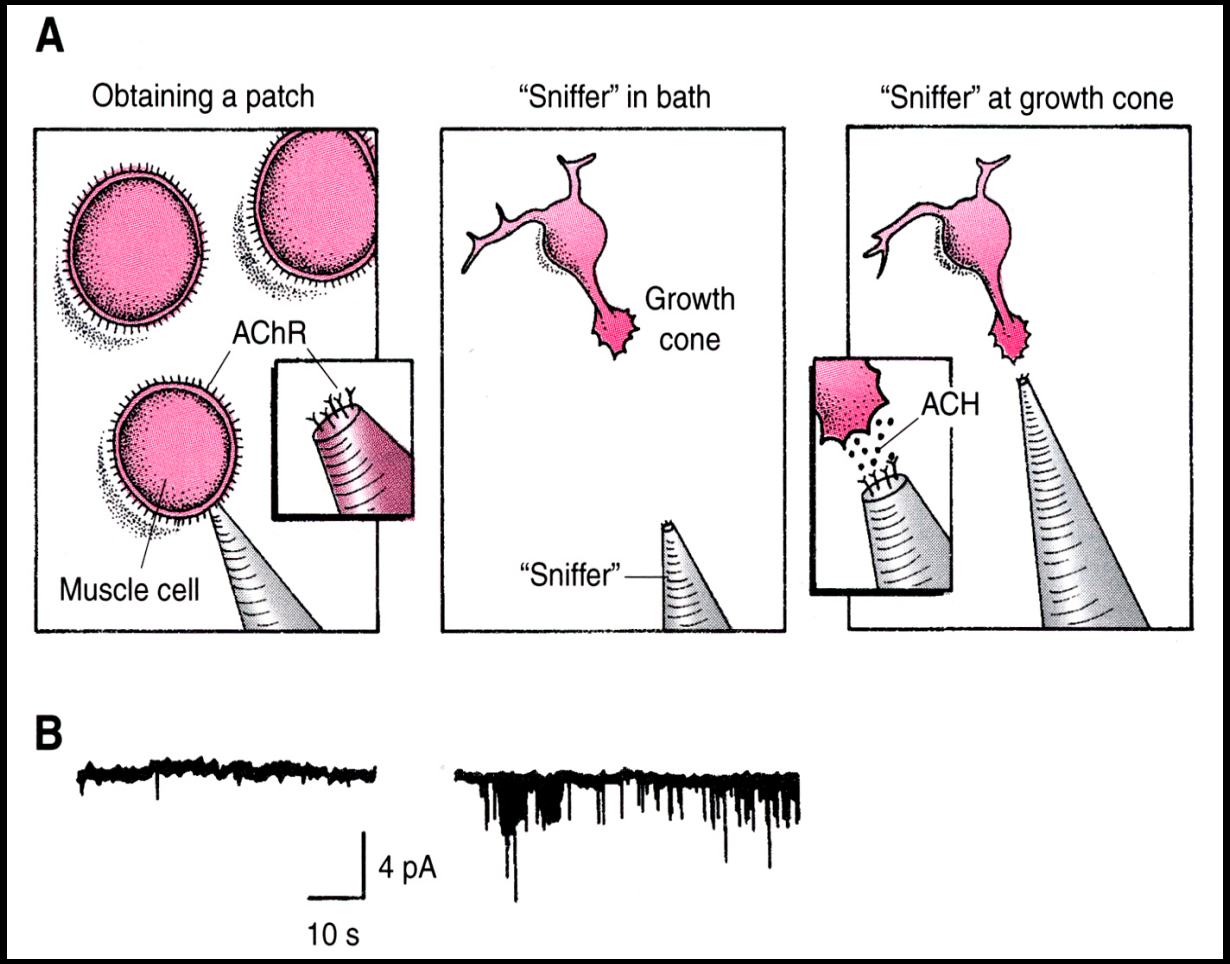
NMJ Formation

(1) Both pre- and postsynaptic cells have the machinery to form synapses BEFORE contact
- isolated axonal growth cones release Ach
- isolated muscle cells express AChRs

(2) Contact serves to cluster presynaptic vesicles and postsynaptic AChRs

(3) Synapses attain their mature structure and physiological properties over the next days to weeks

Spontaneous Release of ACh from Growth Cones



A biological sensor for ACh (a "sniffer") was created by excising a patch of membrane from a muscle cell with a recording pipet. The membrane contained AChRs that were facing outward. Thus, when the pipet was advanced near a growth cone, a current could be recorded when Ach bound to the receptors.

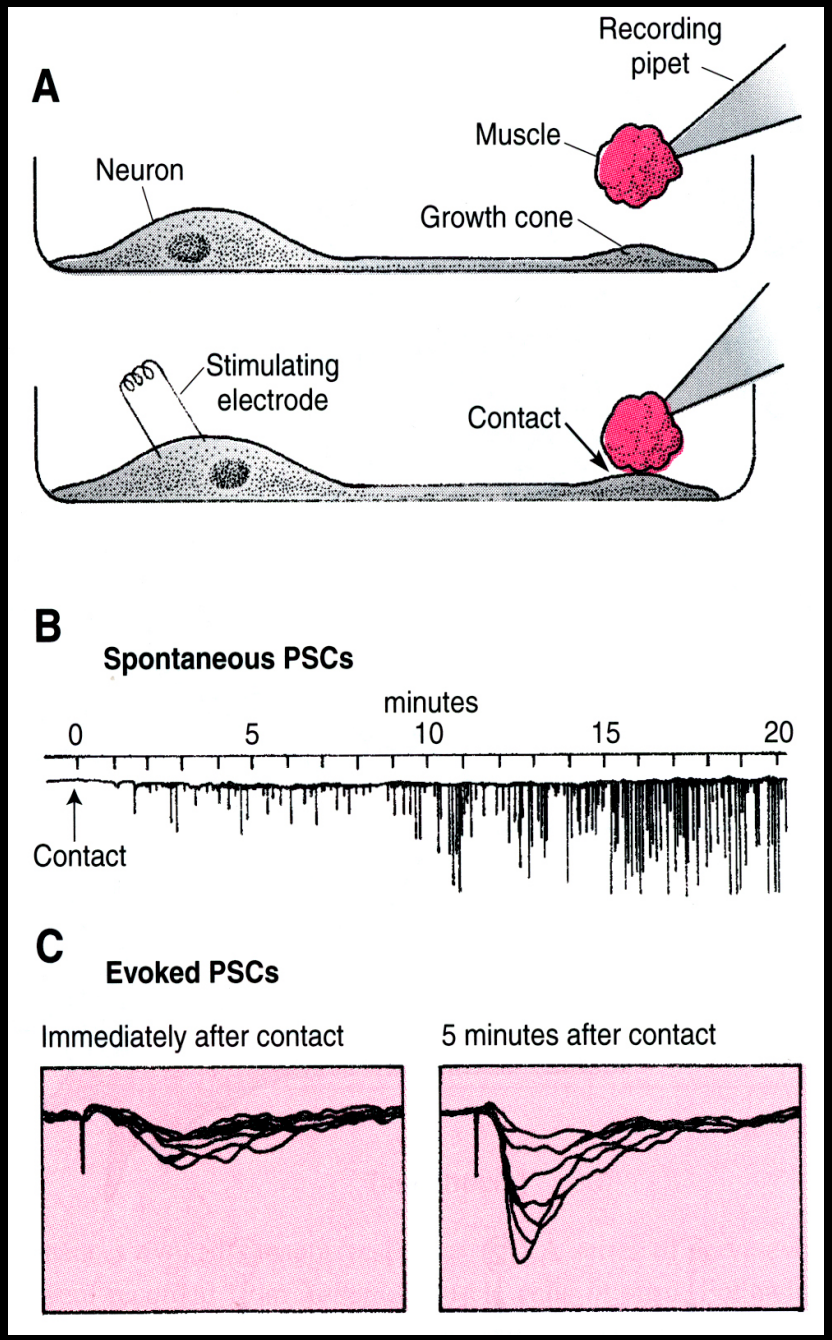
• Recording of ACh-evoked currents (down-ward deflections) when the sniffer patch was distant from the growth cone (left) and when it was within a few microns of the growth cone (right). The increased activity indicates that the growth cone was releasing ACh.

Young and Poo 1983; Hume et al 1983

Presynaptic Differentiation: Contact with a Muscle Cell RAPIDLY Stimulates ACh Release

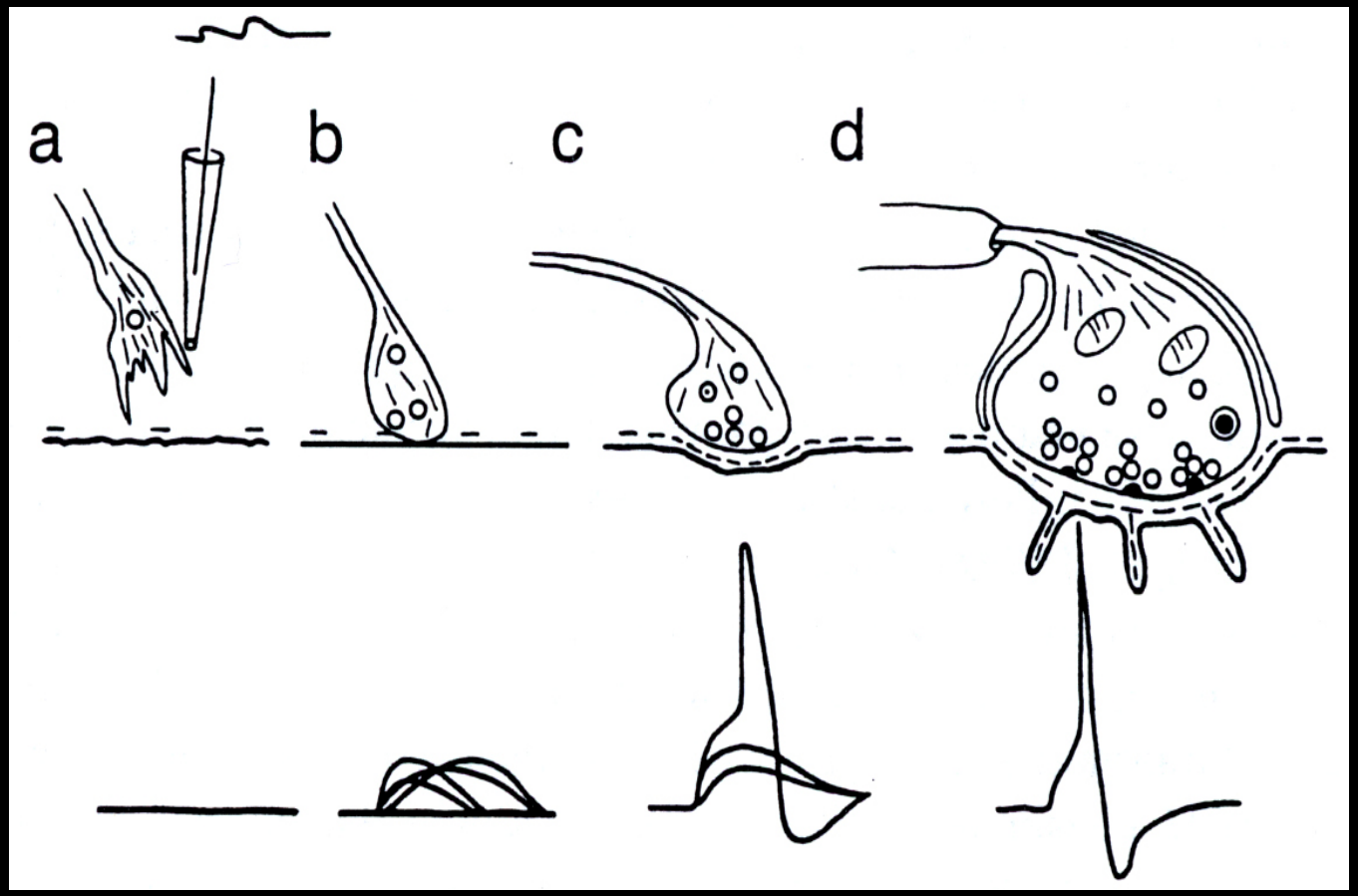
- Cultures of Xenopus spinal neurons were grown and pipets were used to record from round muscle cells and to manipulate them into contact with the neuron
- A continuous recording from a muscle cell shows spontaneous transmission (downward deflections) during the first 20min after contact.
- Nerve-evoked postsynaptic currents increased in amplitude from the moment of contact to 5min later.

**** Increase in synaptic transmission WITHIN MINUTES!!!!!!!



Xie and Poo 1986

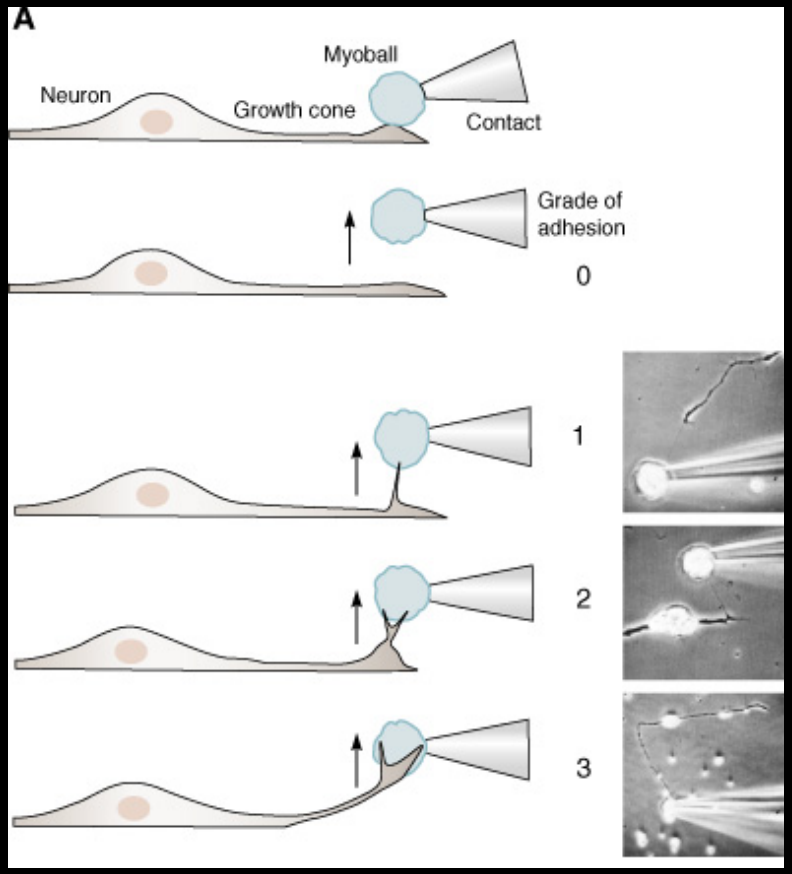
Muscle Signals Presynaptic Differentiation



- Rudimentary synaptic transmission begins immediately, but the efficacy = low: transmission is weak and subthreshold
- After about 1 week, fully functional, but immature synapse forms: synaptic vesicles accumulate and quantal content increases. Transmission is still prone to failures and the action potential is broad.
- After several weeks, mature NMJ is formed: preterminal region is myelinated and transmission has a high safety factor

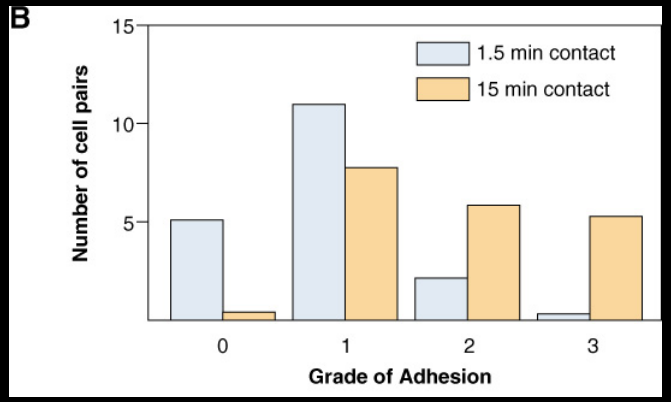
Sanes and Lichtman 1999

Rapid adhesion between growth cone and postsynaptic muscle cell

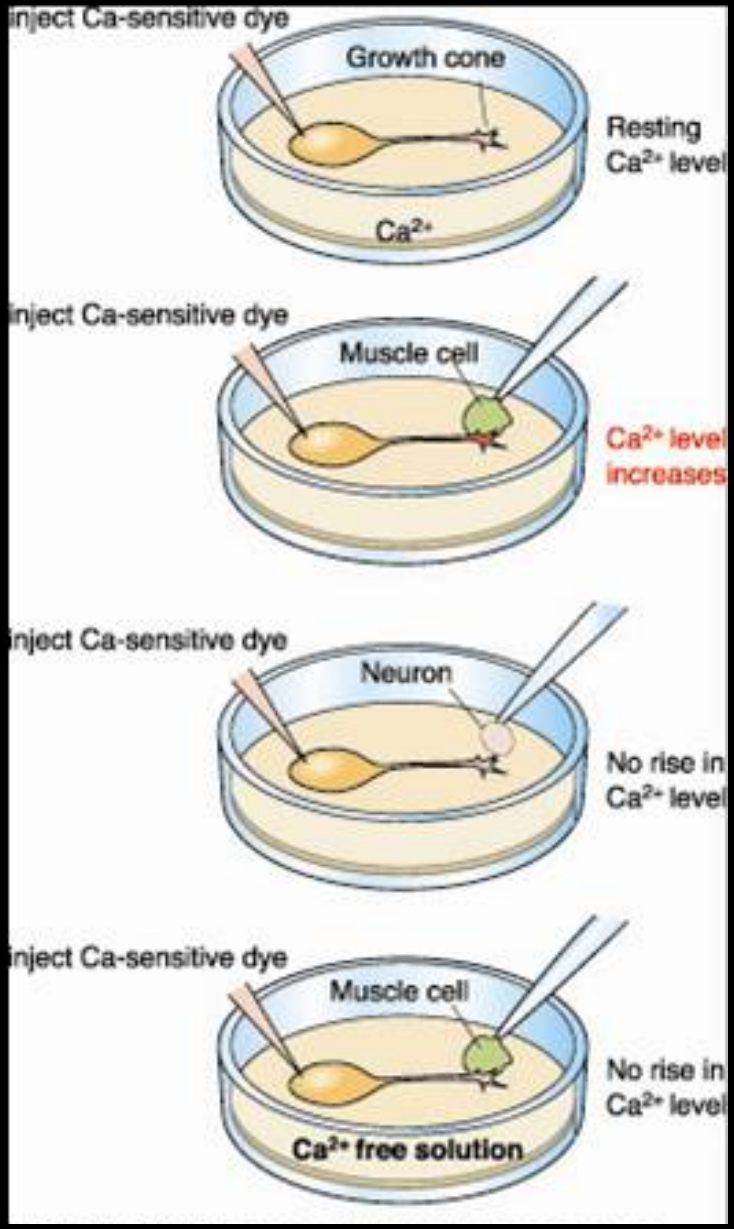


A muscle cell was manipulated into contact with a growth cone in a dissociated culture of *Xenopus* spinal cord. After 1.5 or 15 minutes, the muscle cell was withdrawn, and the degree of adhesion was graded: (0) no attachment, (1) filamentous attachment, (2) deformation of growth cone, and (3) detachment of growth cone from substrate.

After 1.5 minutes of contact, most pairs exhibited only grade 0–1 adhesion. However, after 15 minutes of contact, the level of adhesion shifted to grade 1–3. (Adapted from Evers et al., 1989)

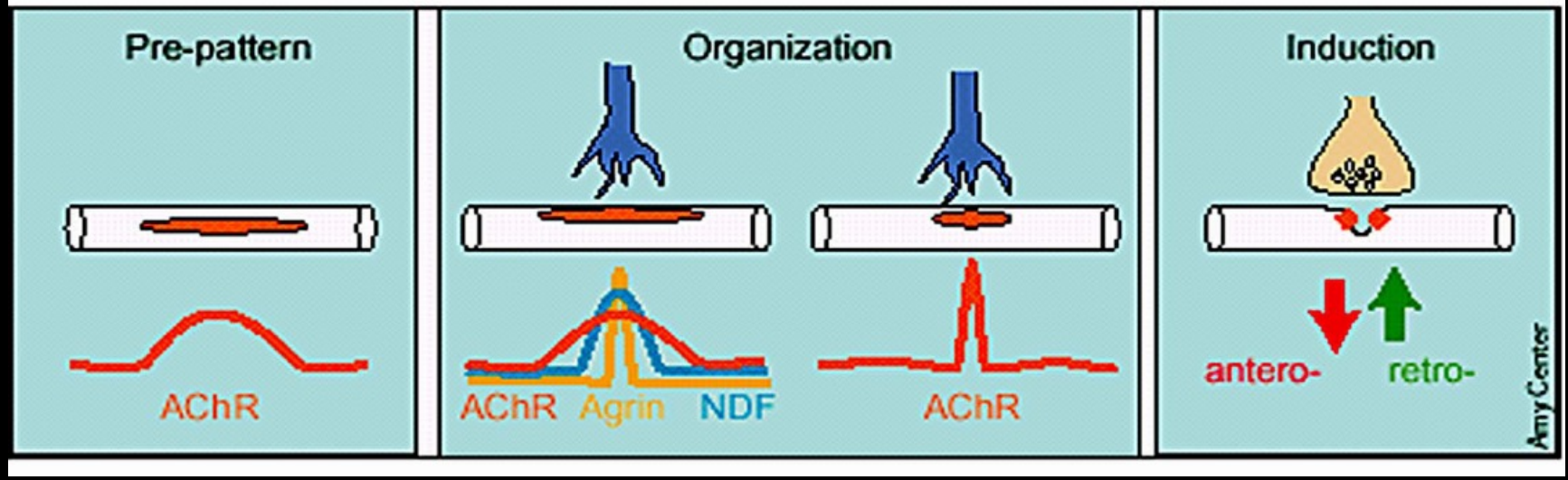


Contact with target increases free calcium in the growth cone.



