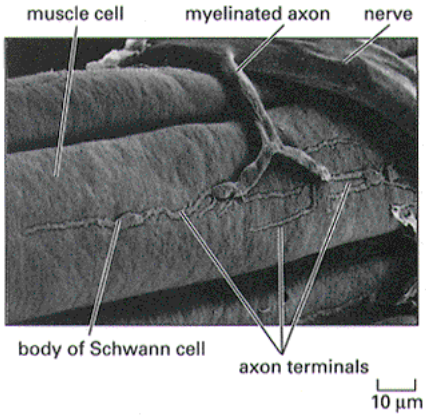
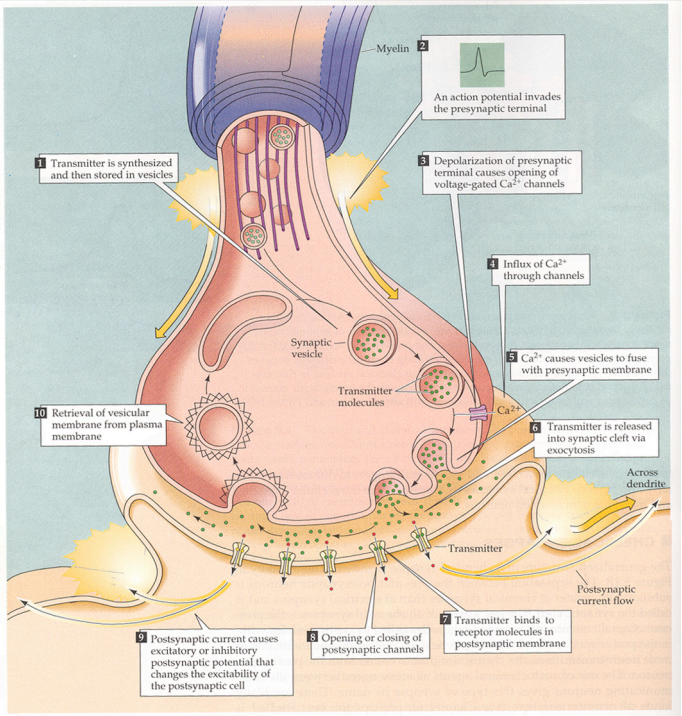
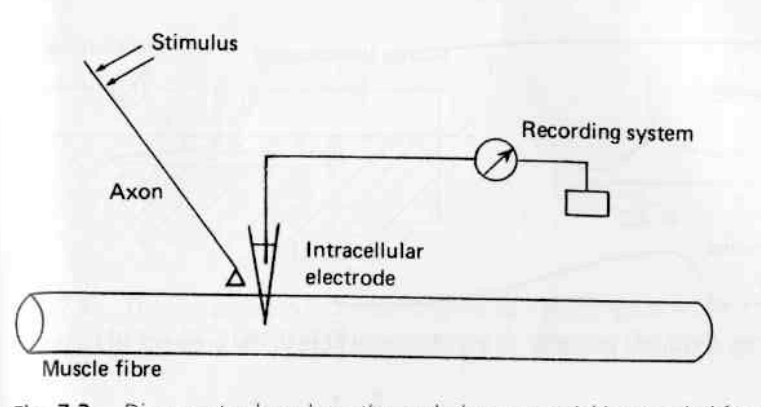
Neuromuscular jxn, S.E.M.



