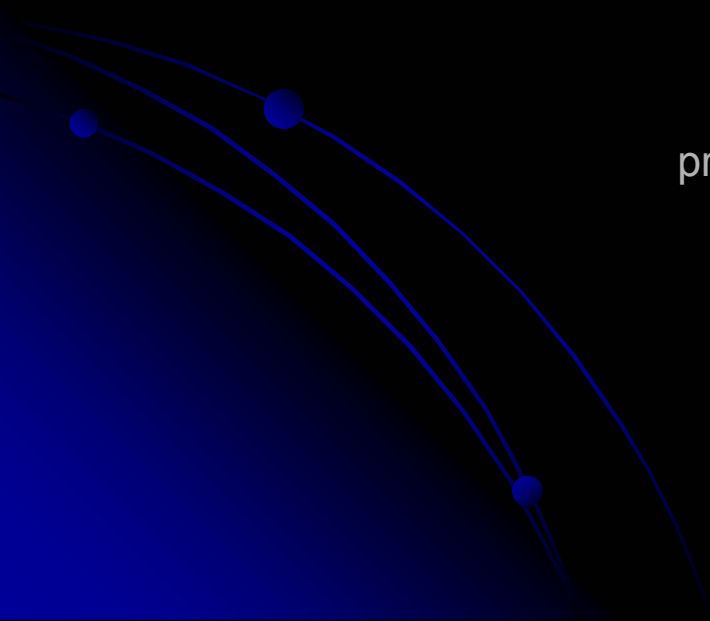


Alterations in Synaptic Strength Preceding Axon Withdrawal

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Synaptic Transmission at the Neuromuscular Junction

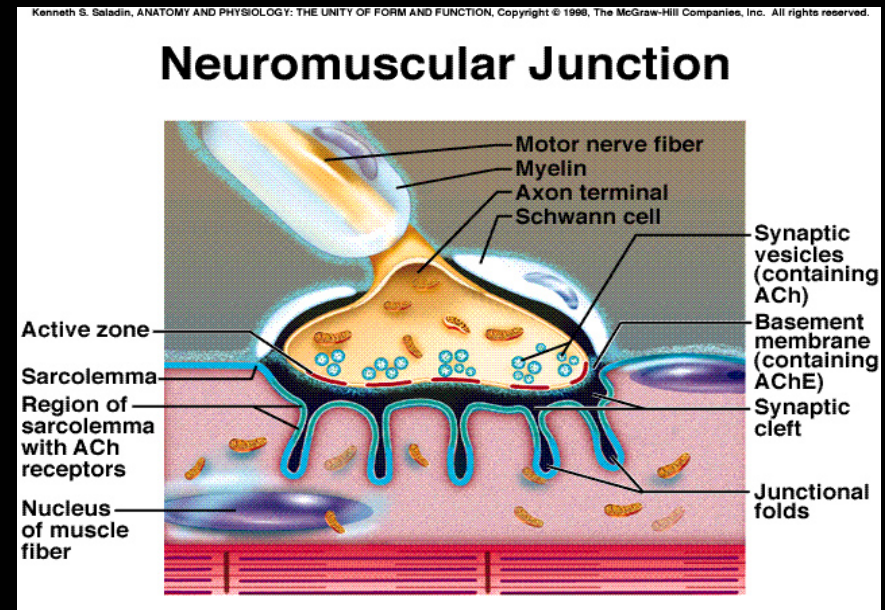
Motor neurons with cell bodies in the spinal cord have long axons that branch near muscles.

Typically, an axon makes a single point of synaptic contact with a skeletal muscle fiber, i.e. the neuromuscular junction (NMJ).

The process of fusion of synaptic vesicles and release of ACh occurs at differentiated regions of the presynaptic membrane called **active zones**.

The end-plate is synonymous with NMJ.

An end-plate potential (EPP) is an example of an excitatory postsynaptic potential caused by the transient opening of AChRs.