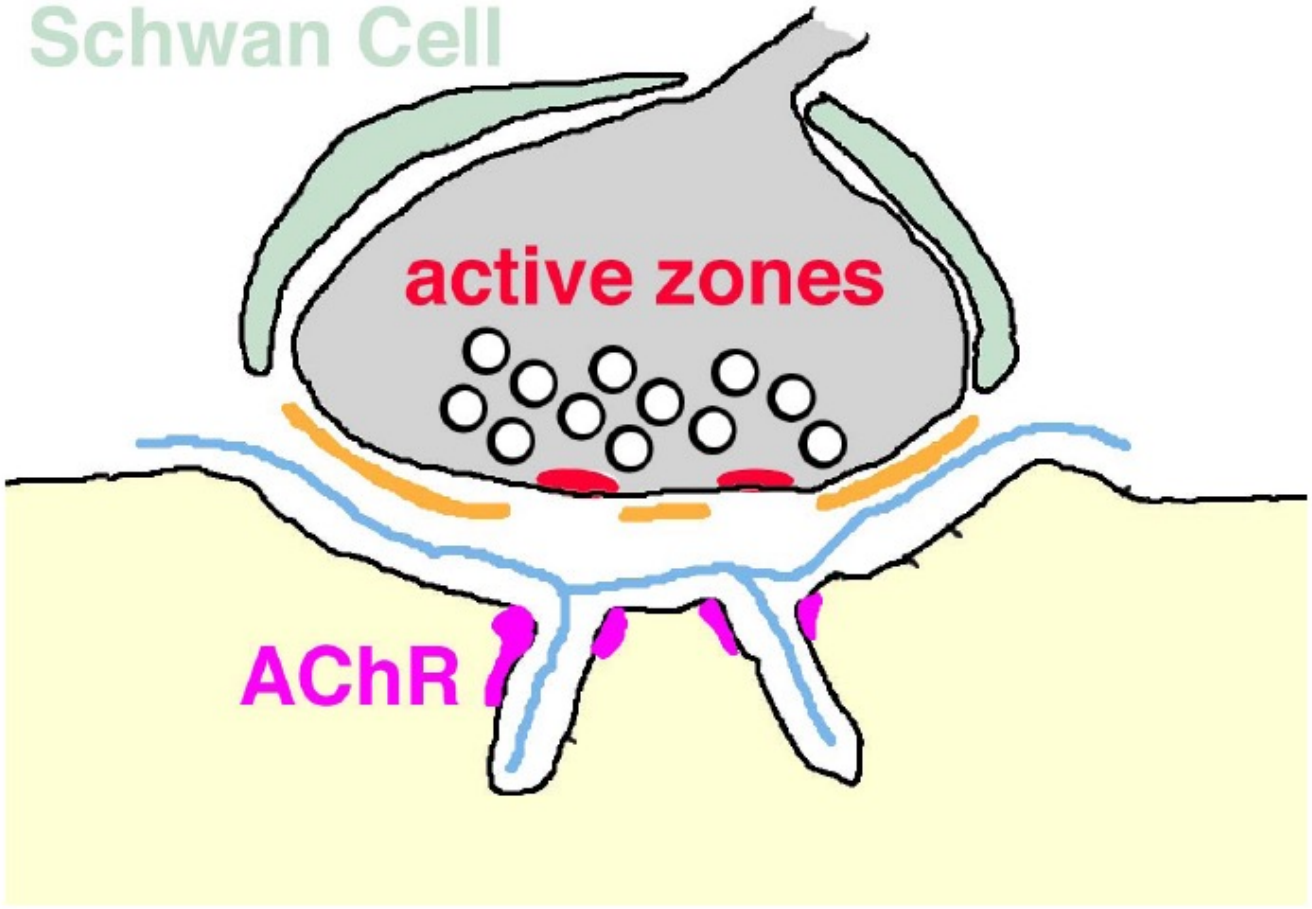
Dissociated neurons were filled with a Ca^{2+}-sensitive dye (top), and the growth cone was imaged while either a muscle cell or a neuron was brought into contact (middle). Intracellular free calcium increased only during contact with the muscle (red). The muscle-evoked rise in Ca^{2+} did not occur when the cells were bathed in a Ca^{2+}-free medium (bottom), indicating the involvement of calcium channels. (Adapted from Dai and Peng, 1993)

Stages of Synapse Assembly

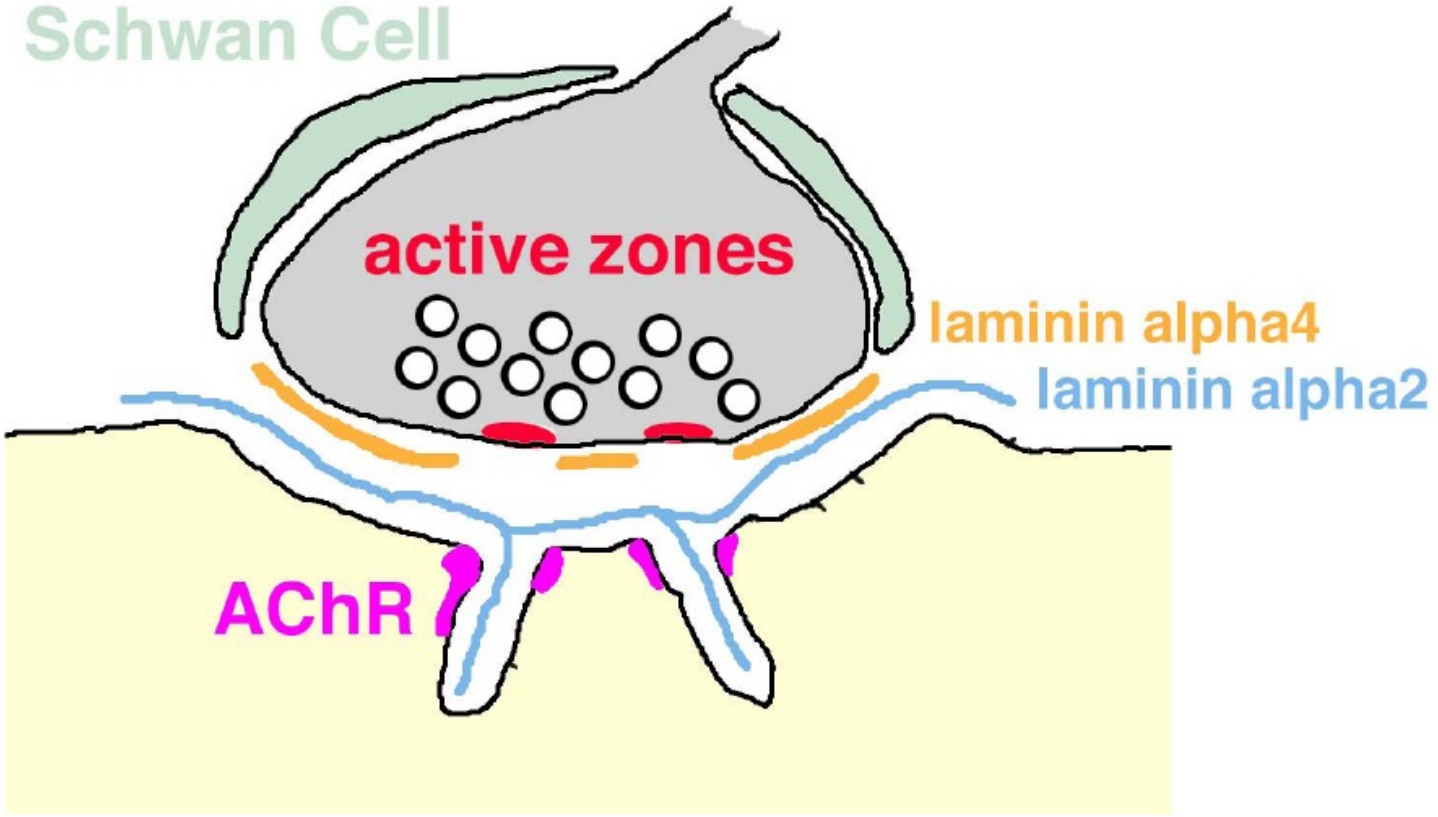


What are the retrograde signals that affect presynaptic differentiation?

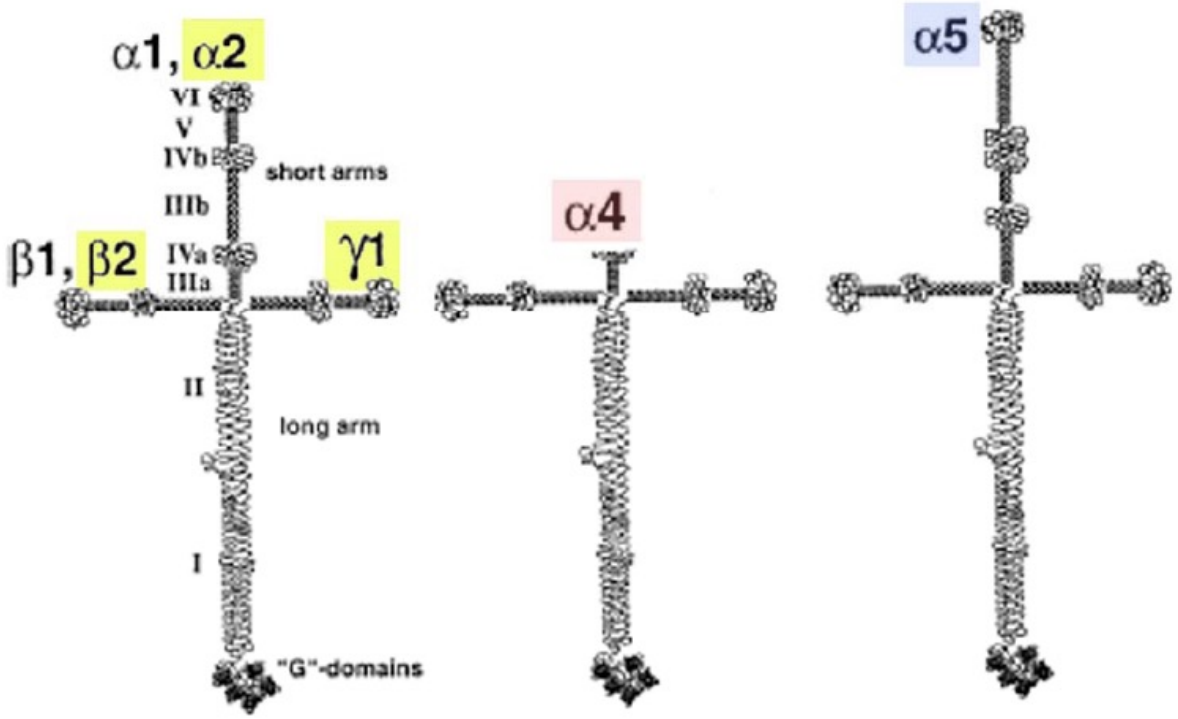
Diagram of vertebrate NMJ



Localization of laminins at the vertebrate NMJ

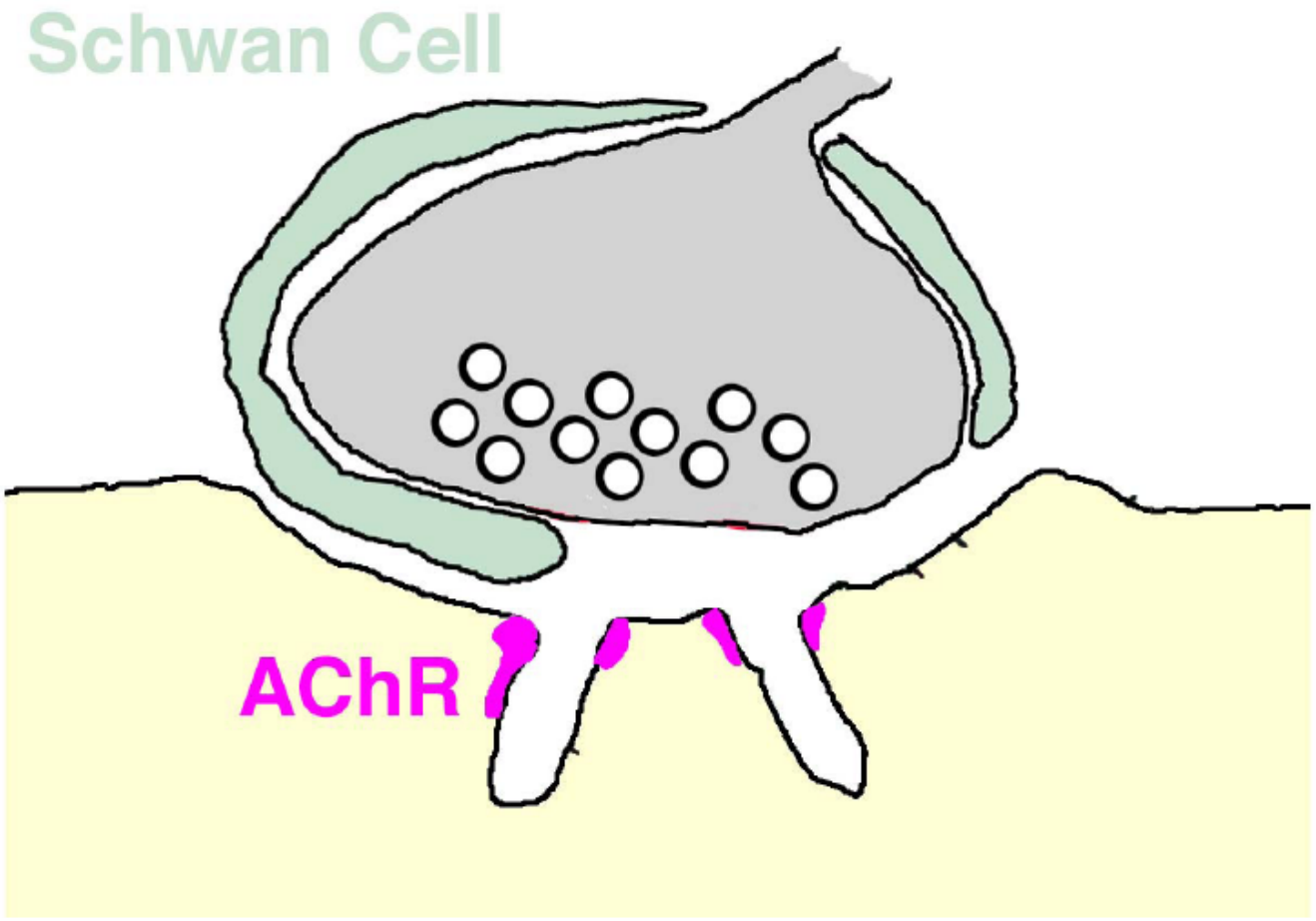


3 Synaptic Laminin trimers

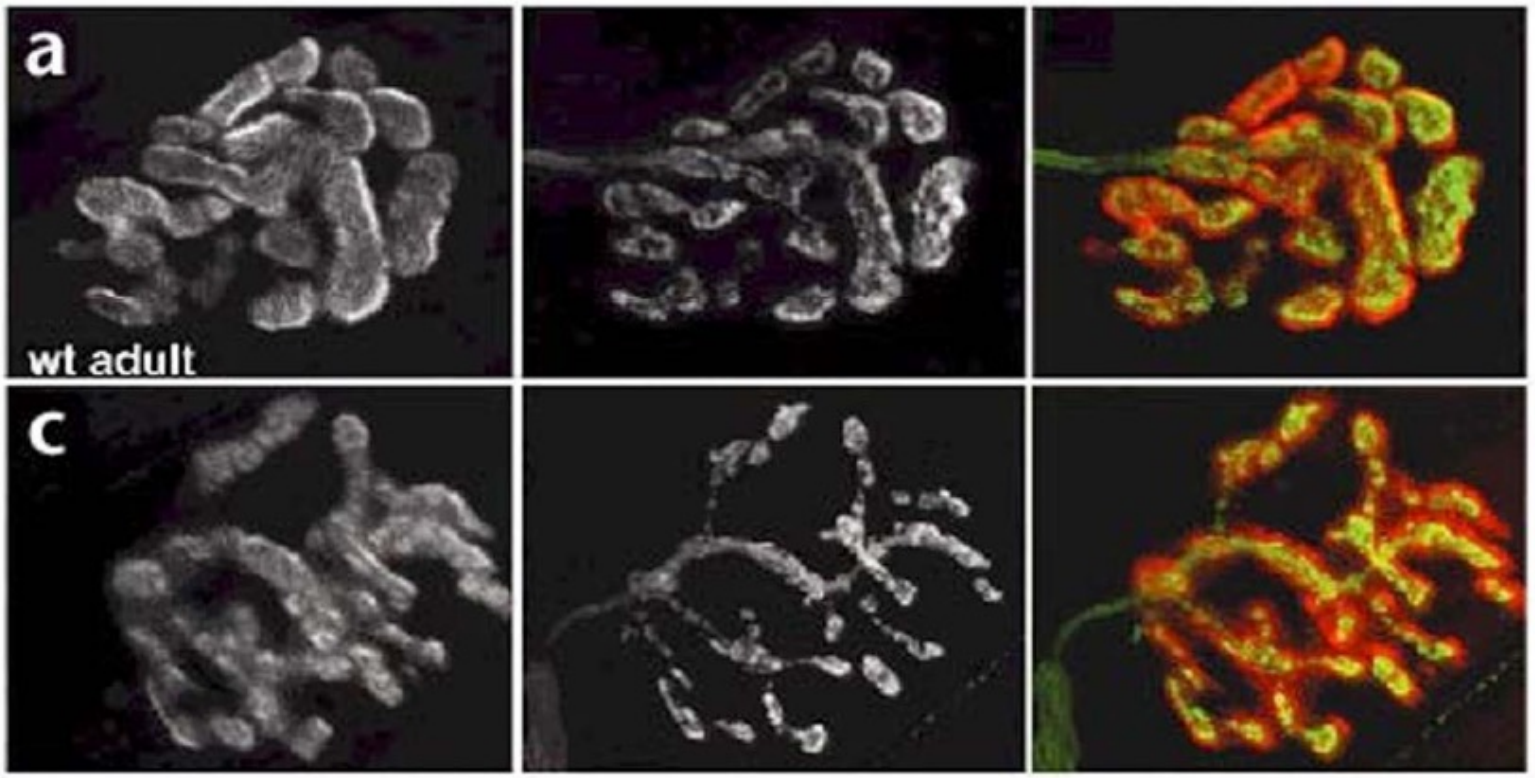


Extrasynaptic and Synaptic Laminins contain:
B1 and B2 (B2 is enriched at synaptic sites)
α2, α4 and α5 chains and only one γ chain (γ1)

Impaired induction of presynaptic specialization at laminin beta2 knockout synapses