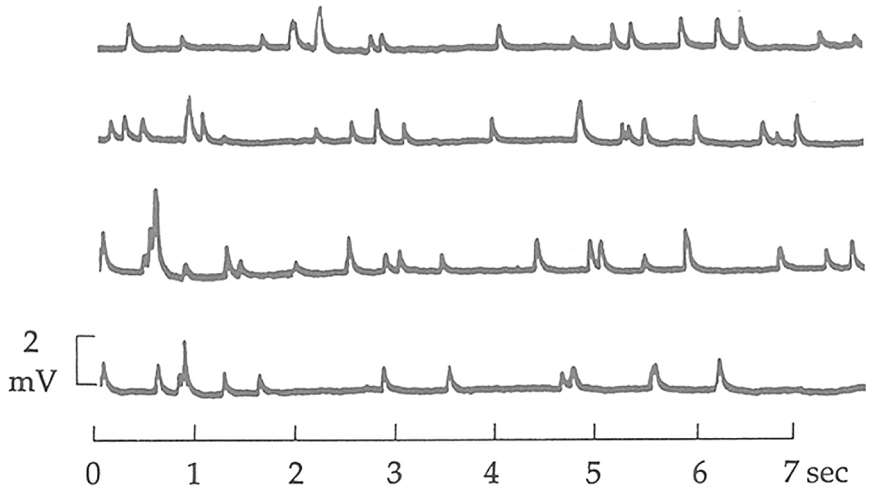


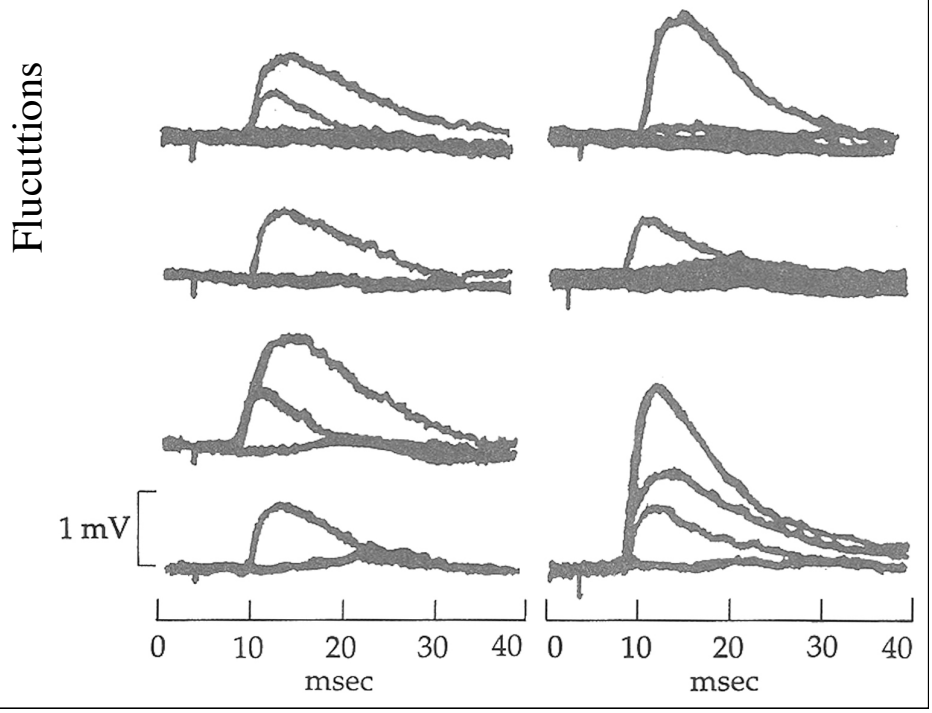
Hypothesis: Acetylcholine is released from the presynaptic terminal by fusion of vesicles which are recycled after fusion.

1. There should be quantal units of Acethylcholine released
2. The size of a quantum should equal the amount of Acetylcholine inside vesicles.
3. There should be vesicles at the synapse
4. These vesicles should fuse with the plasma membrane in response to presynaptic stimulation.
5. Vesicle membrane proteins should be exposed to the outside of the cell following fusion
6. Extracellular fluid should be carried inside the cell by vesicles following fusion.