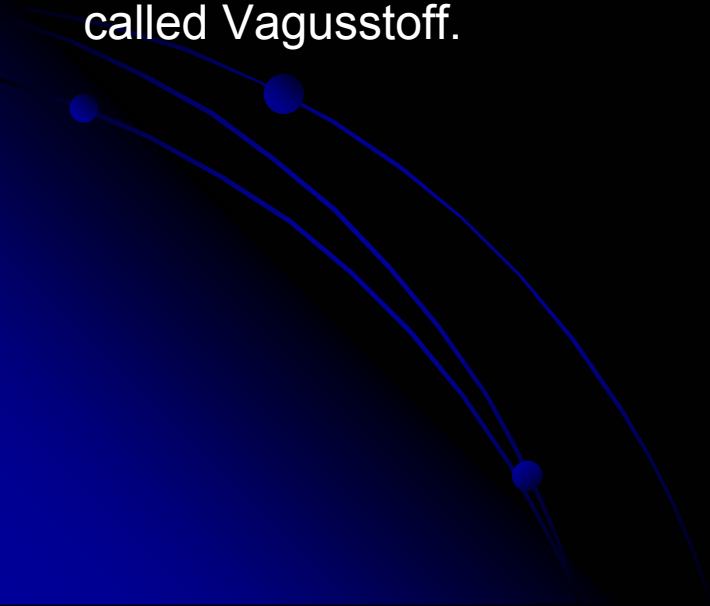
Neat Fact About ACh

A classic experiment performed by Loewi in 1921 is cited as the first definitive evidence for chemical neurotransmission.

He repeatedly stimulated the vagus nerve of a frog heart, which caused a slowing of the heartbeat, and collected the artificial saline that emerged from the ventricle.

When he later perfused the same heart with the fluid previously collected, the fluid alone caused the heart to slow down.

Later the active compound in the fluid was discovered to be acetylcholine, originally called Vagusstoff.



Objective of Experiment

The experimenters examined the functional and structural synaptic changes that occur when muscle fibers transition from polyneuronal to singly innervated cells during postnatal development (P1 – P9).

In adult mammals a one-to-one relationship exists between motor nerve terminals and muscle fibers.

However, this is not true in the embryo and in the postnatal mouse. At intermediate stages of development several axons converge on each muscle fiber, and soon after birth all inputs but one are eliminated.

It is hypothesized that synapse elimination must be part of a competitive process.

What governs this competition?

The purpose of this transient polyneuronal innervation remains a mystery.

- one possibility might be to ensure that each muscle fiber is innervated.
- another is that synapse elimination provides a way by which activity can change the strength of particular connections.

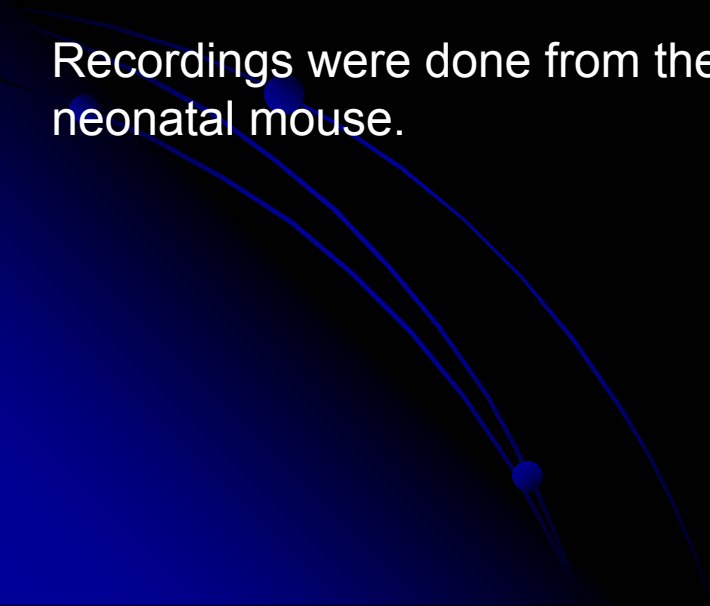
Methods

Intracellular recordings from newborn and adult muscle fibers temporarily innervated by two axons were done.

One suction electrode was applied (for stimulation) to each of the two nearby nerve branches which converged at the same junction. Shown in Figure 1a.

Muscle fibers innervated by two axons were chosen for the study. EPPs were recorded from a common area on the muscle where the two axons converged.

Recordings were done from the trapezius muscle and sternomastoid muscle in the neonatal mouse.



Quantal Content as a Measurement of Neurotransmitter Release

Quantal content is the discrete number of synaptic vesicles that fuse at the presynaptic terminal.

It is thought that EPPs are the summation of many unitary events, each having a magnitude of 0.4mV (discovered by Del Castillo and Katz).

Quantal content is measured under conditions in which there is a high concentration of magnesium and/or a low concentration of calcium.

The increase in magnesium and/or the decrease of calcium greatly reduces the probability that ACh will be released when the motor neuron is stimulated.

A high magnesium concentration partially blocks presynaptic calcium channels which decrease calcium entry into the presynaptic terminal and thus the amplitude of EPPs.