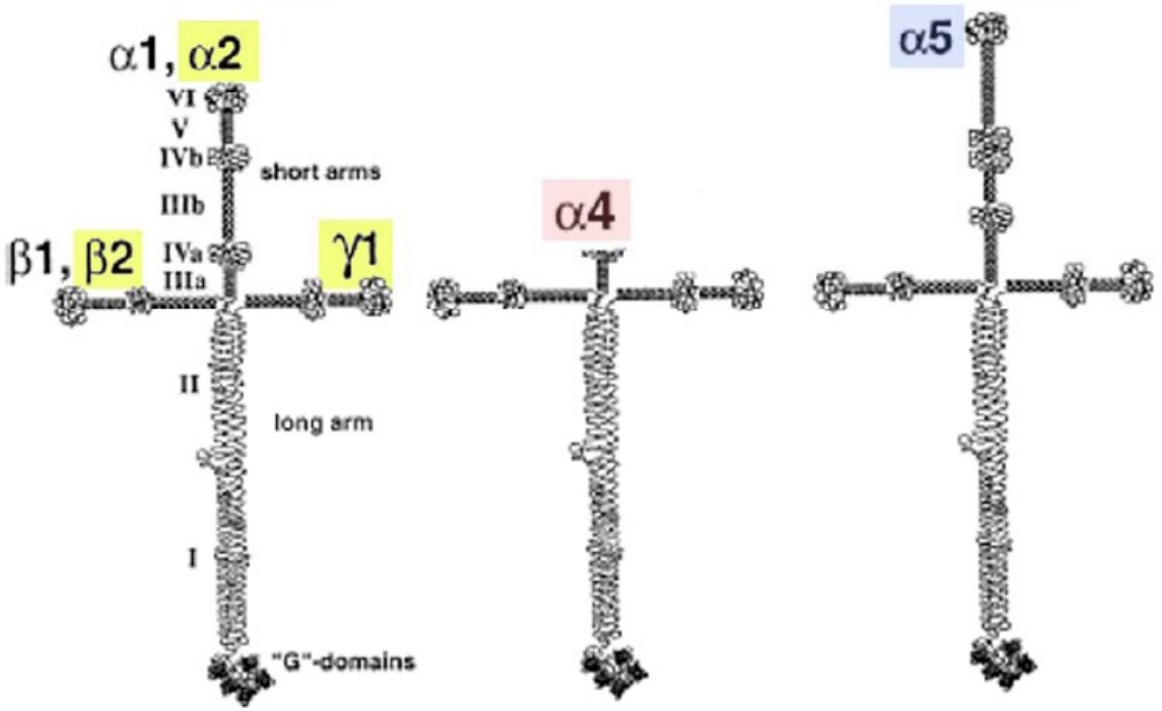


Gross morphology of ***laminin*** mutant synapses



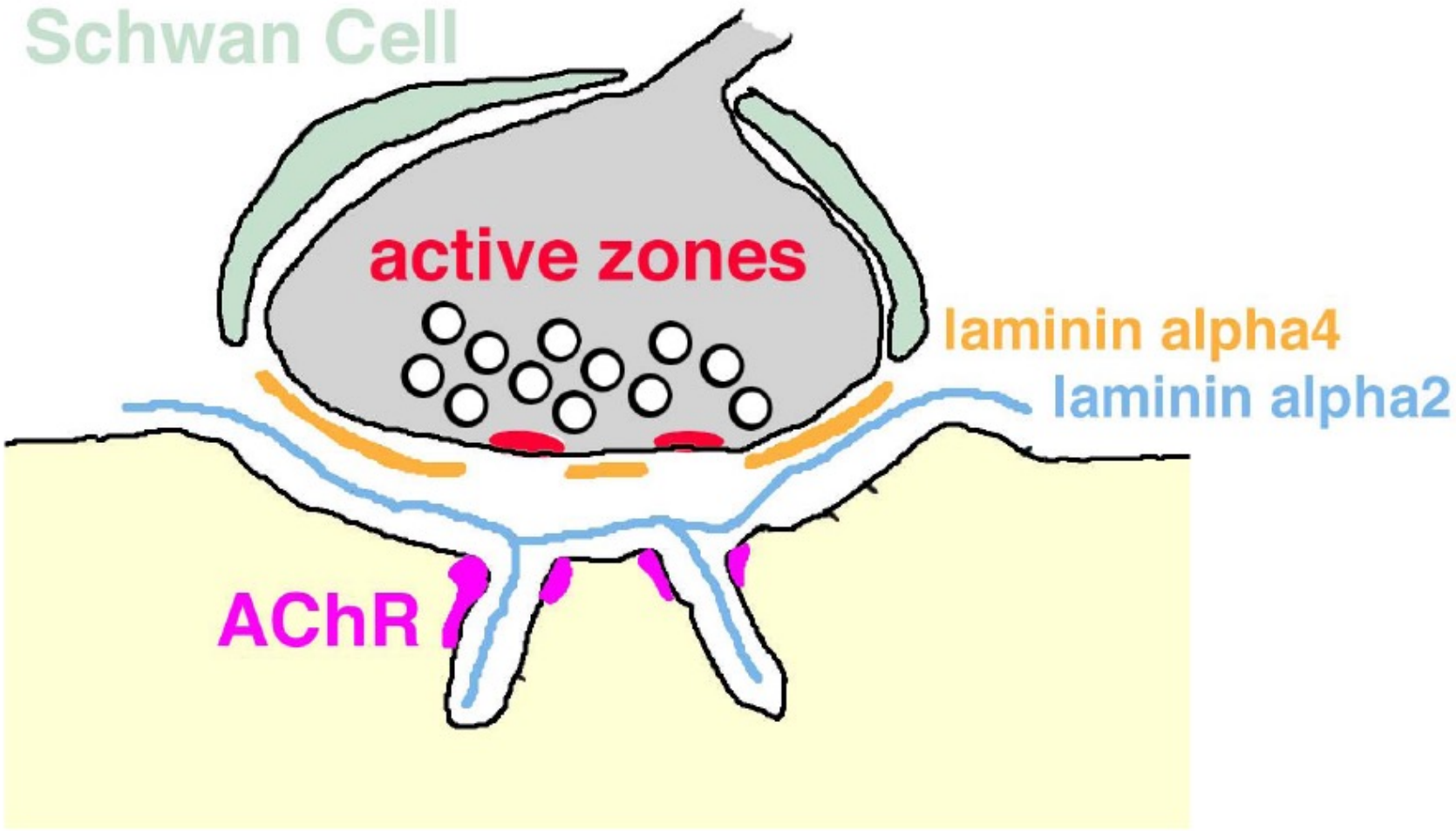
Which Laminins are Important?

3 Synaptic Laminin trimers



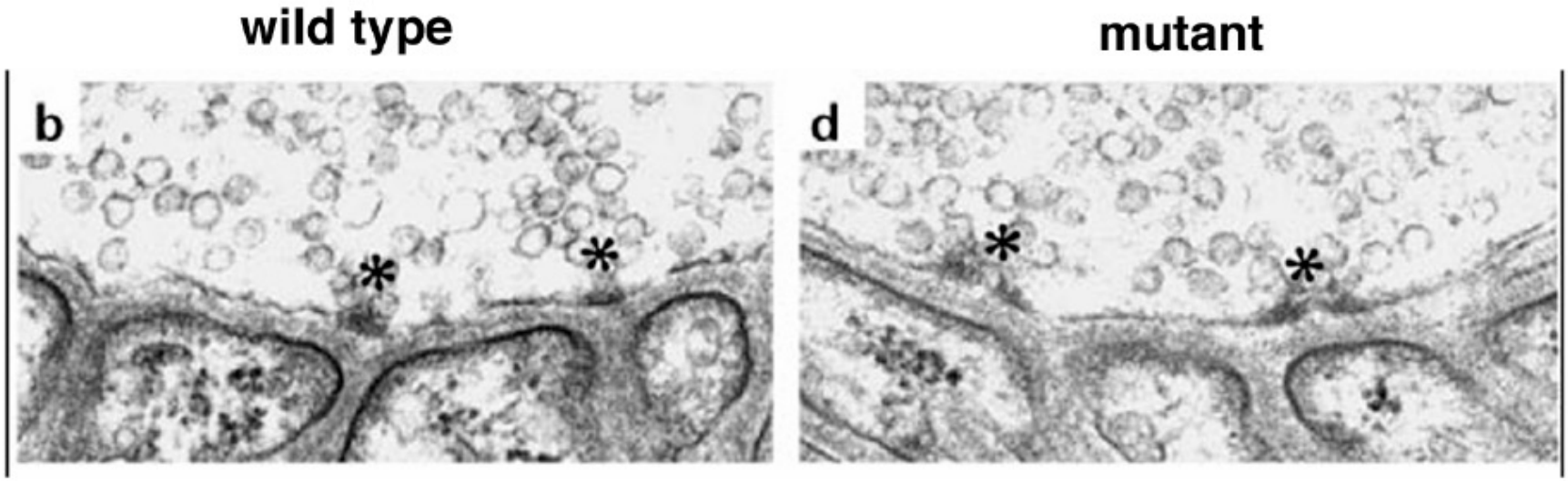
Extrasynaptic and Synaptic Laminins contain:
B1 and B2 (B2 is enriched at synaptic sites)
α2, α4 and α5 chains and only one γ chain (γ1)

Localization of laminins at the vertebrate NMJ

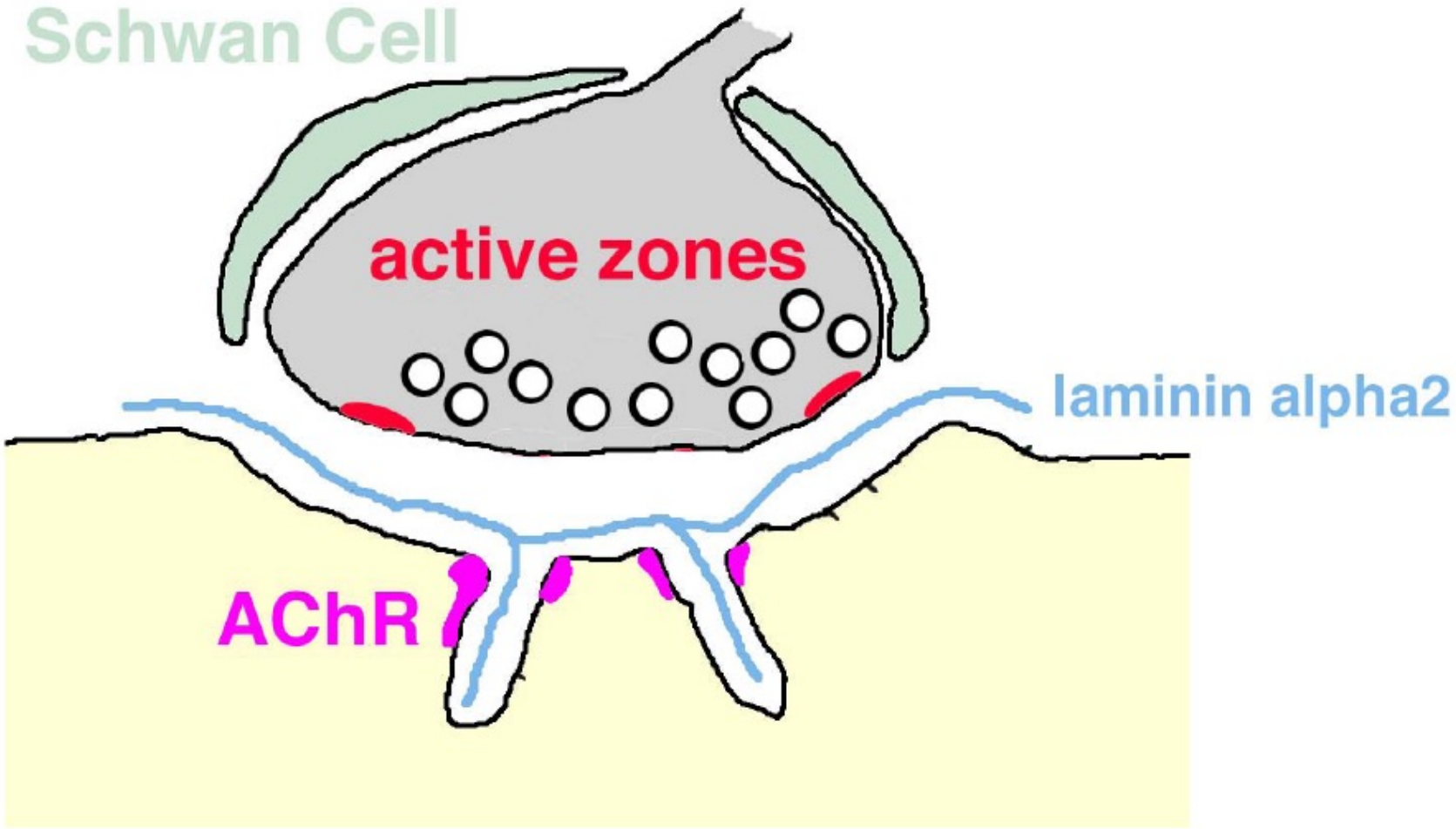


Laminin Alpha-4 KO

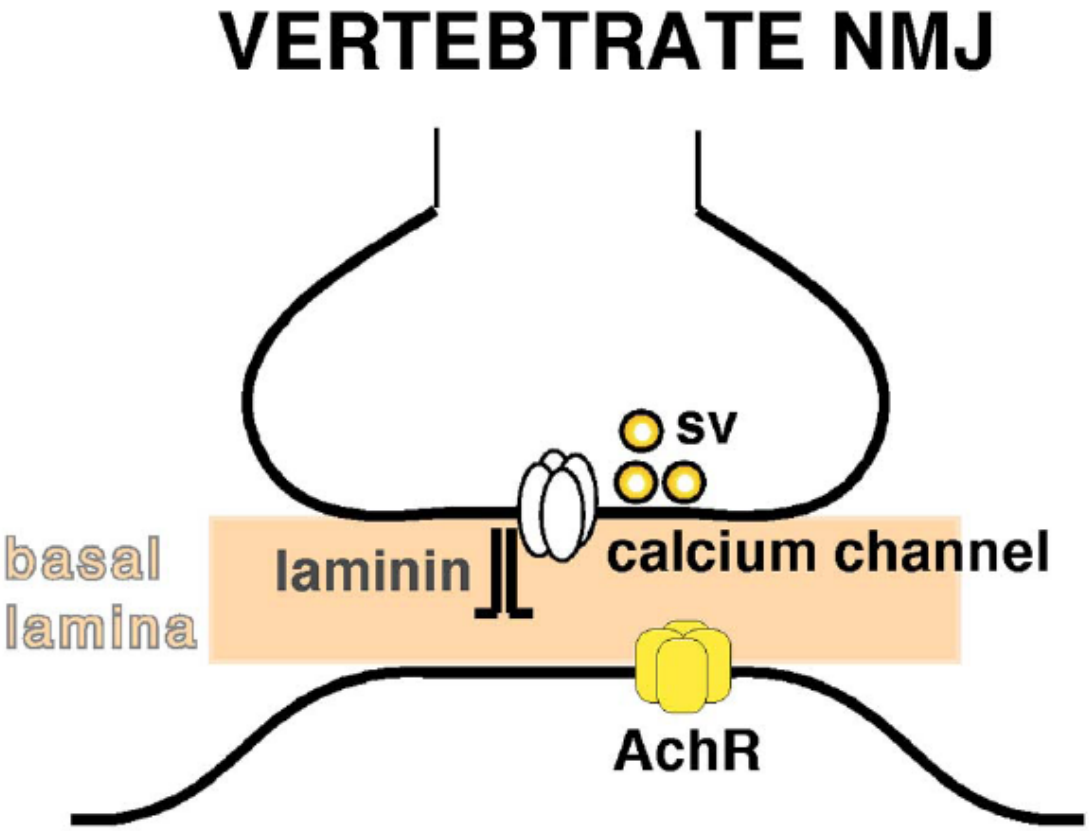
Mis-apposition of pre- and postsynaptic specializations at the NMJ of laminin mutant mice



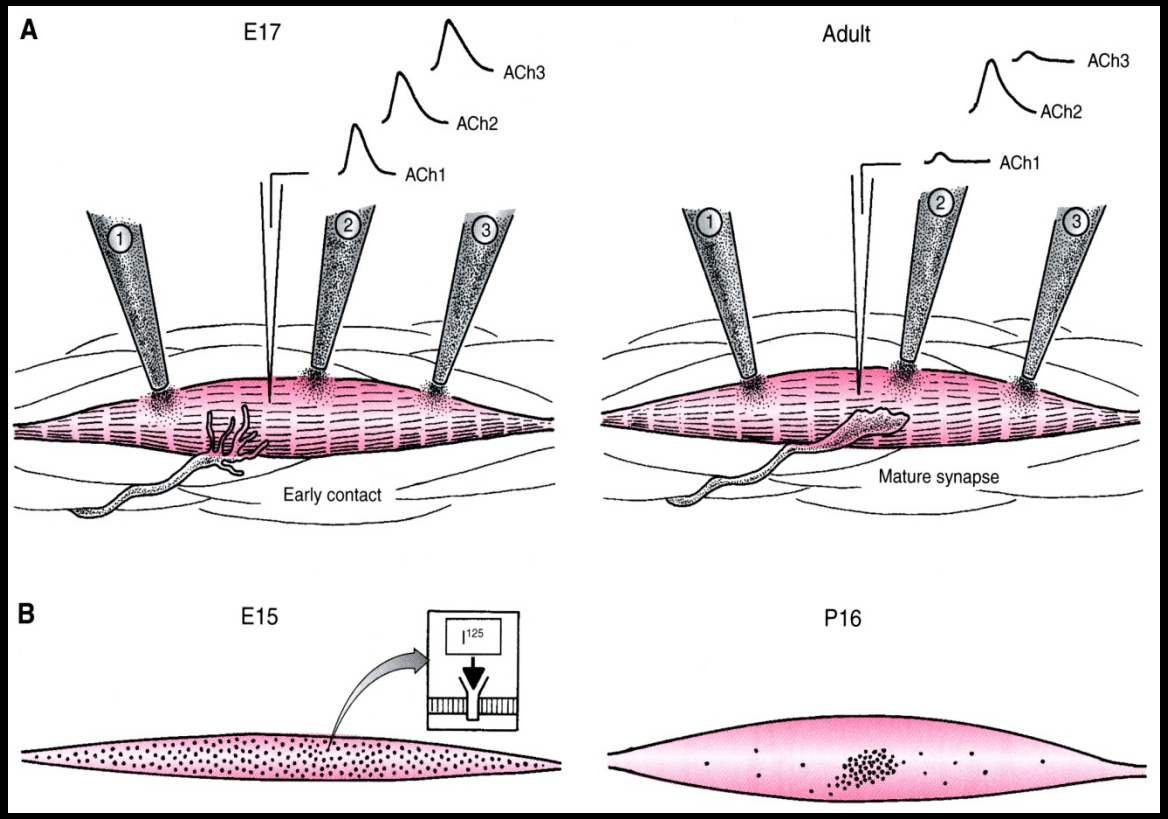
Misalignment of active zones in laminin alpha4 knockout



Linking the basal lamina to presynaptic calcium channels (alpha4, beta2, gamma1)



Postsynaptic Differentiation: AChR clusters form during development



•ACh was applied to different areas of a muscle cell while the evoked response was monitored with an intracellular pipet. At embryonic day 17 (E17), the ACh-evoked a similar response across the entire muscle surface (sites 1, 2, and 3). In adult muscle, an ACh-evoked response can only be obtained close to the synapse (site 2).

• ACh receptors were bound with radiolabeled α-bungarotoxin, and the distribution was assessed autoradiographically.At E15, the label is spread uniformly across the muscle surface, but by P16 the label is restricted to the synaptic region.

Diamond and Miledi 1962; Bevan and Steinbach 1977

Nerve Induces Postsynaptic Differentiation

Accumulation of AChRs in the Postsynaptic Membrane

-nuclei throughout newly formed myotubes express AChR subunit genes, and AChRs are diffusely distributed on the myotube surface (~1000AChRs/um^2)

-the nerve then sends 3 signals to the muscle to affect AChR distribution:

(1) **AGRIN**

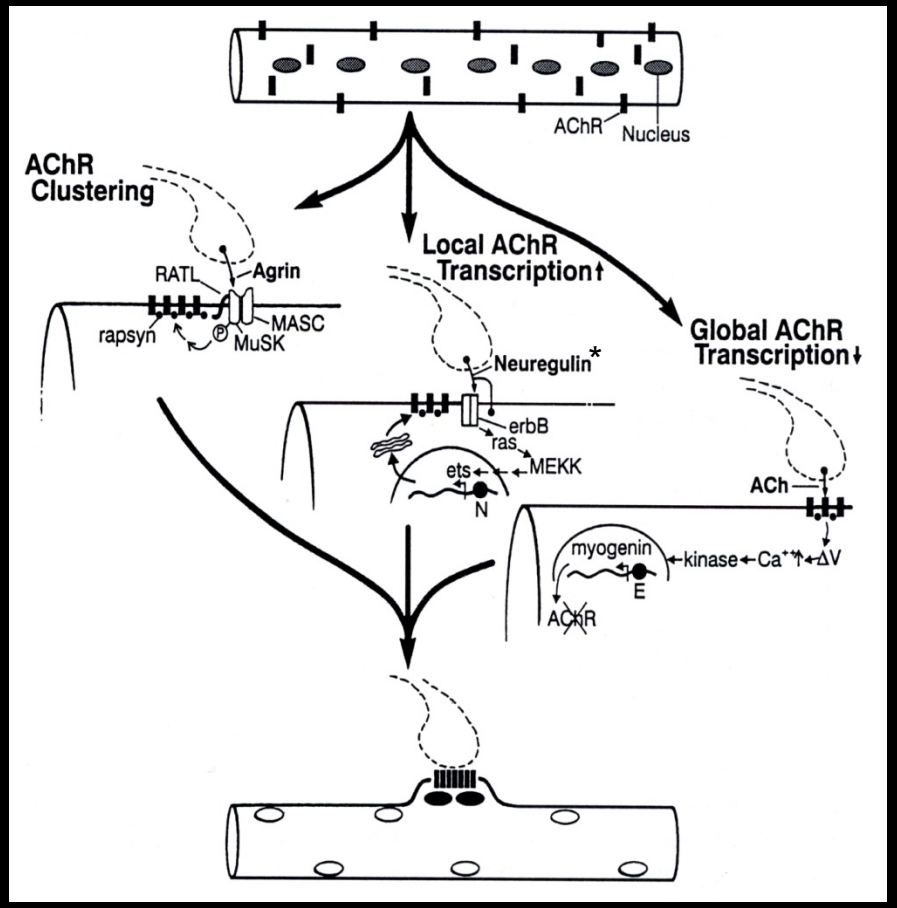
-interacts with MuSK to organize rapsyn-mediated AChR clustering

(2) **NEUREGULIN (ARIA)**

-Affects Schwaan Cell (glial cell) proliferation and survival (not shown)