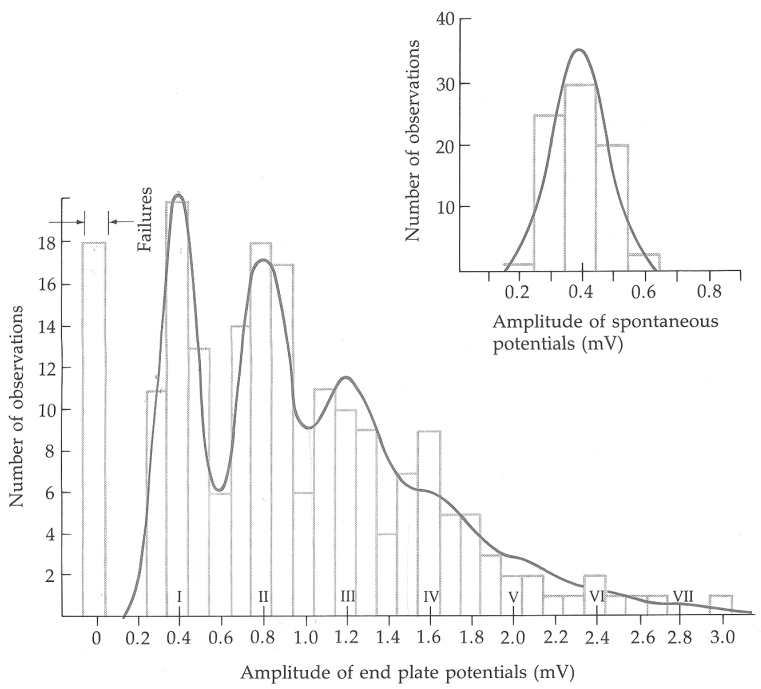


Miniature endplate potentials

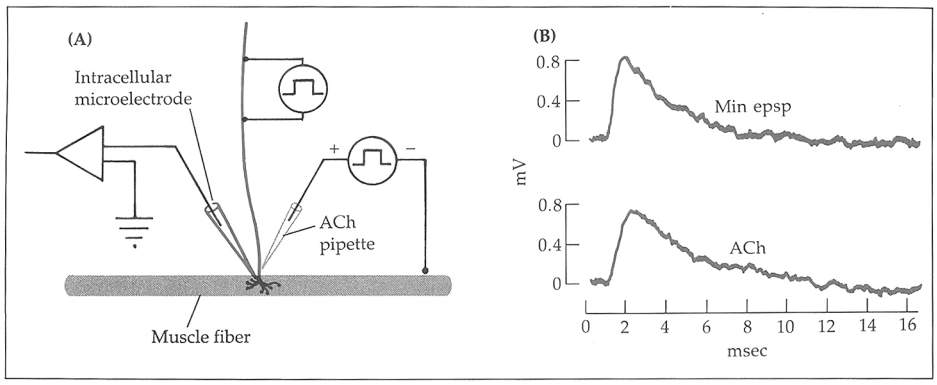


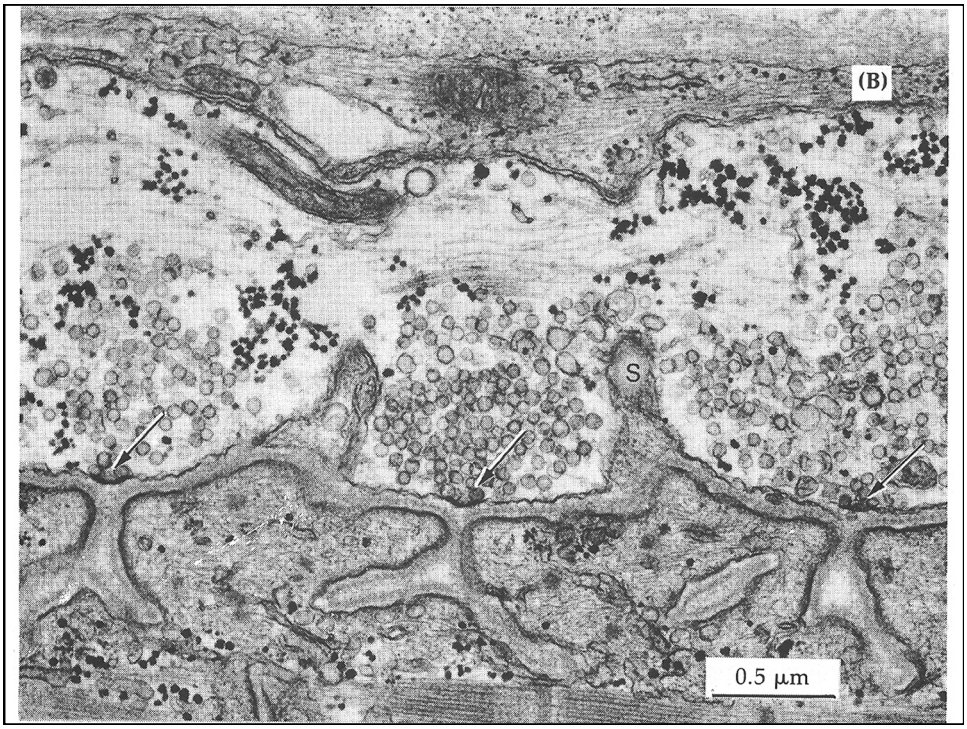


Amplitude distribution of EPPs

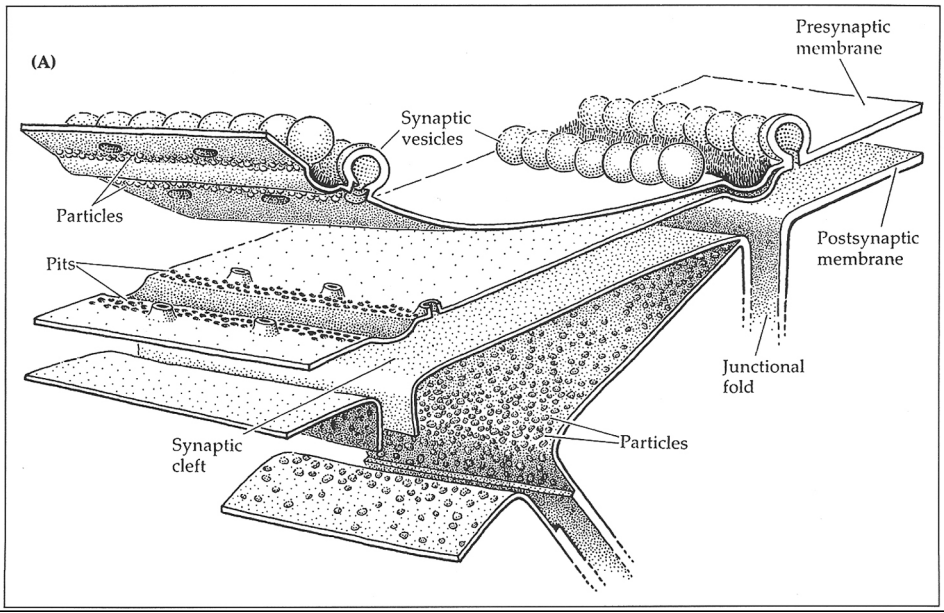


Ach mimics min epsp

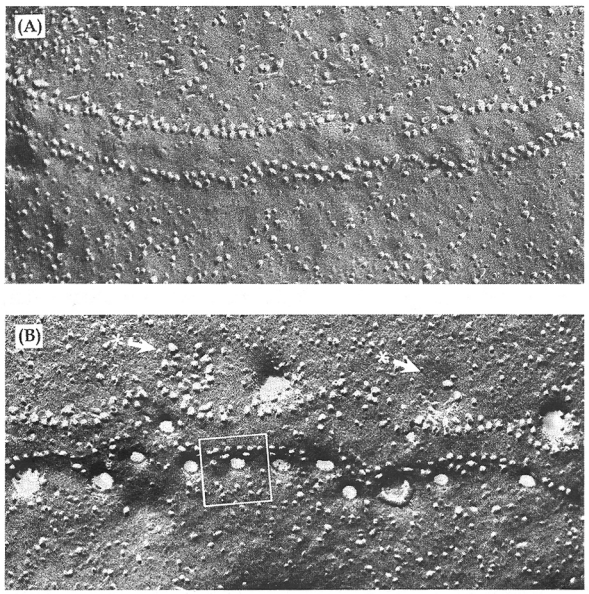


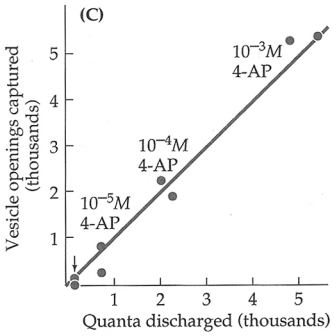


Neuro-muscular Junction, cartoon

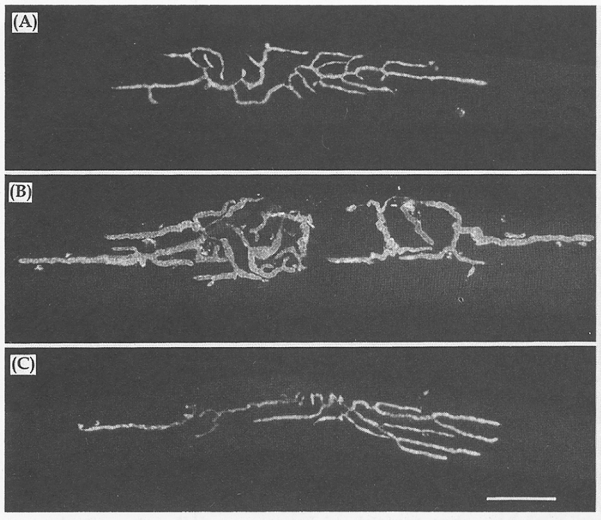


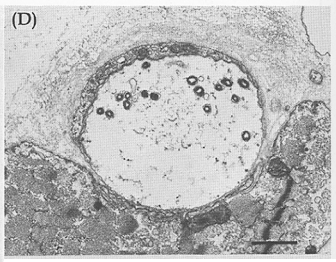
Vesicle fusion



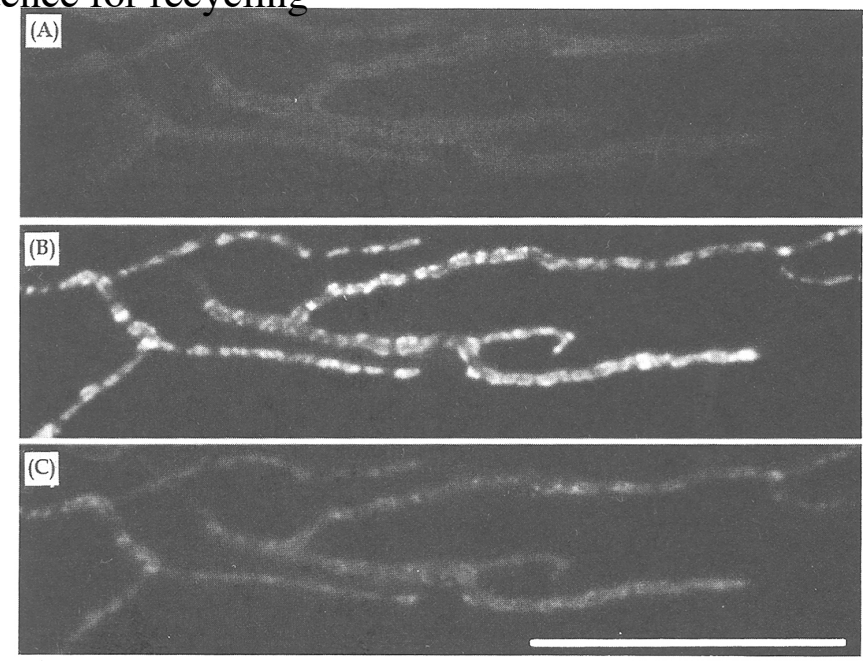


Vesicle proteins exposed on cell surface

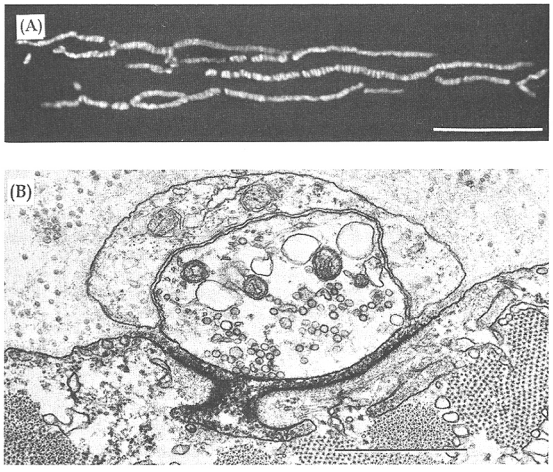


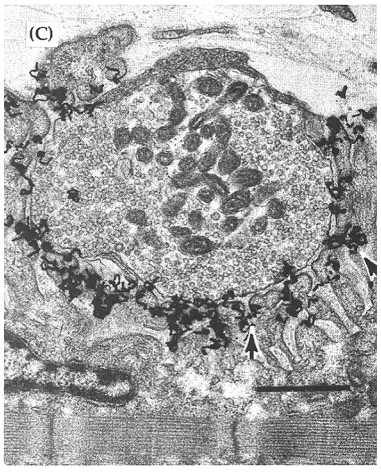


Evidence for recycling