Experiments done by Del Castillo and Katz show that the amplitudes of the peak responses occur in discrete multiples of approximately 0.4mV: 0, 0.4, 0.8, and 1.2mV.

Figure 1

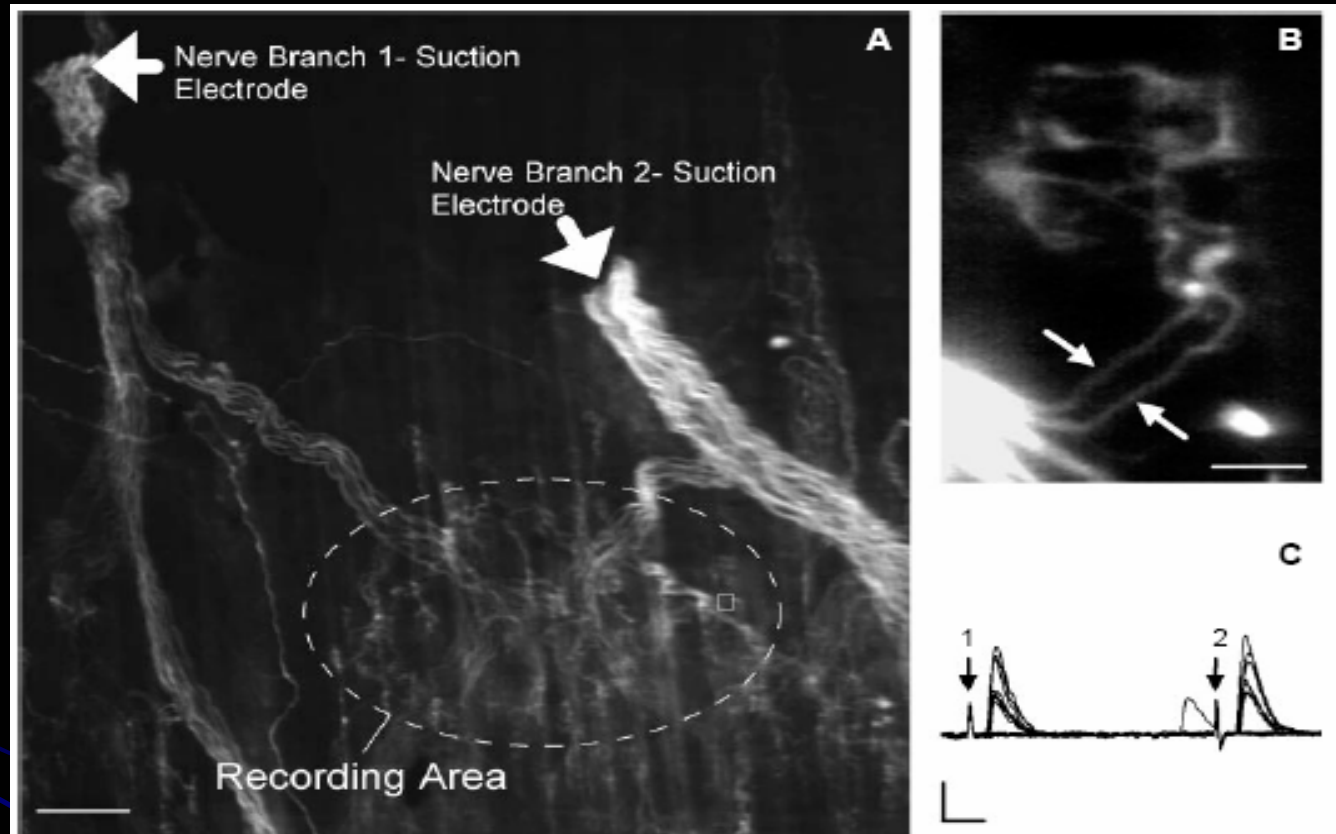


Fig. 1. The neonatal mouse trapezius muscle preparation as used for the physiological studies described. (A) A low magnification view of the dissected muscle and fluorescent staining of the nerves with antibodies to neurofilament 200 (25) and synaptophysin (26) show axons running in two separate nerve branches (large arrows) that converge on a common area of the muscle ("Recording Area"). Normally these branches were left long (1 to 3 mm) to facilitate the placement of suction electrodes on each of the nerve branches (scale bar, 250 μ m). (B) A multiply innervated junction contacted by two motor axons at high magnification [from the boxed region in (A); scale bar, 10 μ m]. (C) Synaptic potentials elicited in high magnesium concentration from a multiply innervated muscle fiber. The numbers 1 and 2 indicate the artifacts from stimulation of the two suction electrodes. Each suction electrode was stimulated once during each trace (scale bars, 1 mV, 6.5 msec).