(3) **ACETYLCHOLINE (ACh)**

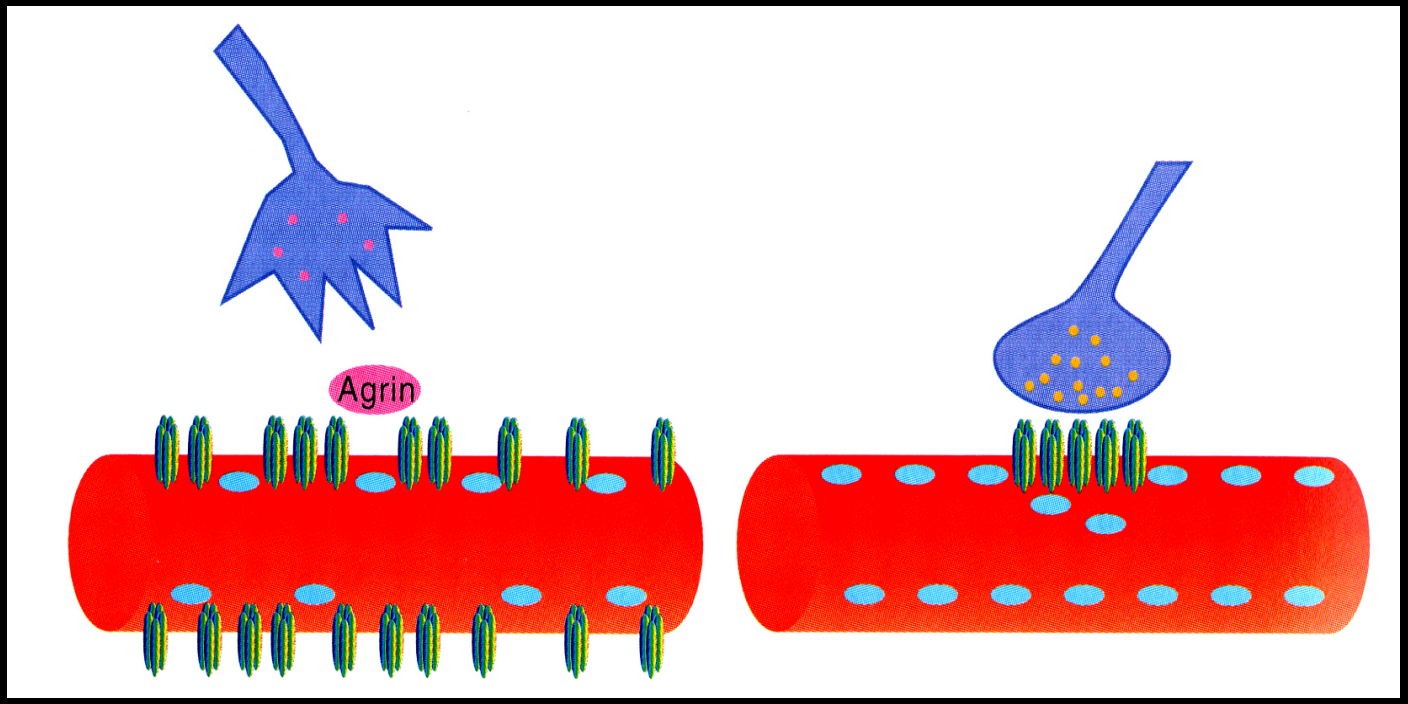
-activates AChRs to generate a voltage- and calcium-dependent signal that represses AChR subunit gene expression in extrasynaptic nuclei



**Together, these signals lead to selective synthesis of AChRs in synaptic areas and precise accumulation of AChRs in the postsynaptic membrane

Sanes and Lichtman 1999

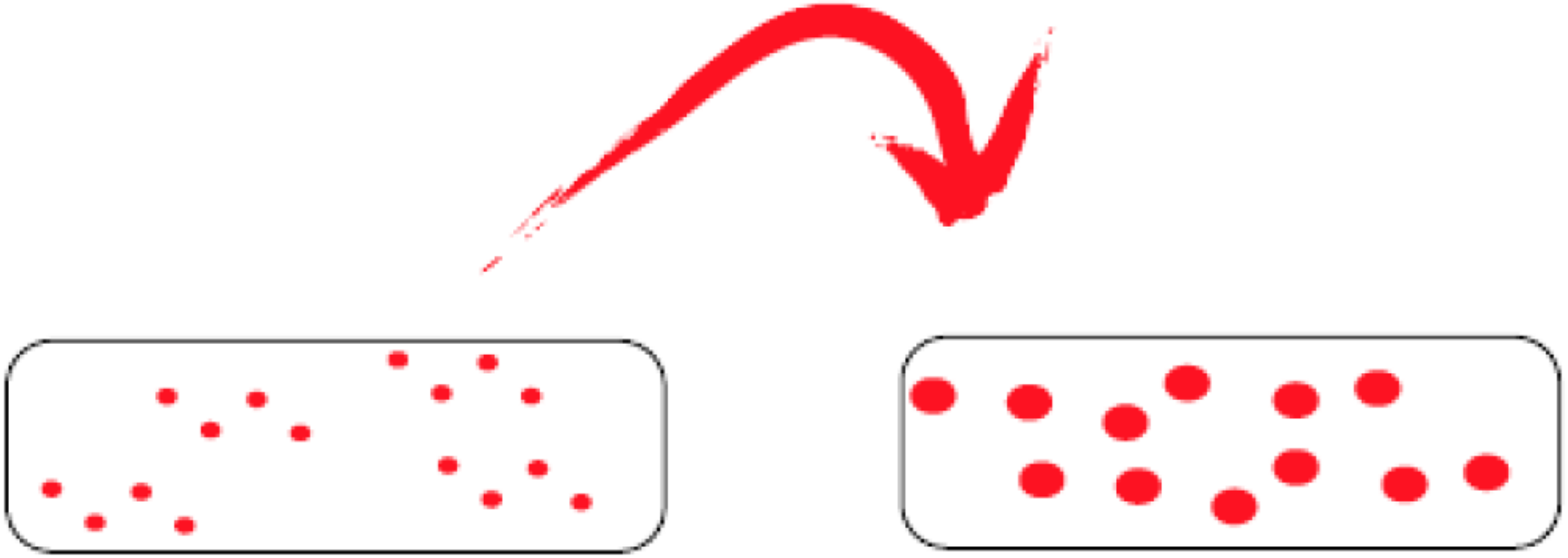
Postsynaptic Differentiation:
(1) Agrin Induces Synaptic Clustering of AChRs



Motor neurons synthesize and secrete agrin into the synaptic basal lamina.

- Before innervation, AChRs (green) are spread diffusely over the surface of the myotube.
- The release of agrin by the motor neuron results in redistribution of previously unclustered AChRs to synaptic sites, immediately adjacent to the nerve terminal.

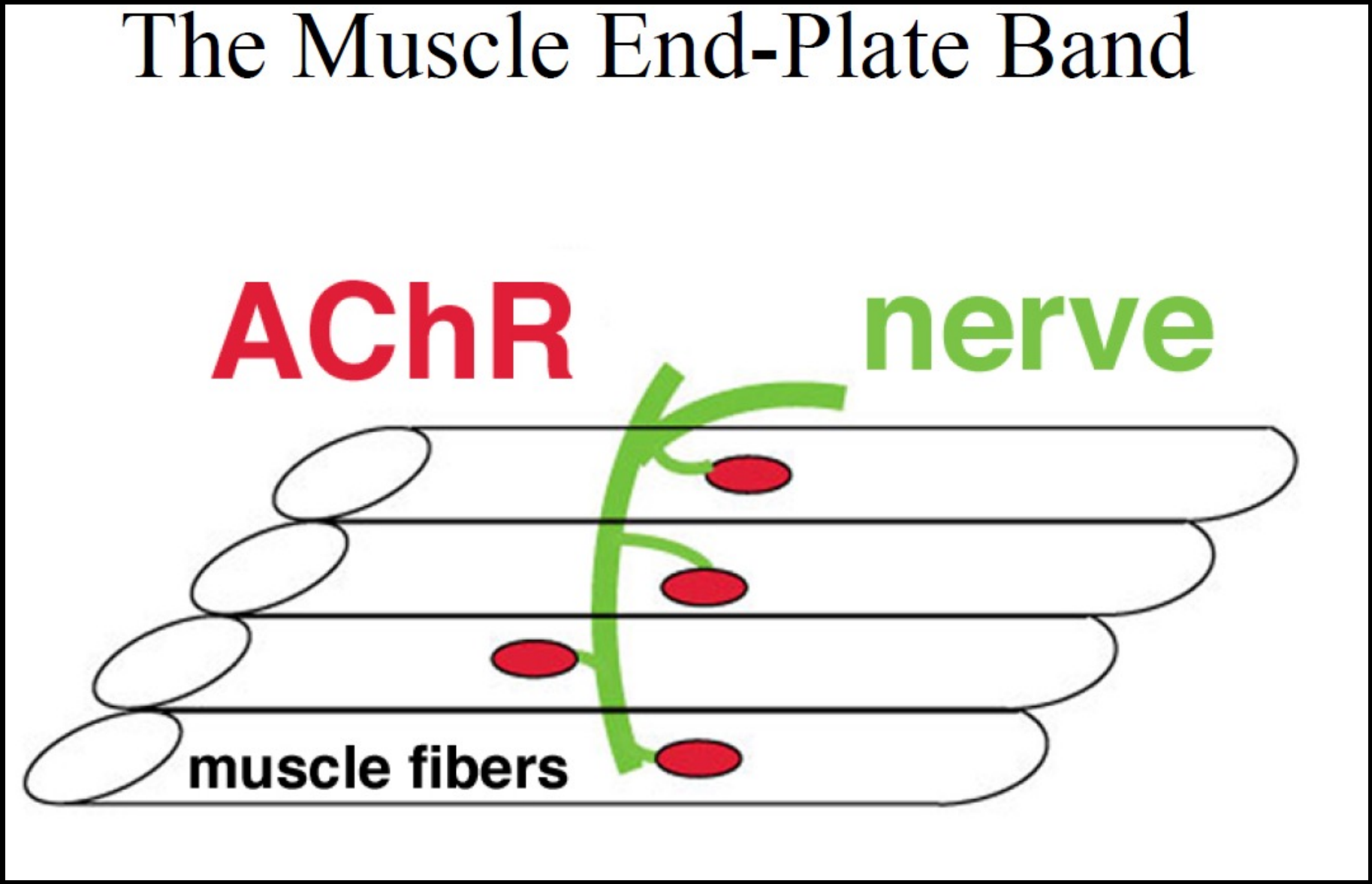
Add agrin purified from Torpedo electroplaque



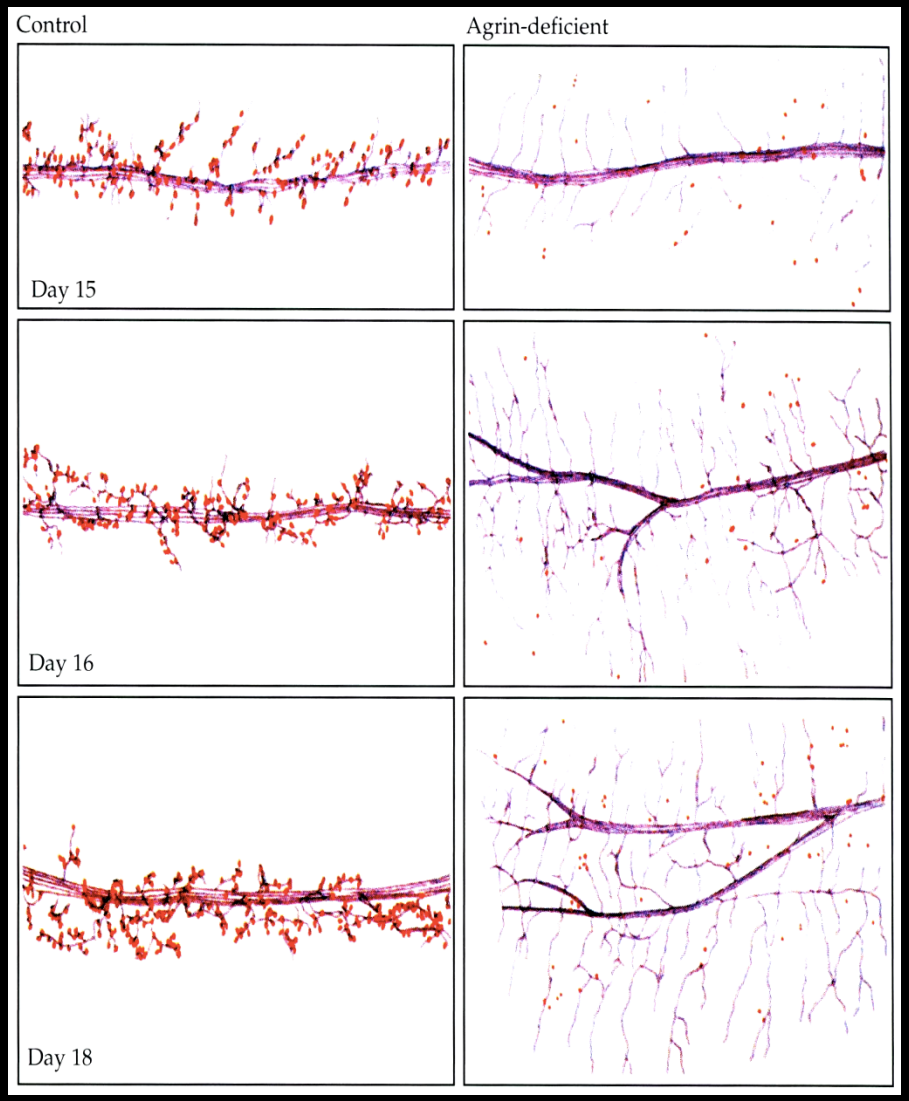
cultured myotubes
AChR clusters

Screen for increased AChR clustering

How does Agrin work? Let's look at mutant mice NMJs



Agrin Induces Synaptic Clustering of AChRs



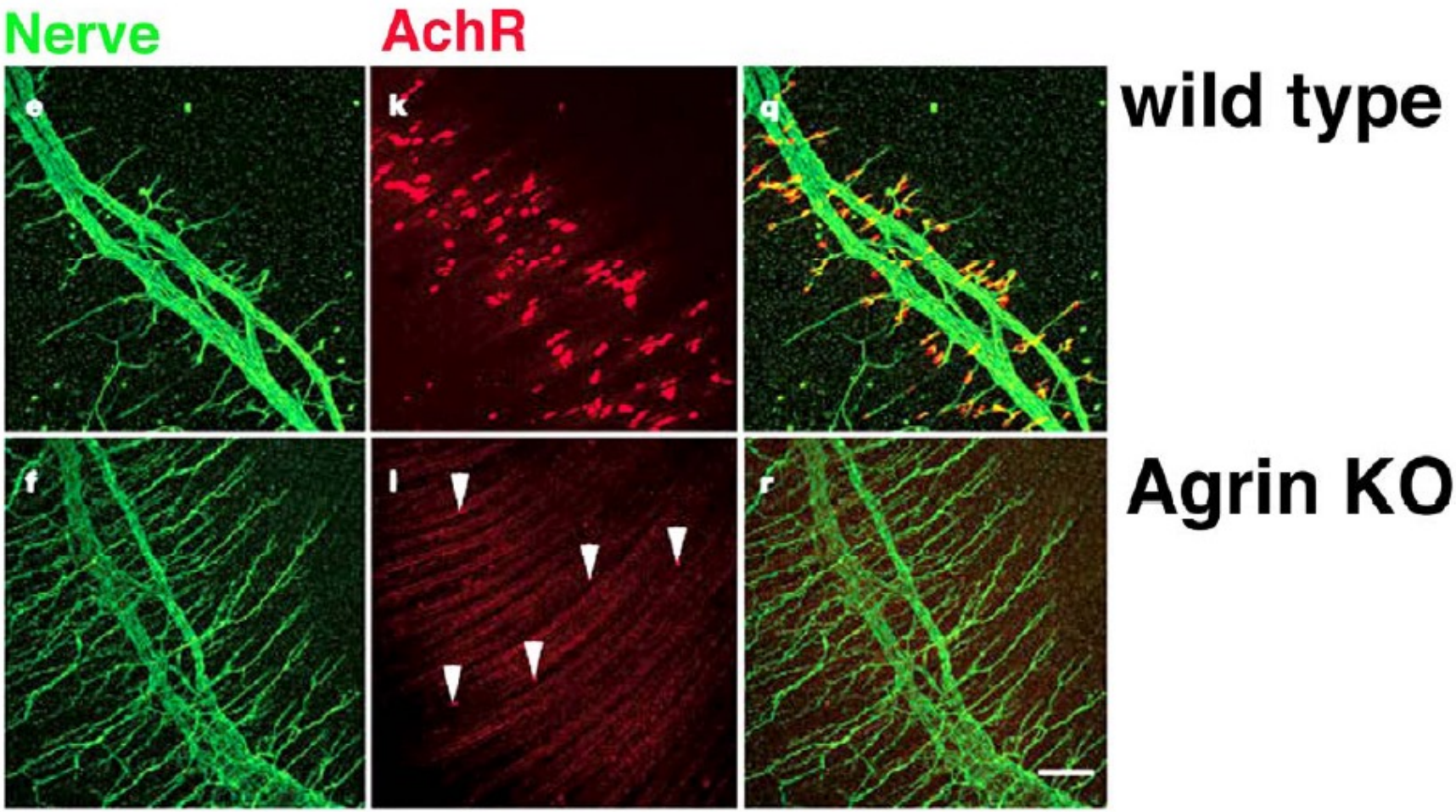
Gautam et al 1996

Development of NMJs in agrin-deficient mice

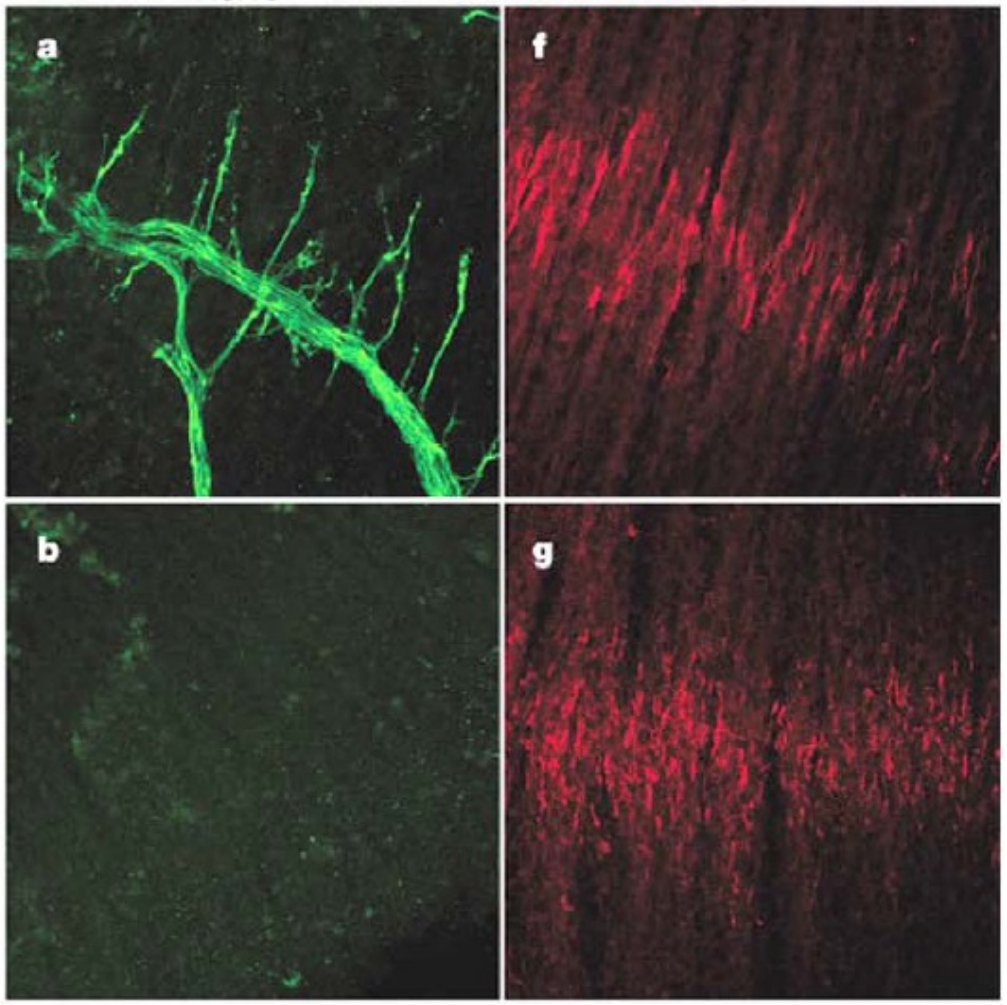
- Diaphragm muscles from control (left) and agrin-/- mice (right) at E15, 16, and 18 were double-stained for AChRs and axon, and then were drawn. The developing muscle fibers run vertically.
- In both control and mutant muscles, an intramuscular nerve (black) and aggregates of AChRs (red) are present by E15.
- In controls, axonal branches and AChR clusters are confined to a band at the central end plate at all stages.

- Mutant AChR aggregates are smaller, less dense, and less numerous; axons form fewer branches and their synaptic relationships are disorganized.