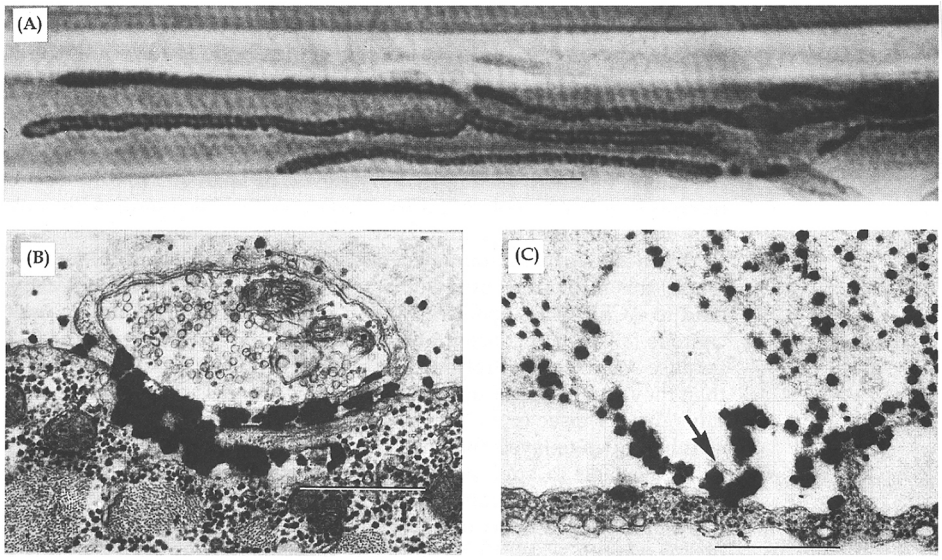


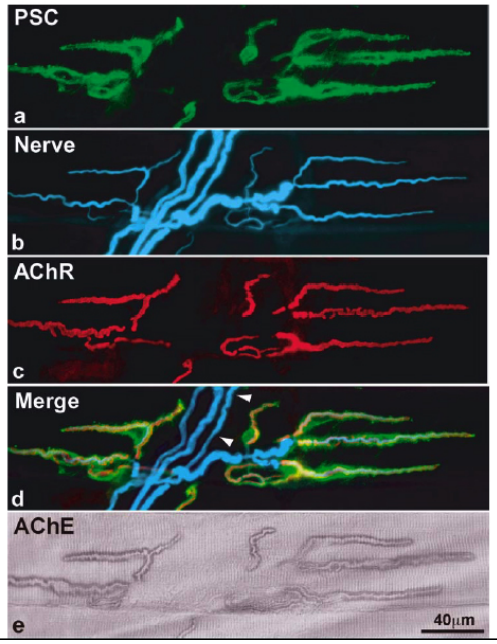
Ach receptors cluster at NMJ





Acetylcholinesterase concentrated at NMJ



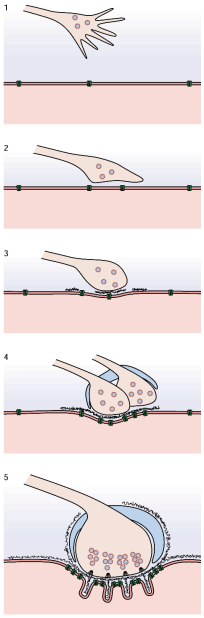


Presynaptic Schwann Cells

neurofilament

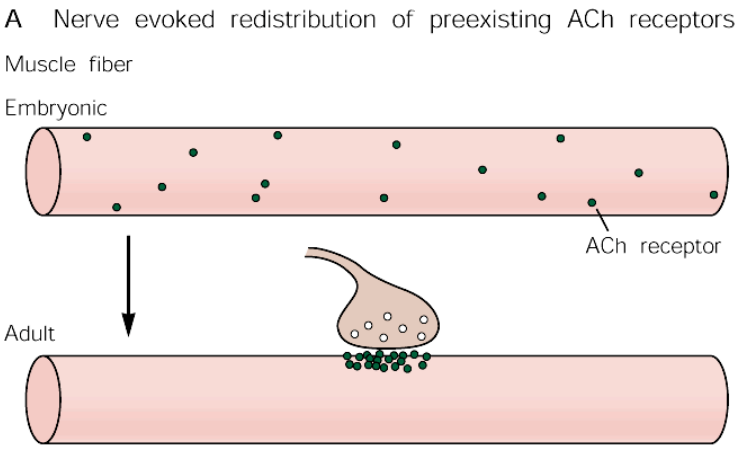
Bungarotoxin

Stages of NMJ Development

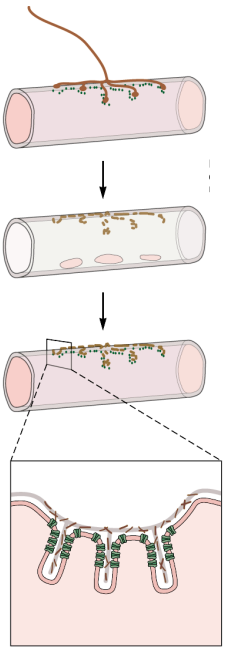


- growth cone approaches
- non-specialized but functional contact
- immature specializations
- multiple innervation
- elimination of additional axons, maturation

Clustering of ACh-R: A) Aggregation of existing receptors



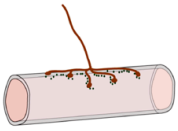
The basal lamina directs clustering of ACh-Rs