Quantal Content Ratios as indicators of synaptic strength

Synaptic strength is the ratio of the quantal content of one input vs. the other input

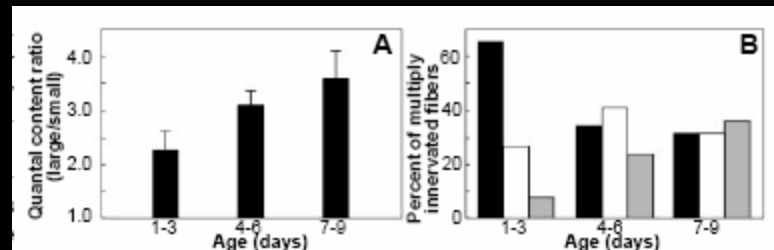


Fig 2A shows how initially synaptic strengths were similar (quantal content ratio <2), but over time there is an increase in disparity in quantal contents of inputs co-innervating a muscle fiber.

Fig 2B (black bars indicate q.c.r. of < 2, white bars q.c.r. of 2 to 4, gray bars q.c.r. of >4) demonstrates that over time there is a shift in the percentage of multiply innervated junctions with low quantal content ratios towards quantal content ratios of more than 4.

Quantal content disparity predicts single innervation

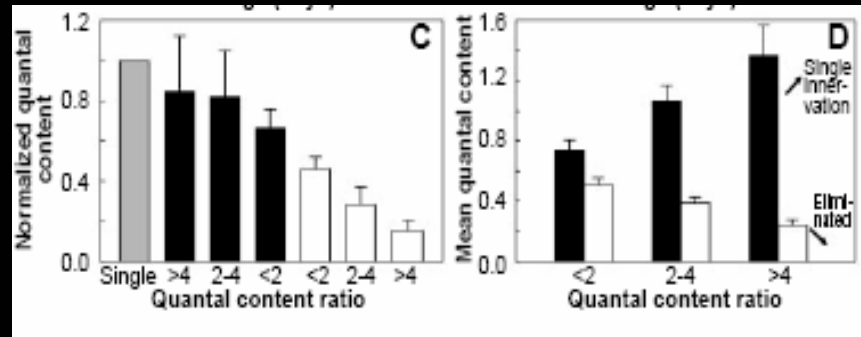


Fig.2C shows the comparison of the synaptic strengths of multiply innervated fibers with the synaptic strength of an input of a singly innervated fiber on the same muscle.

For each pair of axonal innervations converging at a NMJ there is a weaker and a stronger input. For the case where the quantal content ratio with respect to each other is <2 , when the stronger's quantal content is taken in proportion to the quantal content of the singly innervated you get a black bar with <2 .