Altered AChR clustering and synapse morphology in agrin KO



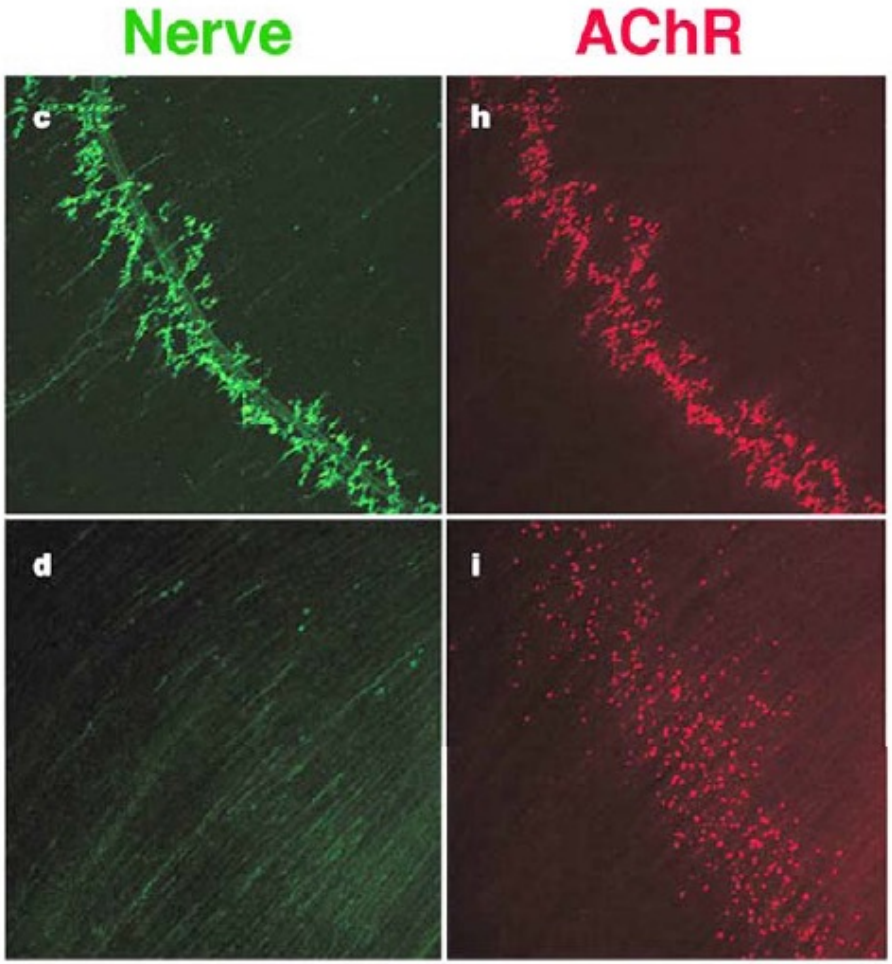
Clustering of AChRs at early stages in the absence of the nerve



wild type

NO Nerve

Clustering of AChRs is initiated in the absence of the nerve



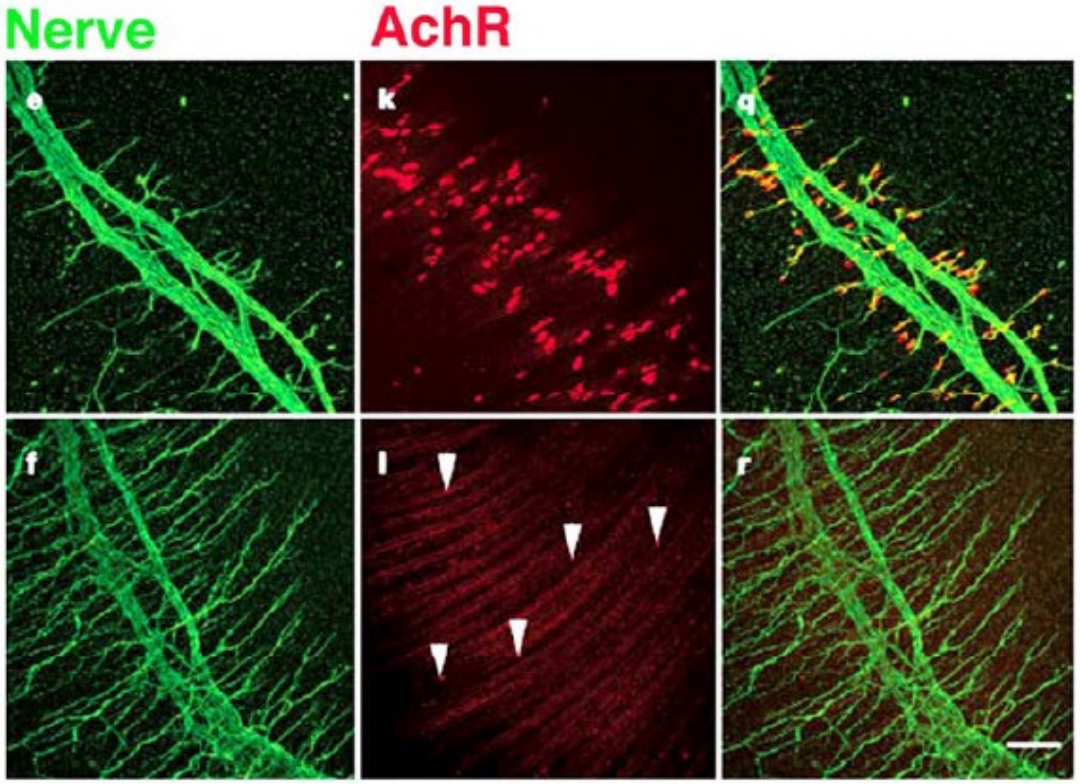
WILD TYPE

Clusters of AChRs at NMJ

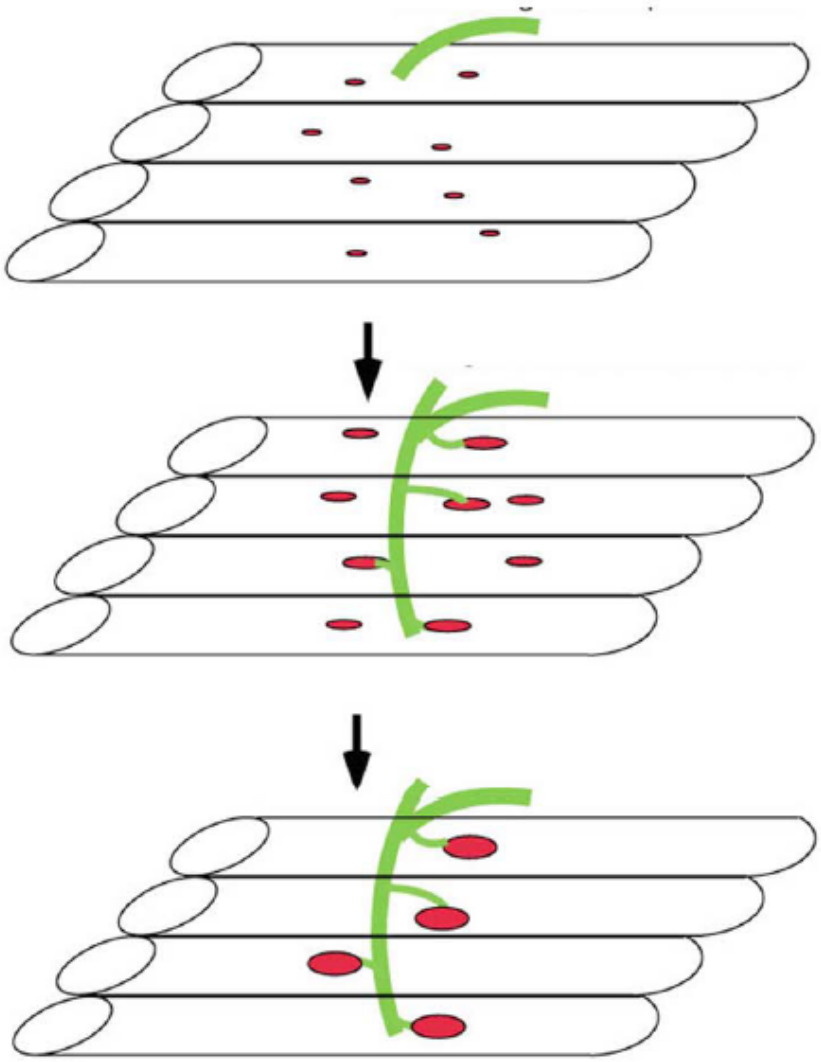
NO NERVE PRESENT

AChR clusters persist in the absence of the nerve

AchR clusters are abolished in the presence of the nerve, but the absence of Agrin



Summary: Agrin Hypothesis Revisited



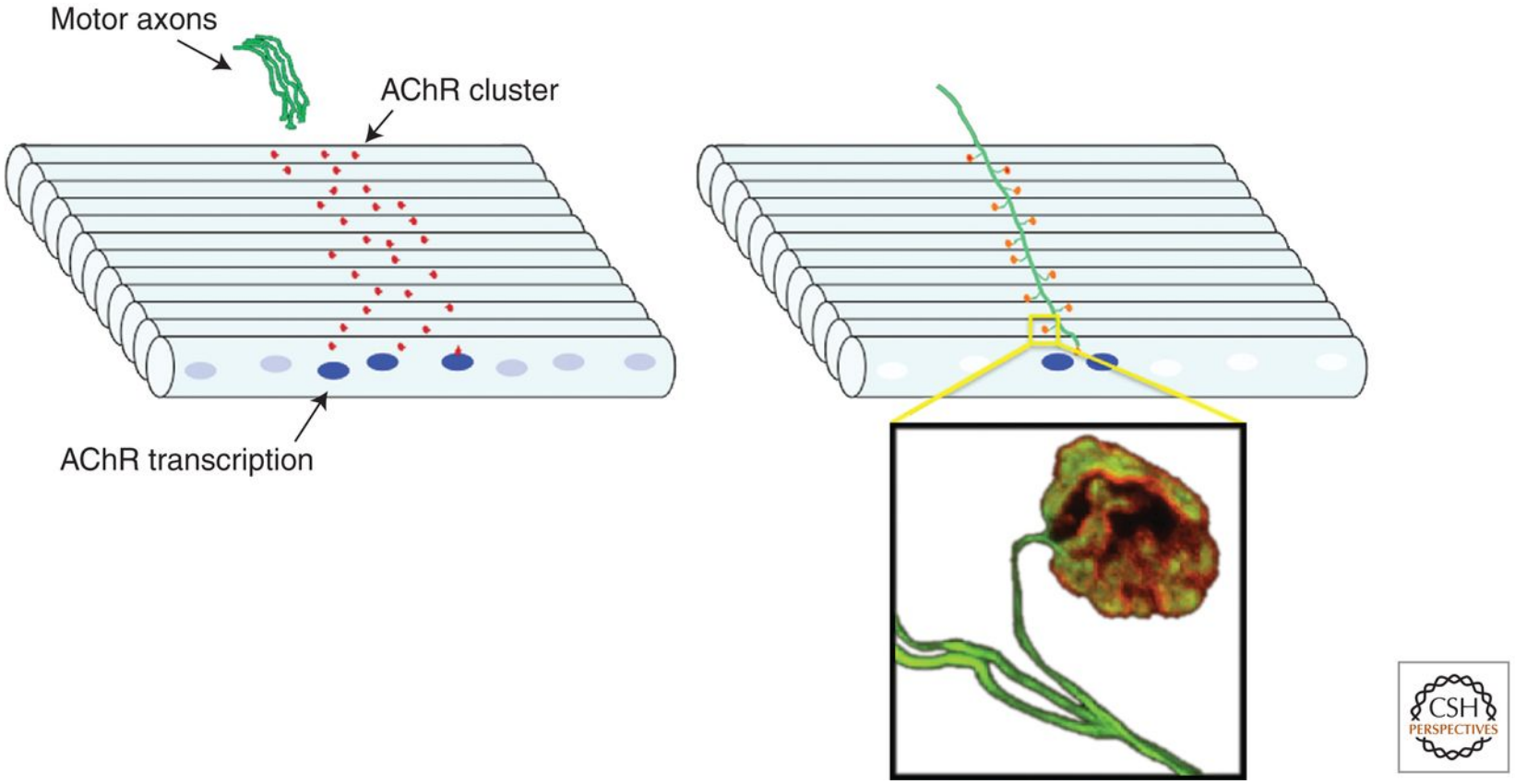
1. Muscle pre-patterning of AChRs (requires MuSK)

2. Agrin/MuSK-dependent stabilization and growth of AChR clusters in endplate region

3. Dispersion of aneural AChR clusters (nerve-dependent)

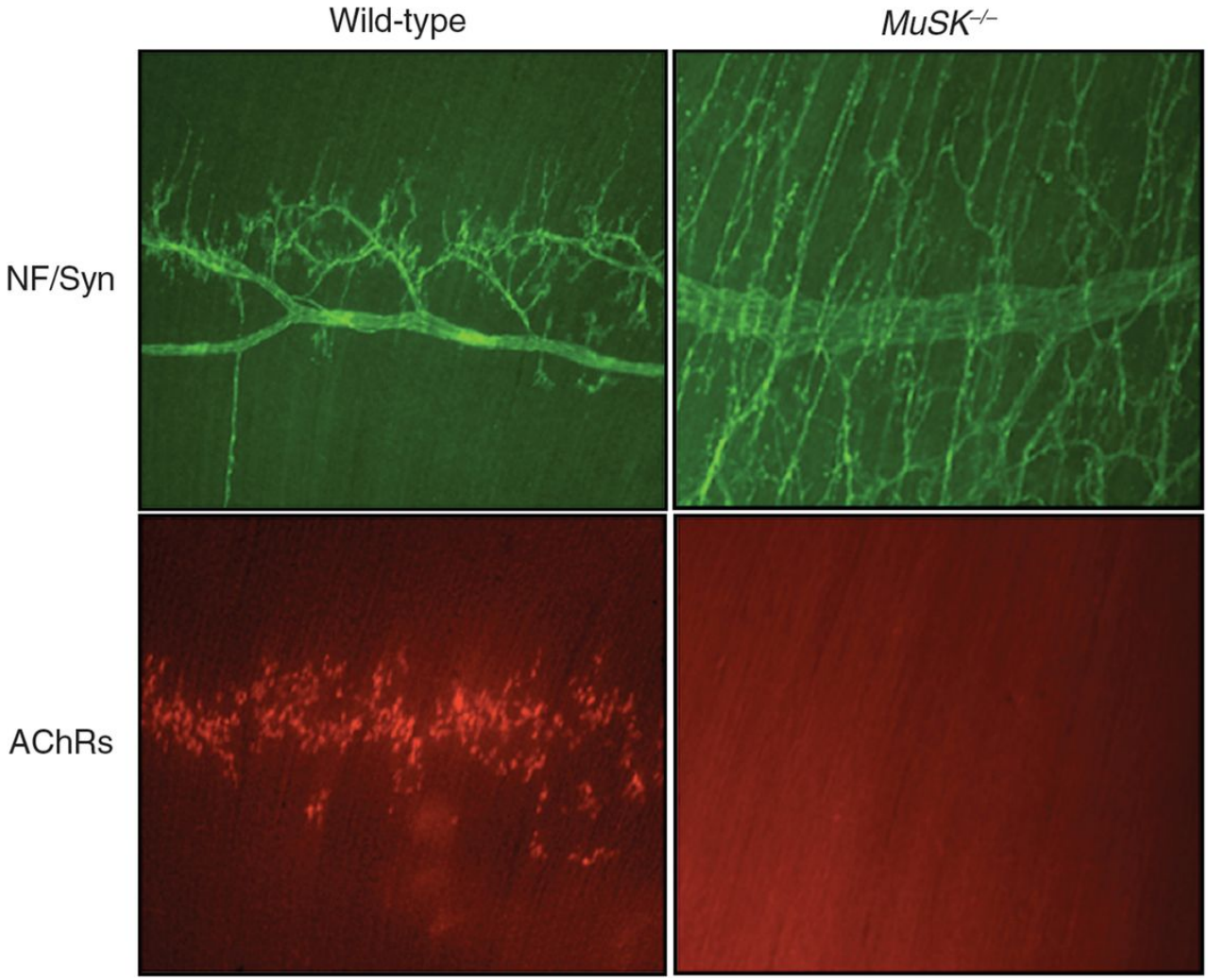
Ideas for establishment of pre-pattern?

During development, motor axons (green) approach muscle in which AChR gene expression (blue) and clustering of AChRs (red) is enhanced in the prospective synaptic region of muscle.



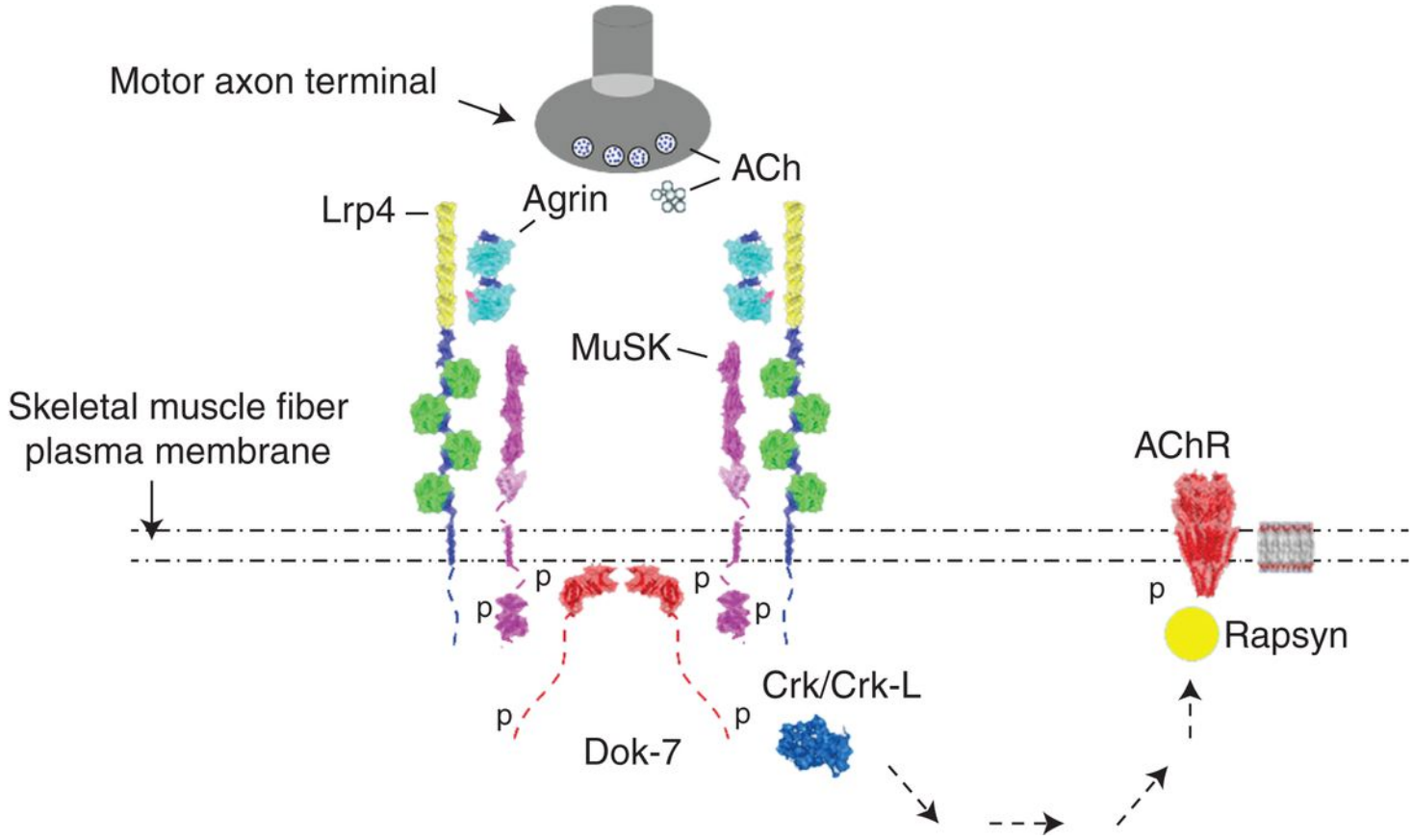
Burden S J et al. Cold Spring Harb Perspect Biol 2013;5:a009167

In the absence of MuSK, AChRs (red) fail to cluster and motor axons (green) fail to stop and differentiate.