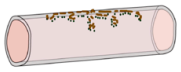
Denervation and muscle elimination
(but preservation of muscle satellite cells which will form new myotubes)

In the absence of nerve, ACh-Rs cluster at the original synaptic site

Components of the basal lamina can organize the nerve terminal

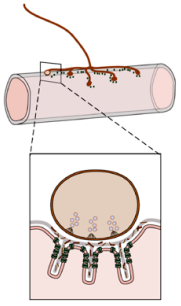


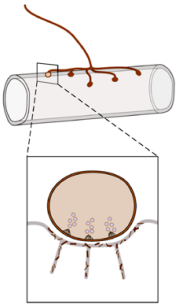
Denervation



Denervation +
Muscle elimination

Regeneration

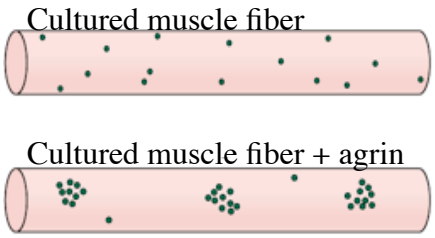




Regeneration

Agrin

- Component of the basal lamina
- 400 kDa proteoglycan
- Secreted from motor neuron and muscle
- Neural form potently induces clustering of ACh-Rs in myotubes