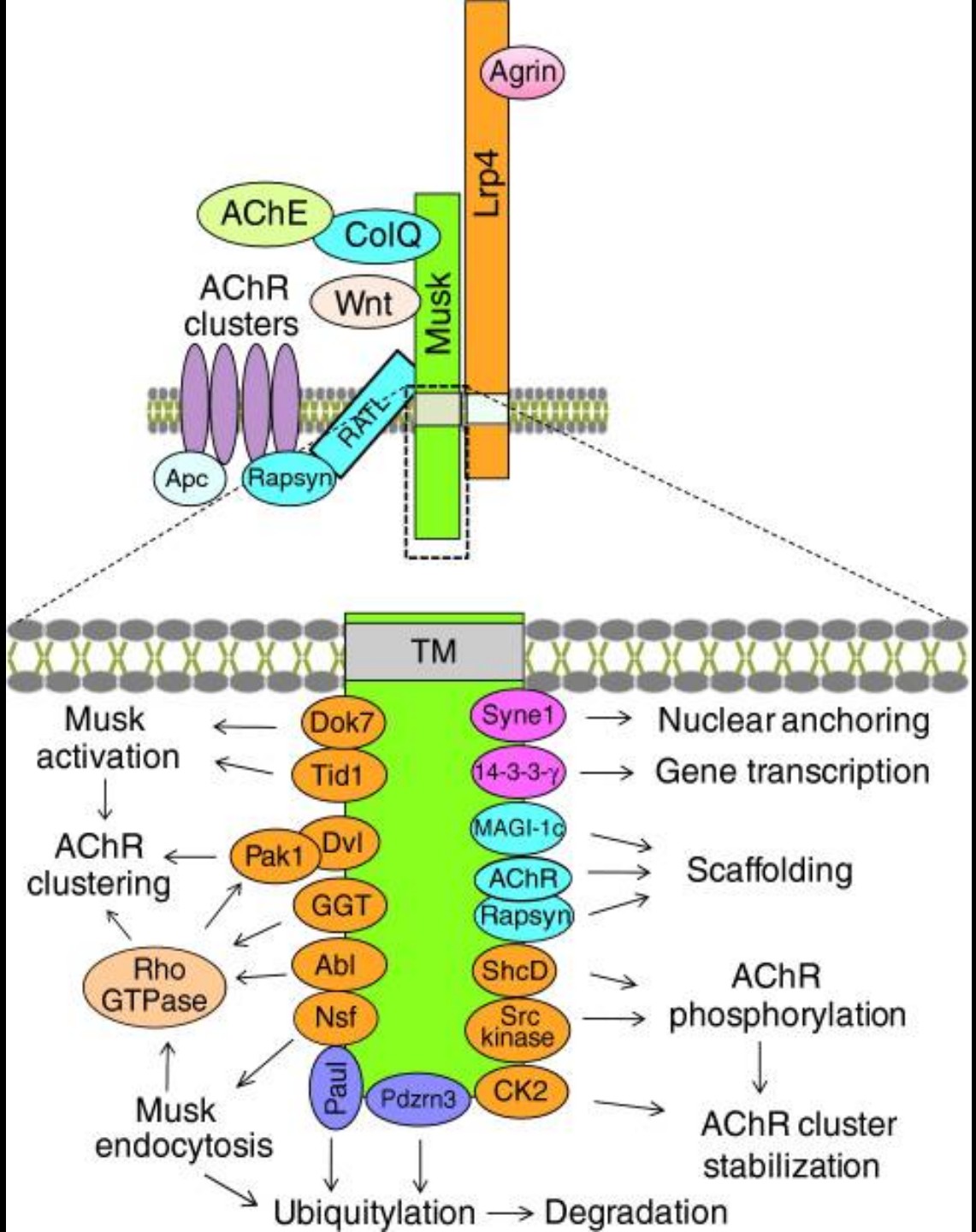


Burden S J et al. Cold Spring Harb Perspect Biol 2013;5:a009167

Motor axons release Agrin and ACh. Agrin binds to Lrp4, which stimulates association between Lrp4 and MuSK and MuSK phosphorylation.

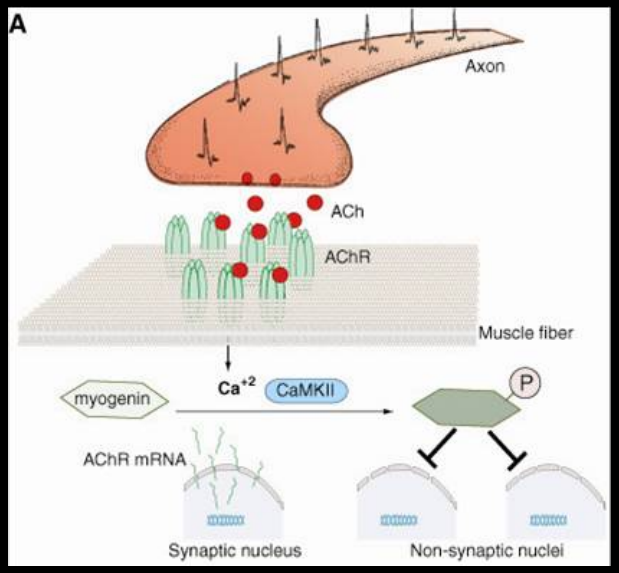


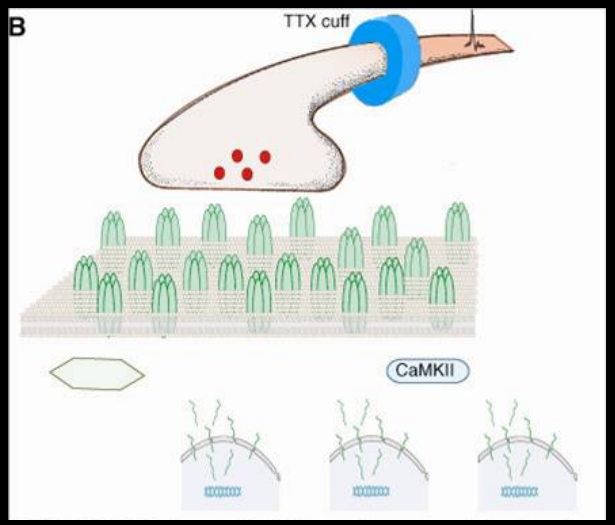
Burden S J et al. Cold Spring Harb Perspect Biol 2013;5:a009167



Wu et al.
Development 2010

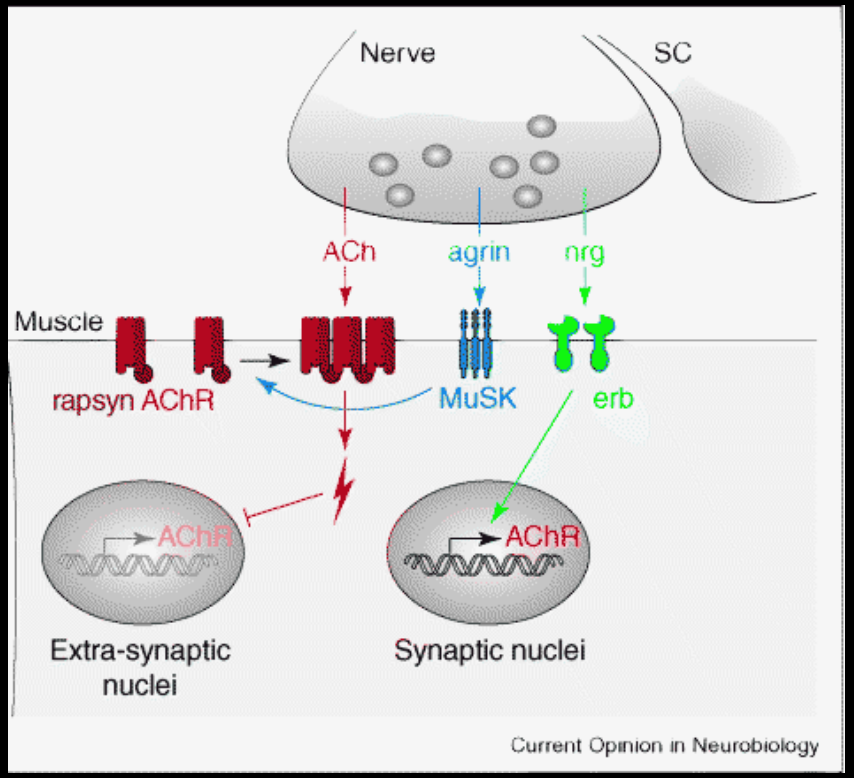
Postsynaptic Differentiation:
(3) ACh decreases transcription of extrasynaptic AChRs



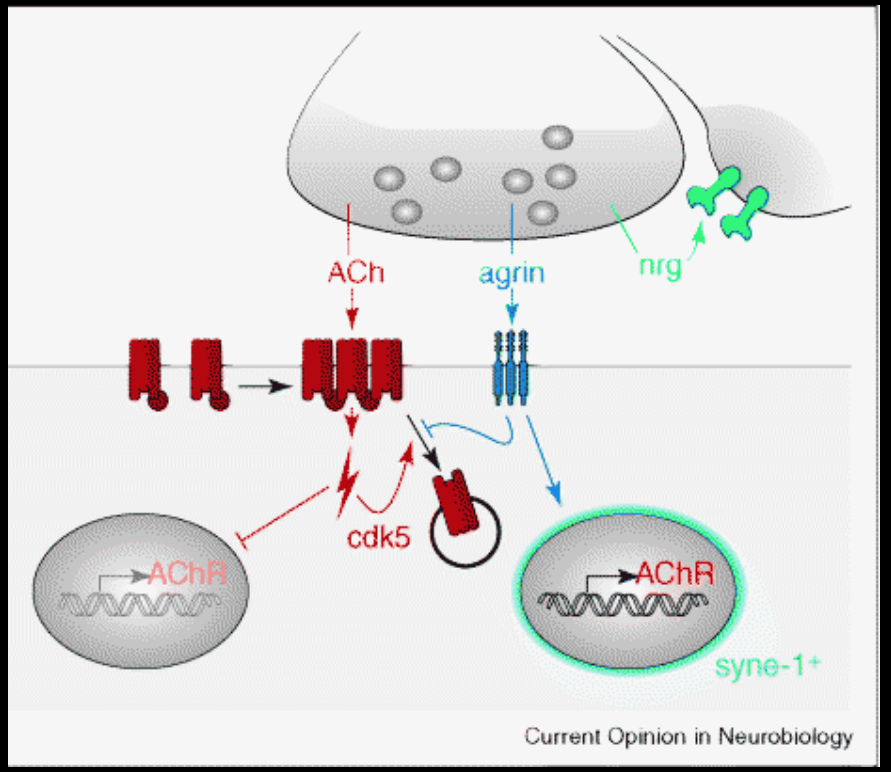


Extrasynaptic ACh receptors accumulate when the nerve is inactive. A. At the control nerve–muscle junction, the electrically active terminal releases ACh and the receptors are clustered at the postsynaptic membrane. The activity-dependent signal that suppresses extrajunctional receptors involves calcium influx and activation of the calcium calmodulin-dependent protein kinase II (CamKII). A transcription factor found in muscle (myogenin) is phosphorylated and blocks transcription in extrasynatpic nuclei. B. When motor axon activity is blocked with the sodium channel blocker, tetrodotoxin (TTX), extrajunctional ACh receptors are distributed over the entire muscle surface. (Adapted from Lømo and Rosenthal, 1972)

Revised View of NMJ Formation



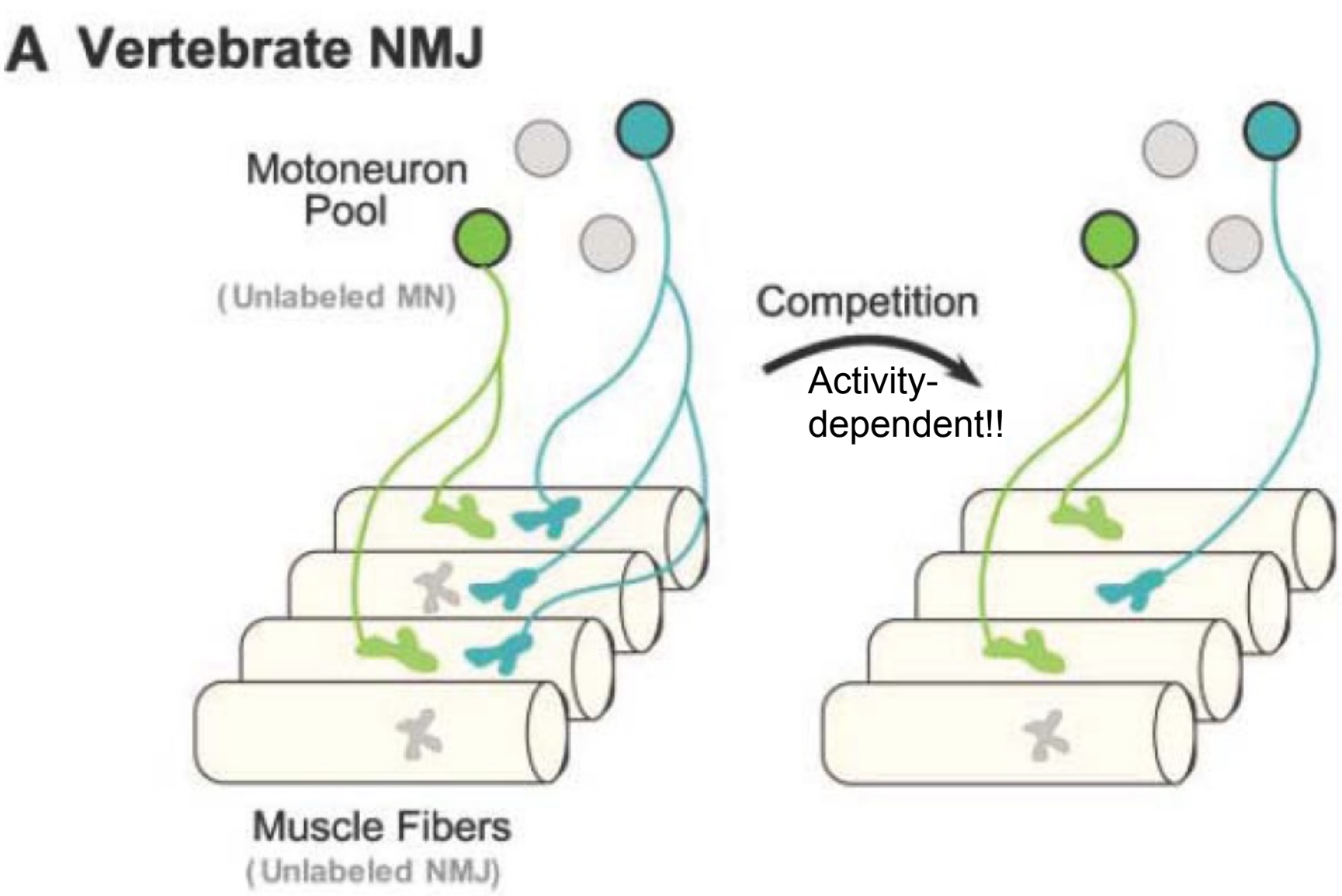
1990 Model

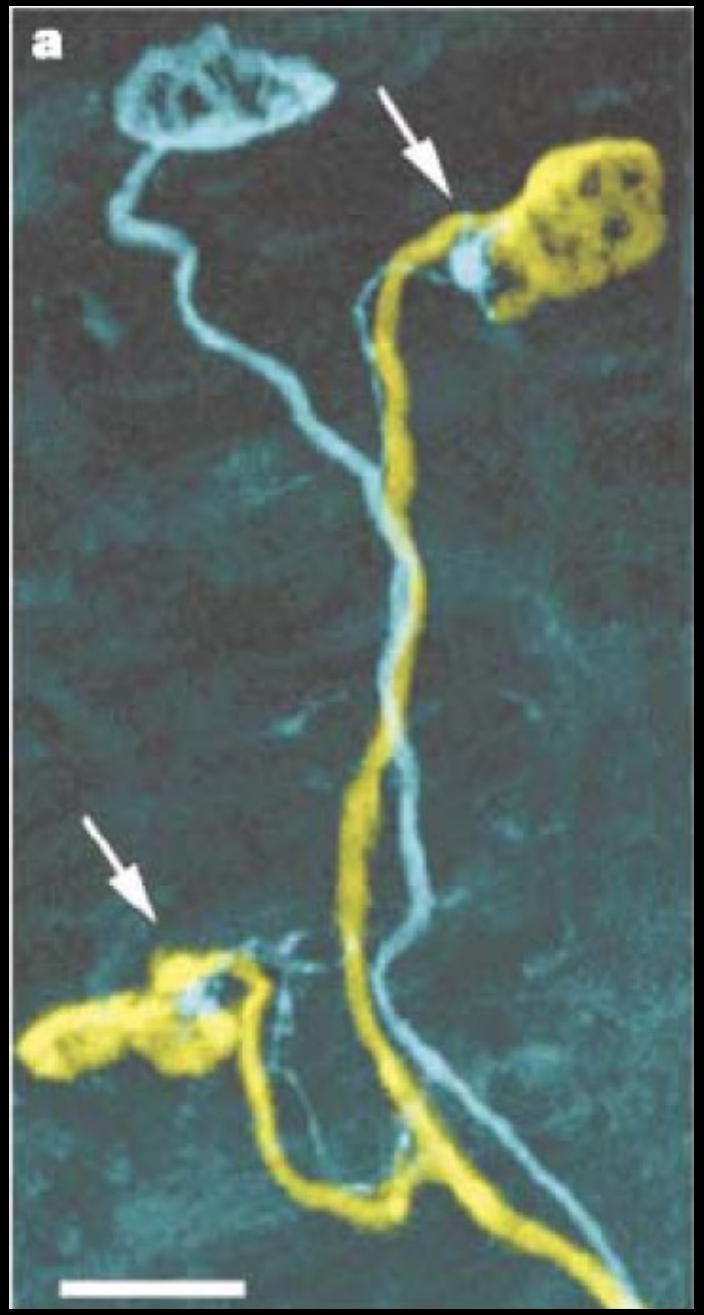


Revised Model

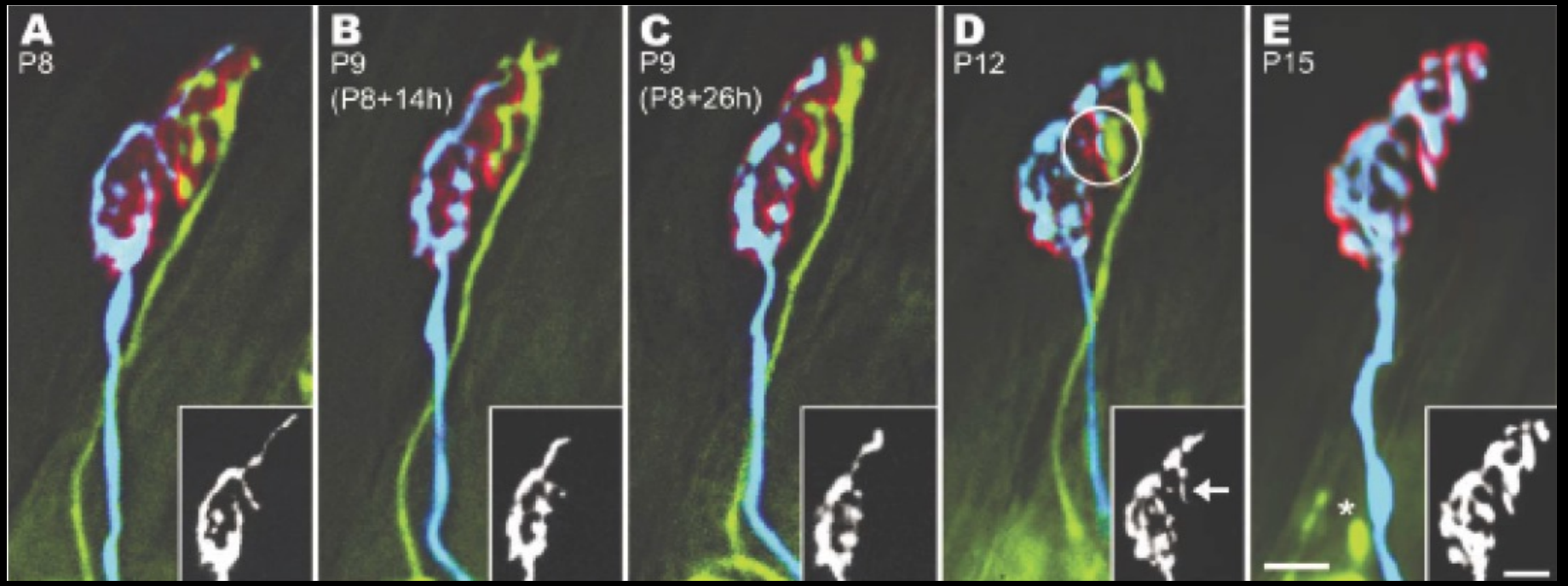
Synaptic Competition during synapse formation

Synaptic Competition at the Vertebrate NMJ

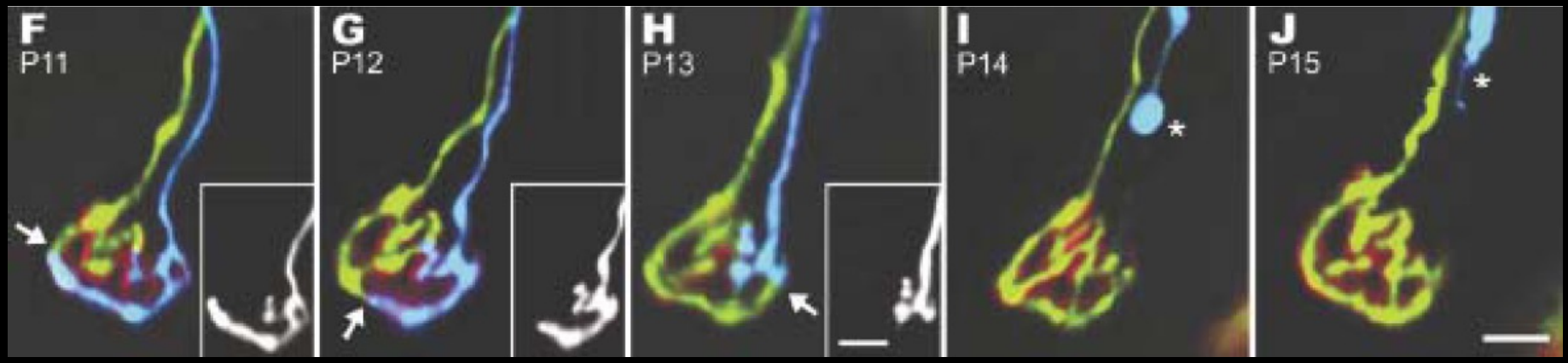



a

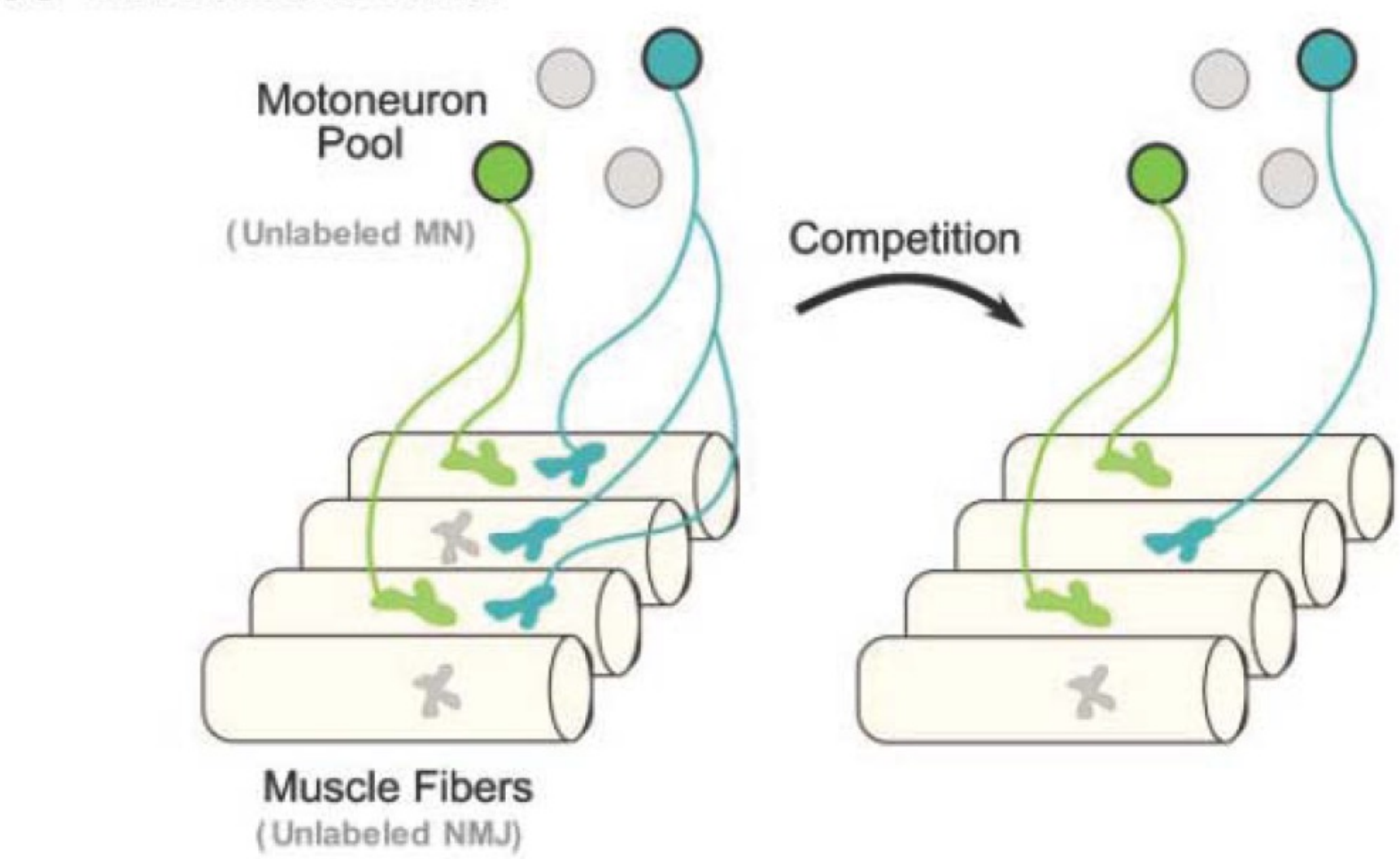
Presynaptic terminals fight over the same postsynaptic space

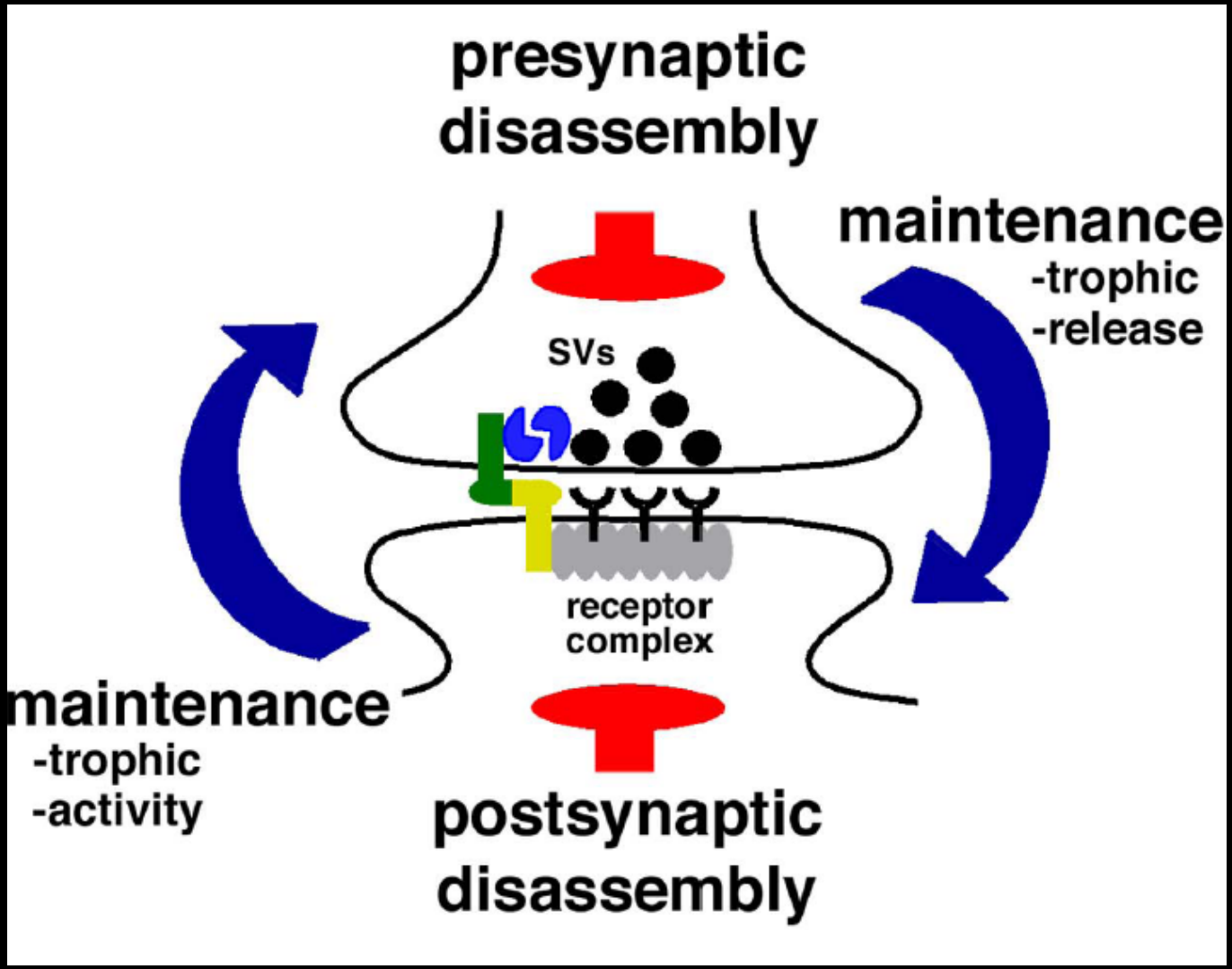


The losing axon retracts



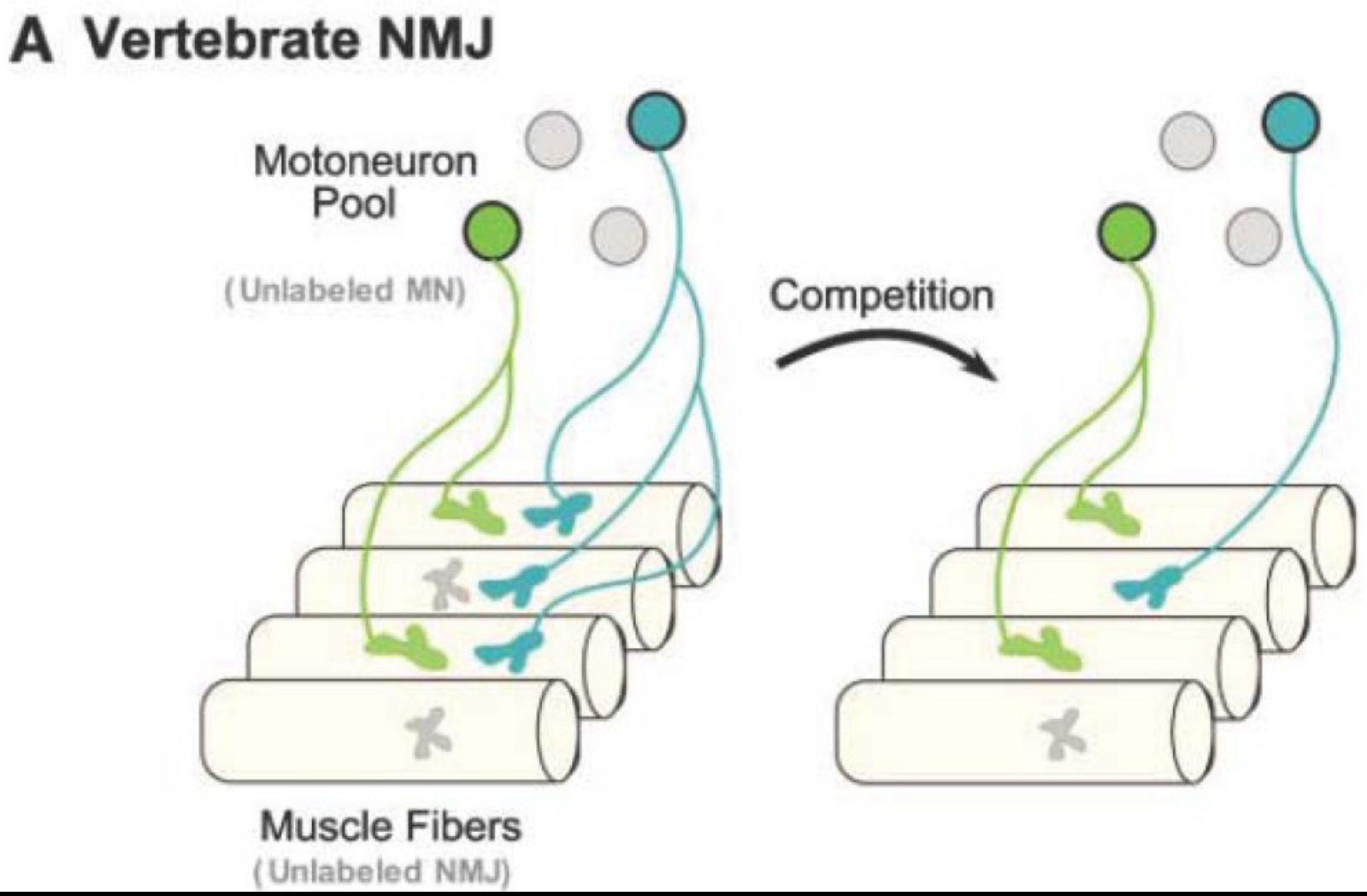
Input Elimination:
Synapse Disassembly is Necessary for Competition

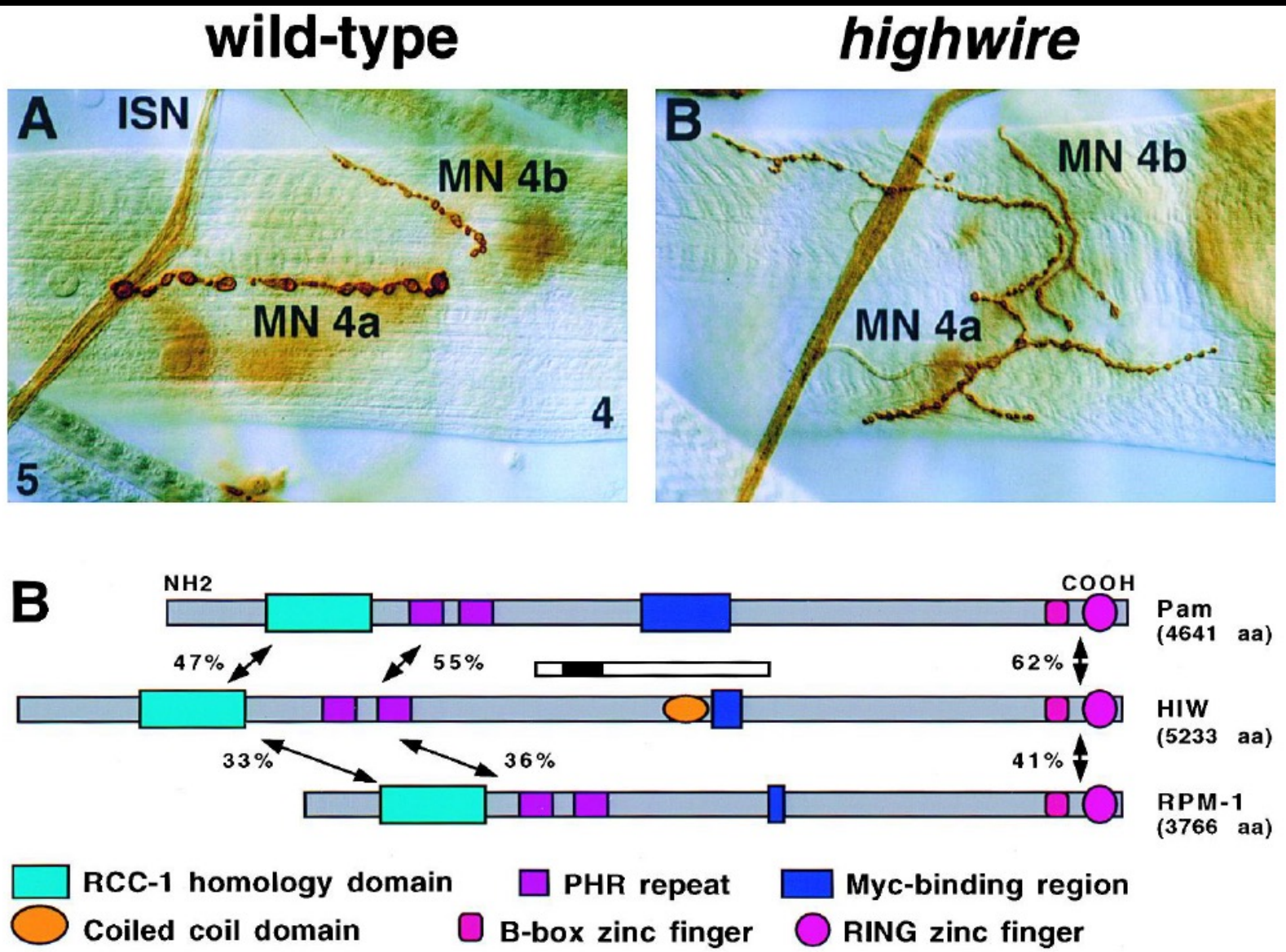



presynaptic
disassembly
maintenance
-trophic
-release
SVs
receptor
complex
maintenance
-trophic
-activity
postsynaptic
disassembly

During Synaptic Competition, a MN with Larger Territory is at a Competitive Disadvantage: Consequences for Growth and Synapse Assembly/Disassembly

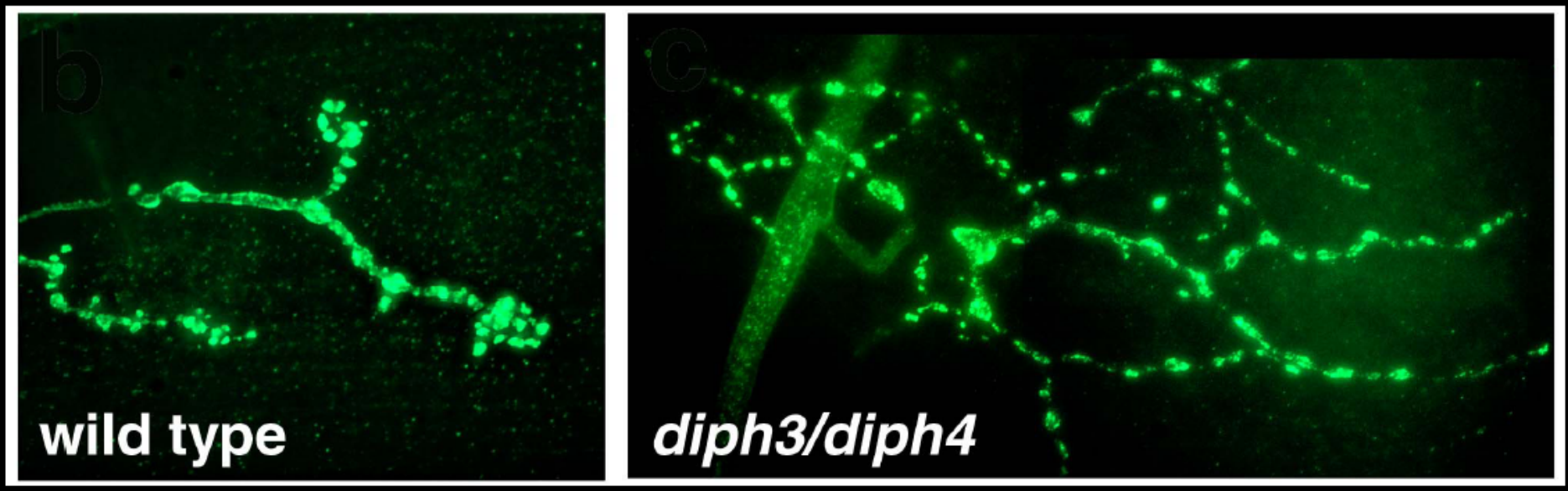
Why would that be?