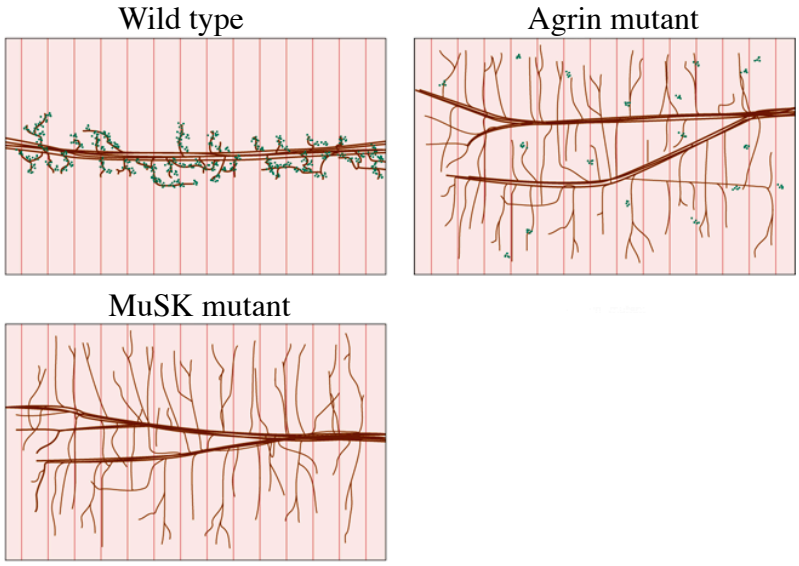


Summary of mutant phenotypes

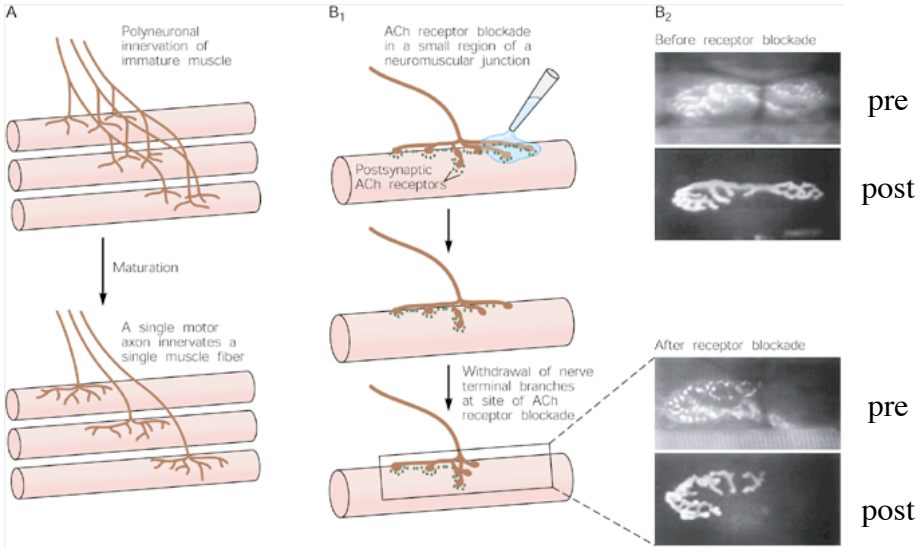
- **Agrin -/-:** few ACh-R clusters, overshooting of axons
- **MuSK -/-:** no ACh-R clusters, overshooting of axons

- Pre-synaptic defects **in all mutants**, due to the lack of retrograde signals from the muscle

Mouse mutants confirm essential roles for agrin, MuSK

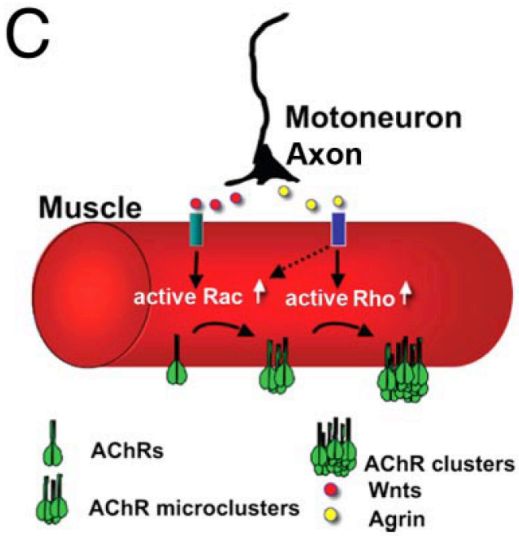


Synaptic inactivity can lead to synapse elimination



Wnt signaling promotes AChR aggregation at the neuromuscular synapse in collaboration with agrin

Juan P. Henriquez[a,1,2], Anna Webb[a,1], Matthew Bence[a], Heidi Bildsoe[b], Macarena Sahores[a], Simon M. Hughes[b], and Patricia C. Salinas[a,3]



Henriquez et al., 2010

Reading a scientific research paper. TITLE

Wnt signaling promotes AChR aggregation at the neuromuscular synapse in collaboration with agrin

Juan P. Henriquez[a,1,2], Anna Webb[a,1], Matthew Bence[a], Heidi Bildsoe[b], Macarena Sahores[a], Simon M. Hughes[b], and Patricia C. Salinas[a,3]

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The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0806300105/DCSupplemental.

Reading a scientific research paper. INTRO

Wnt proteins regulate various aspects of neuronal connectivity, from axon guidance to dendritic development and synapse formation (1). At central synapses, Wnts act as retrograde signals that regulate terminal axon remodeling and presynaptic differentiation (2, 3). At peripheral synapses, a role for Wnt signaling was first identified in invertebrate systems. In *Drosophila,* the Wnt homologue Wingless (*Wg*) positively regulates the correct assembly of presynaptic active zones and clustering of post-synaptic components (4). In contrast, the *Caenorhabditis elegans* Wnt homologue lin44 inhibits the formation of synapses at specific areas along the axon (5). Therefore, in invertebrates, Wnt factors can promote or inhibit the formation of peripheral synapses. However, a role for Wnt signaling at vertebrate peripheral synapses is less understood.