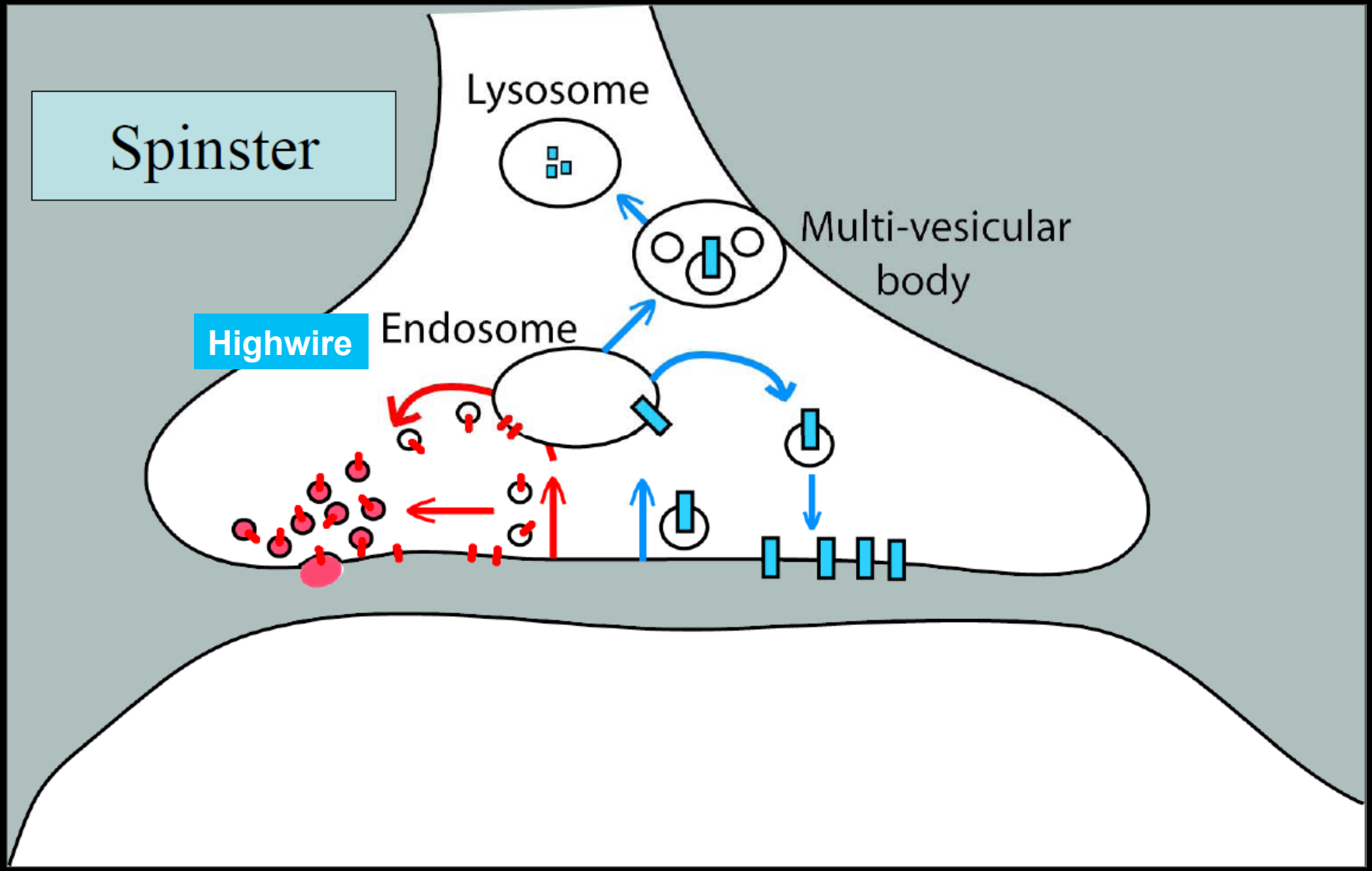




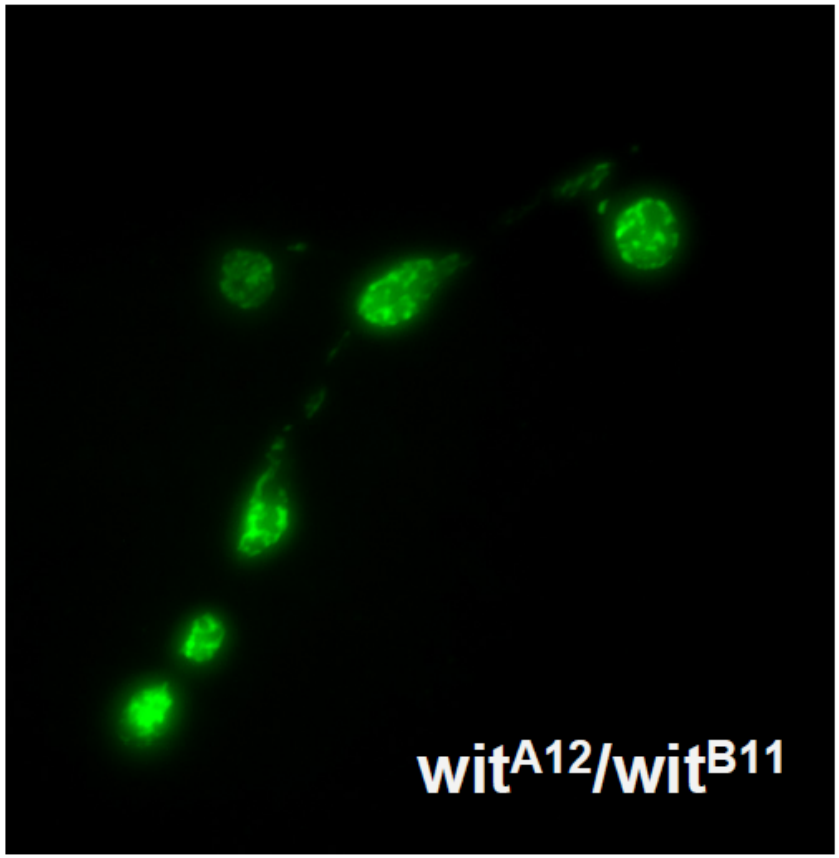
Wan et al., (2000) Neuron 26; 313-329

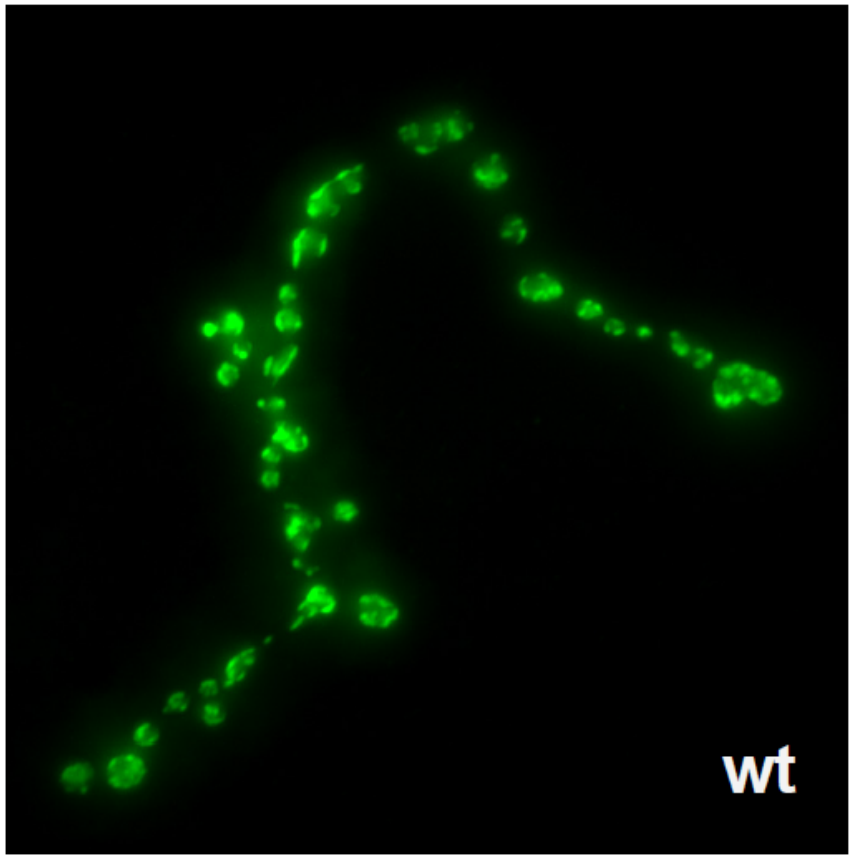
spinster controls synapse growth




Spinster
Lysosome
Multi-vesicular body
Highwire
Endosome

The TGF-ß type-II Receptor *wishful thinking* (*wit*) is Necessary for Normal Synaptic Growth

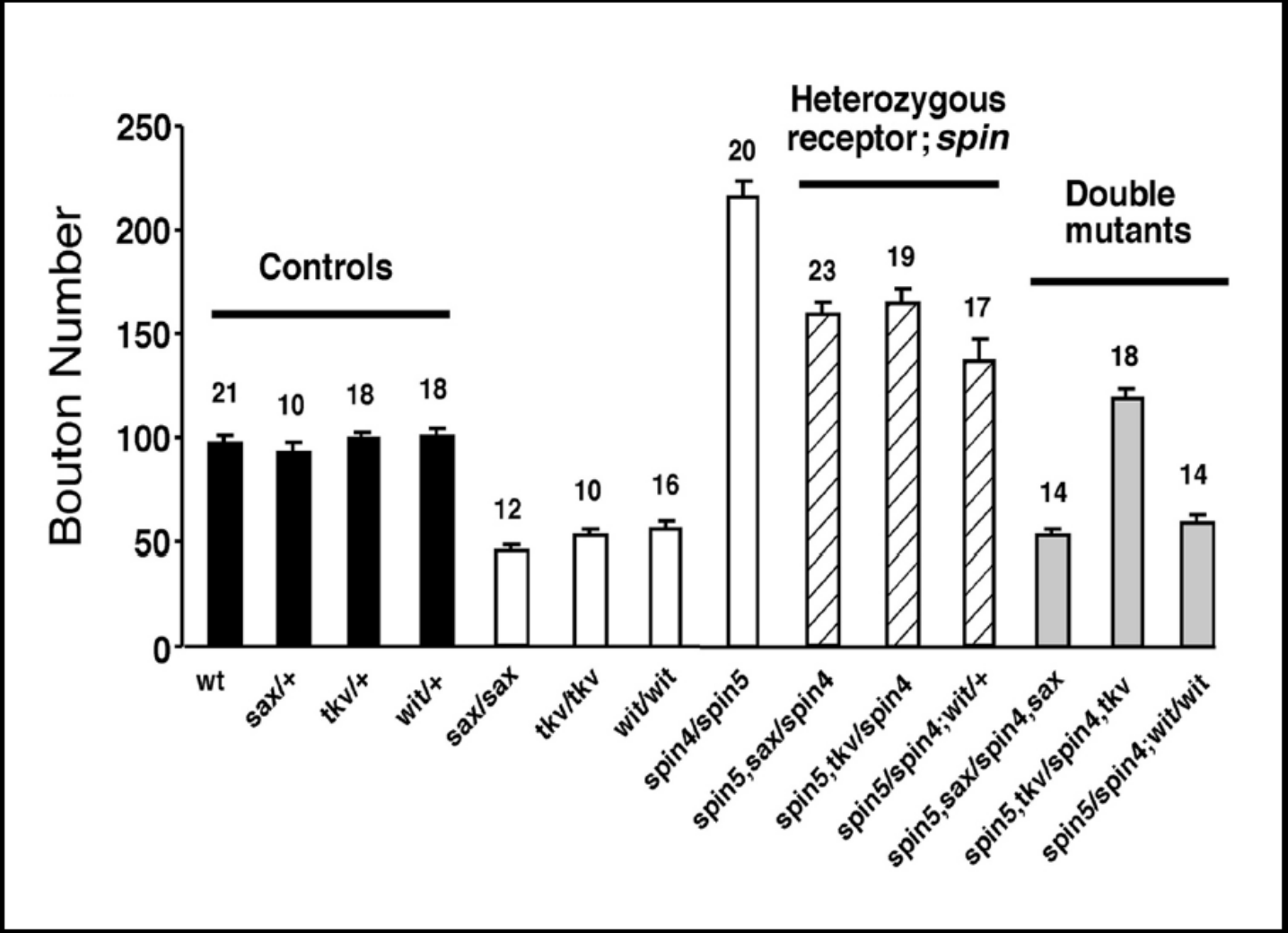




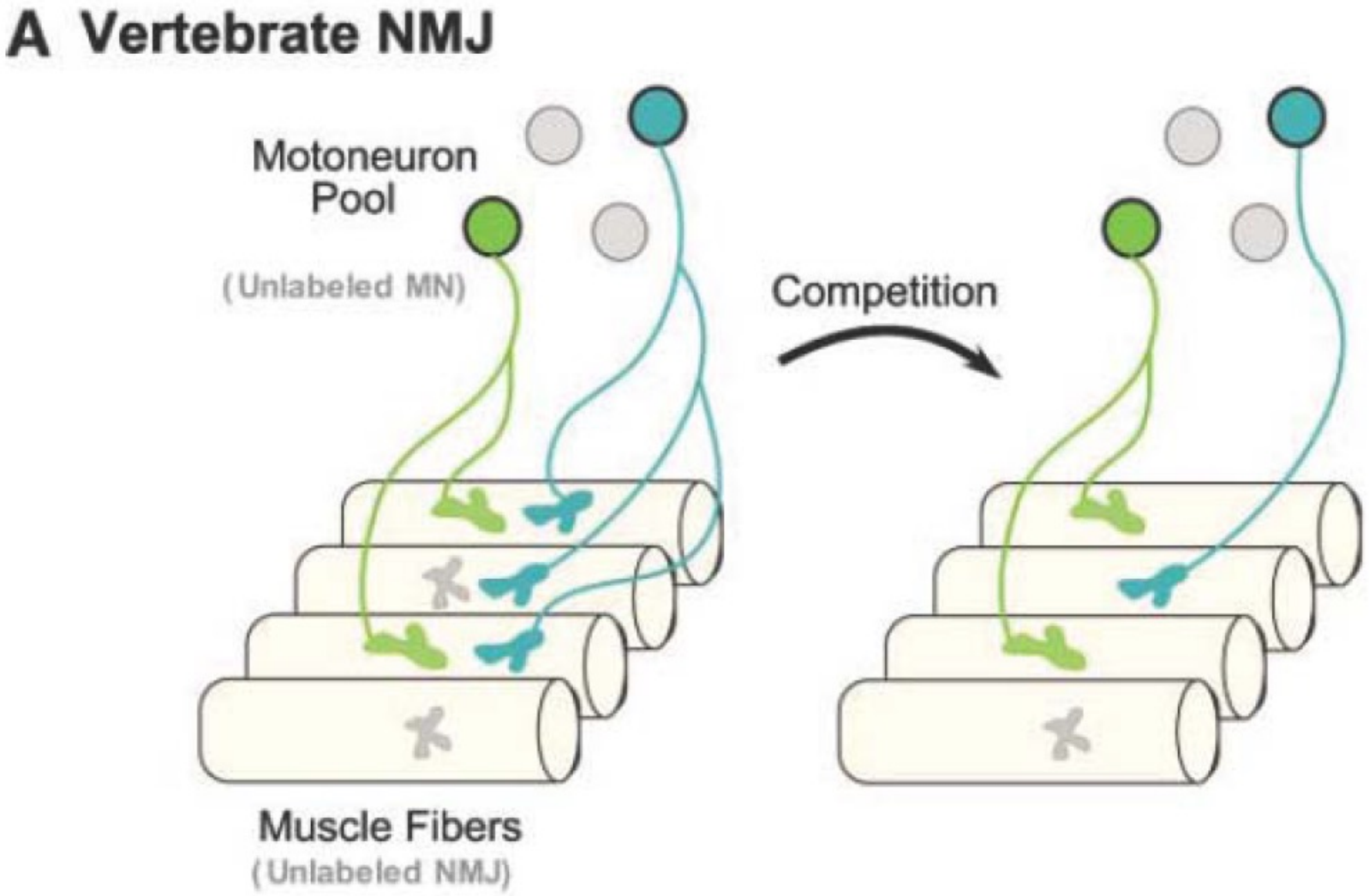
Aberle et al., (2002) Neuron *33*, 545-558

Marques et al.,(2002) Neuron 33, 529-543

Loss of TGF-ß receptors reduces *spin* overgrowth in a dose dependent manner

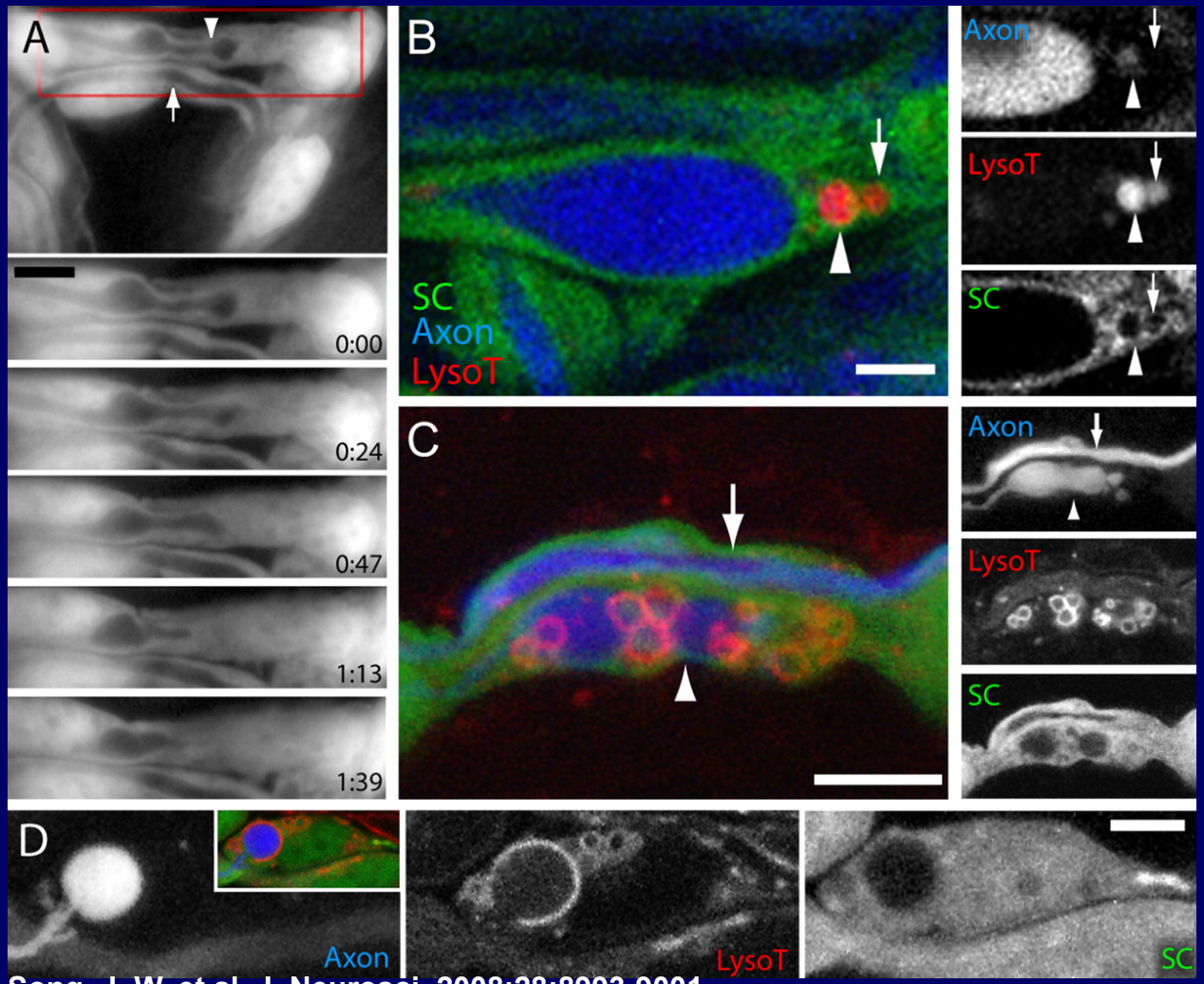


During Synaptic Competition, a MN with Larger Territory is at a Competitive Disadvantage

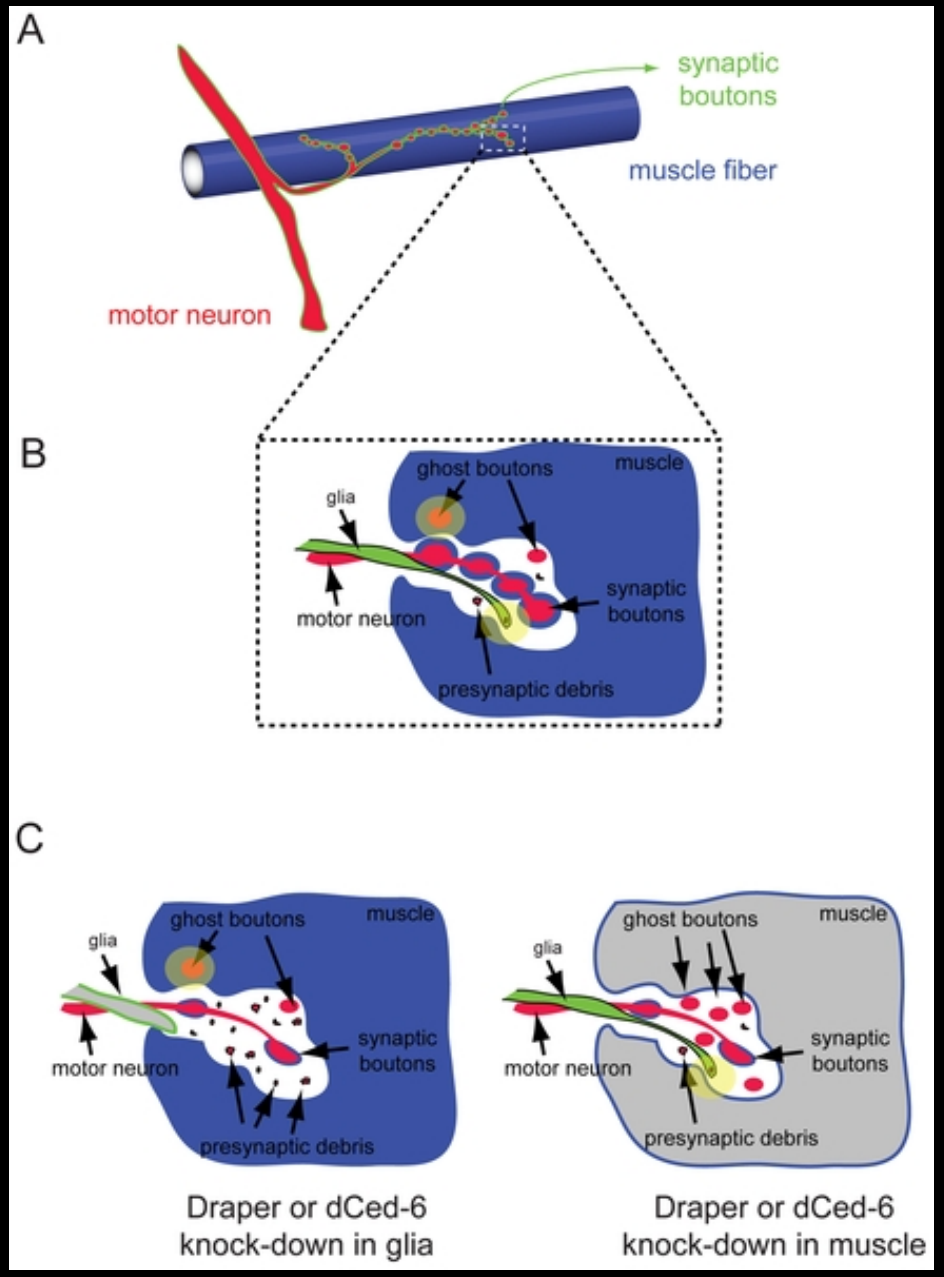


Glia are also involved in synapse elimination at the NMJ

Schwann cells seem to be eating parts of retracting axons



Song, J. W. et al. J. Neurosci. 2008;28:8993-9001


A
synaptic boutons
muscle fiber
motor neuron
B
ghost boutons
muscle
glia
synaptic boutons
motor neuron
presynaptic debris
C
ghost boutons
muscle
glia
synaptic boutons
motor neuron
presynaptic debris
Draper or dCed-6 knock-down in glia
ghost boutons
muscle
glia
synaptic boutons
motor neuron
presynaptic debris
Draper or dCed-6 knock-down in muscle