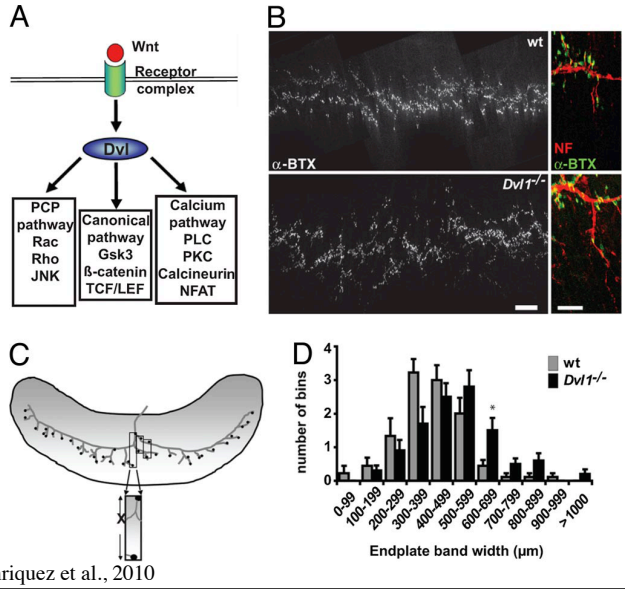
At the vertebrate cholinergic neuromuscular junction (NMJ), agrin, a heparan sulfate proteoglycan secreted by motoneurons (6, 7), induces post-synaptic differentiation by aggregating acetylcholine receptors (AChR) and other proteins at the post-synaptic membrane (8–10). This effect is mediated through sequential activation of Rho GTPases; agrin induces a rapid and transient activation of Rac1 that is necessary for the initial phase of AChR micro-cluster formation, whereas the subsequent RhoA activation is crucial for the coalescence of the micro-clusters into full-sized AChR clusters (11, 12). Although initial evidence suggested that agrin was crucial for initiation of post-synaptic development (6, 7), agrin also plays a later maintenance role (13, 14). **These various functions of agrin at different developmental stages might be achieved through other factors that influence agrin activity.**

Here we report that Wnt3, which is expressed by motoneurons at the time when they invade muscle regions in the limb (3), induces the clustering of AChRs during early stages of NMJ assembly in chick wing muscles. Conversely, exposure to the Wnt antagonist Sfrp1 dramatically reduces the number of AChR aggregates in the chick limb, suggesting that endogenous Wnts are required for AChR clustering during neuromuscular innervation. Importantly, diaphragms from mice lacking *Dishevelled-1* (*Dvl1*), a scaffold protein required in all Wnt pathways (15) (Fig. 1*A*), exhibit abnormal AChR cluster distribution, indicating a requirement for Wnt signaling in post-synaptic differentiation at the mouse NMJ. In myotubes, Wnt3 induces a rapid activation of Rac1 and the accumulation of AChR micro-clusters, which are converted into fullsized clusters in the presence of agrin. **Our findings demonstrate** a function for Wnts as modulators of post-synaptic differentiation at vertebrate peripheral synapses by collaborating with agrin.

Reading a scientific research paper. DISCUSSION

Here we provide evidence that Wnt signaling plays a positive role in post-synaptic differentiation at the vertebrate NMJ. Gain- and loss-of-function studies demonstrate that Wnt signaling is required *in vivo* for the proper clustering of AChRs, a hallmark of post-synaptic differentiation at the NMJ. In cultured myotubes, Wnt3 alone induces the formation of AChR micro-clusters through Rac1 activation, which fail to aggregate into large clusters. In the presence of agrin, however, Wnt3 promotes the formation of large clusters, thus enhancing agrin activity. **We propose that** Wnt factors collaborate with agrin by increasing the number of micro-clusters, which are subsequently converted into large AChR clusters by agrin.

Loss of Wnt signaling changes AChR cluster distribution



Dvl 1 mutant:
No wnt signaling

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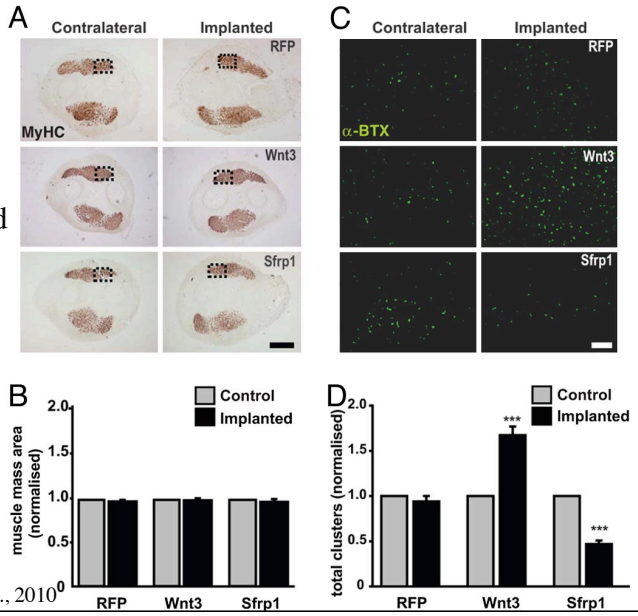
Wnt3 and Sfrp1 implantation, chick wing

Muscle is unaffected



Synapses

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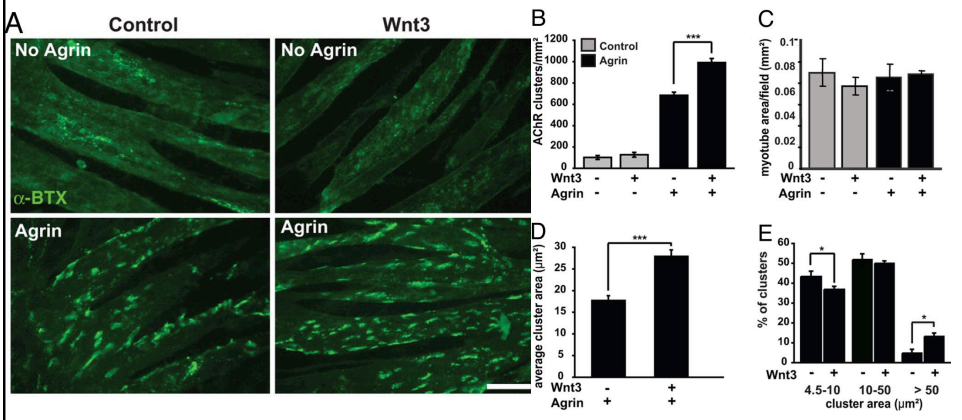
Wnt3 in tissue cultured muscle fibers



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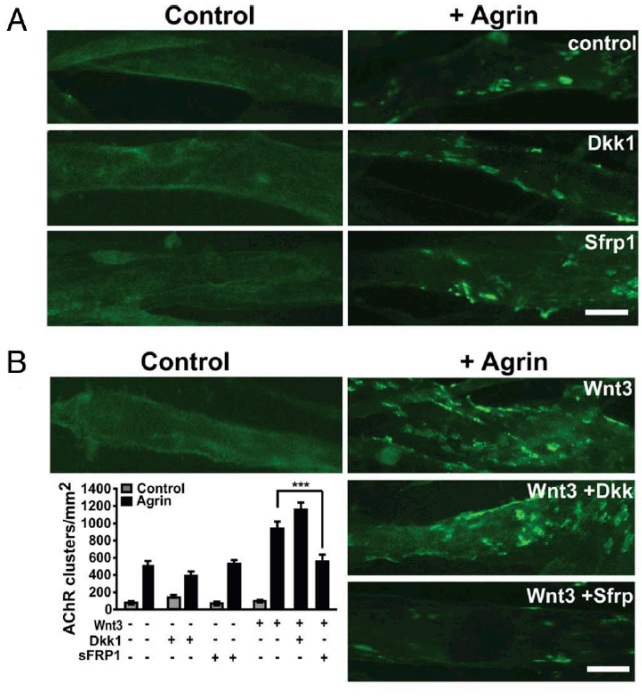
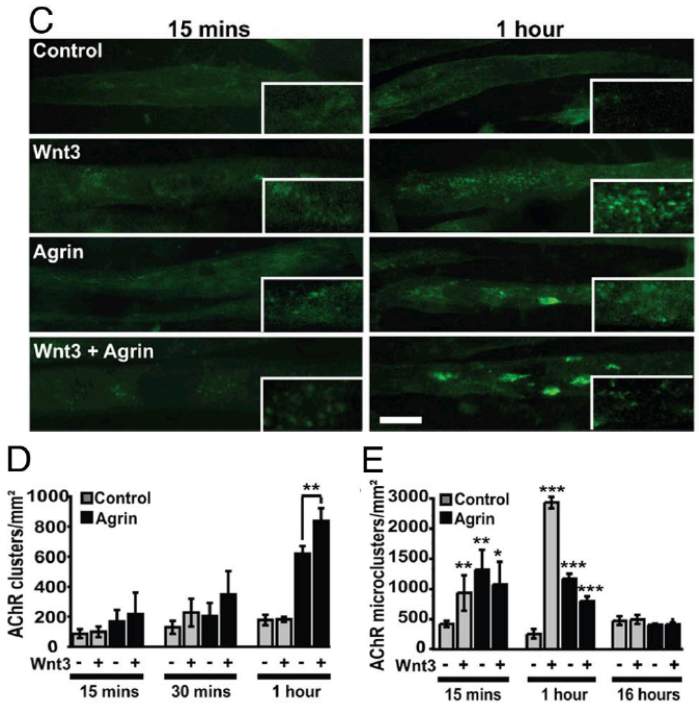
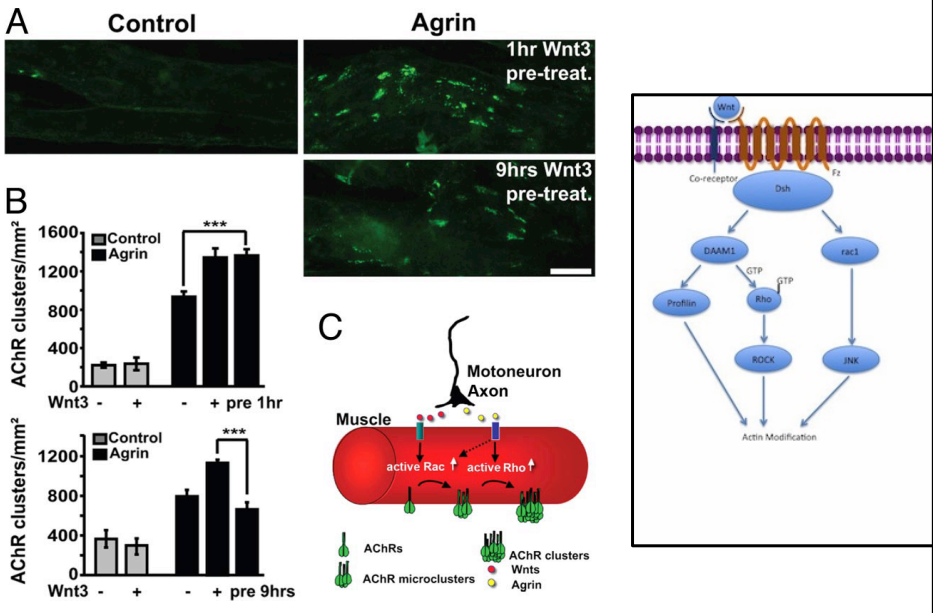
No effect on agrin alone, wnt comes from nerve.



Microclusters



Transient effect of wnt.



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