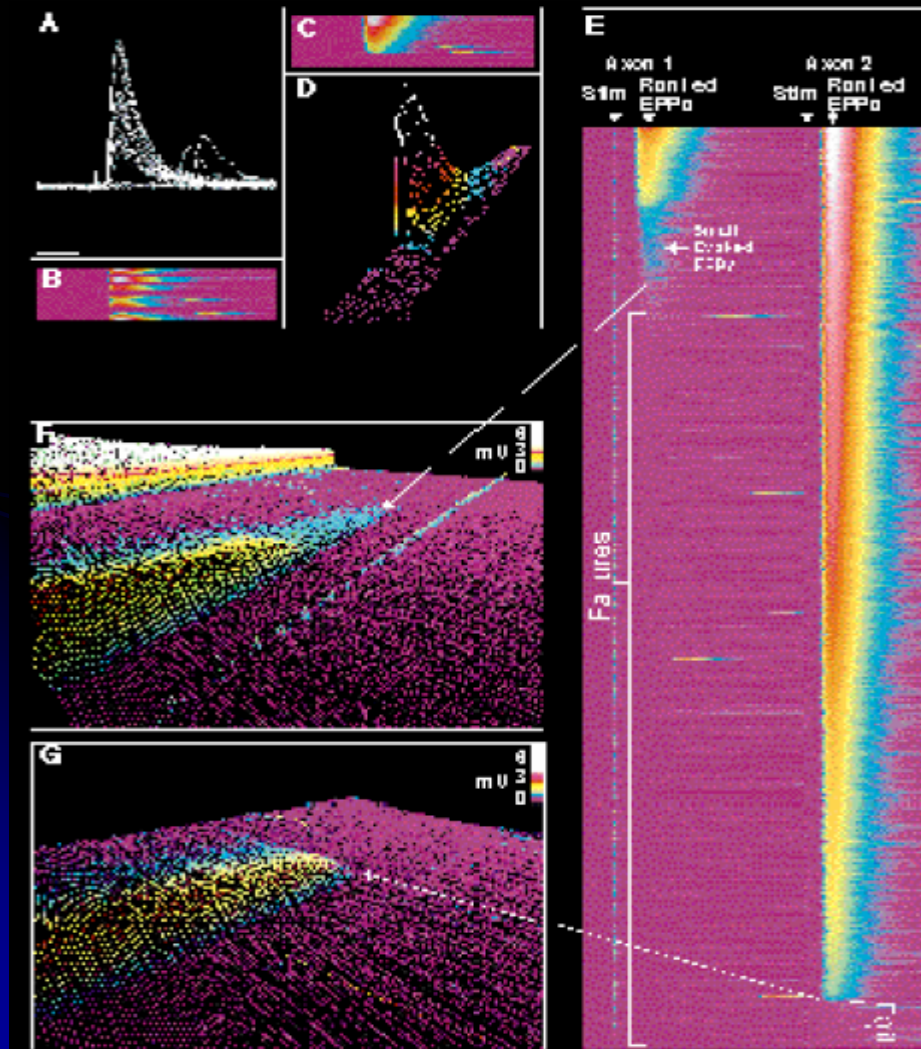
In conclusion, the stronger input from the pair with a quantal content ratio >4 has a synaptic strength similar to that of a singly innervated NMJ.

Therefore, fibers with the widest disparities of quantal content ratios are the direct predecessors to singly innervated muscle fibers.

Fig.2D – Increasing disparity in quantal content of the inputs co-innervating a muscle fiber is due to **both** an absolute increase in the quantal content of the stronger input and the decrease of quantal content of the weaker input.

Changes in Quantal Efficacy

Under the conditions of high magnesium concentrations, depolarization induced by individual quanta (quantal efficacy) were observed. Fig.3A – G shows that one input gives rise to a significantly smaller quantal responses than the other input.



A - response to nerve stimulation

B - amplitude of synaptic response in color.

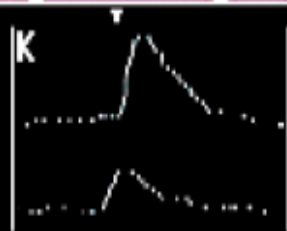
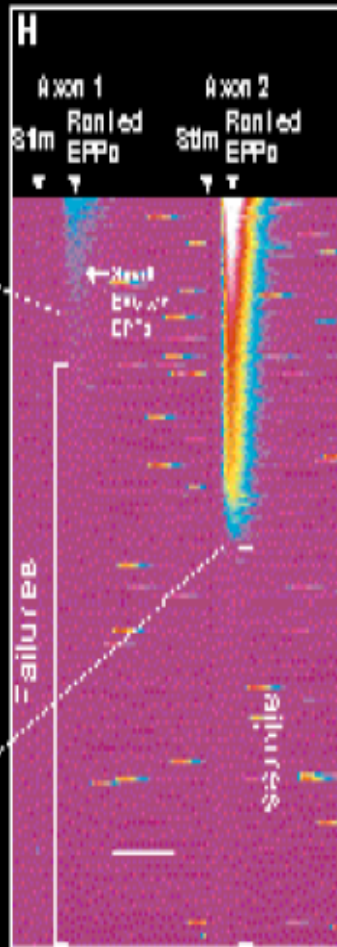
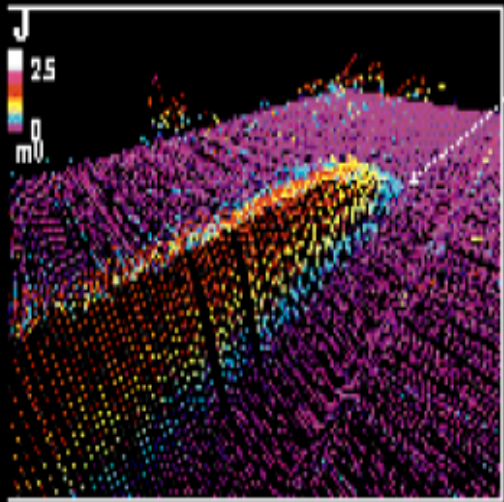
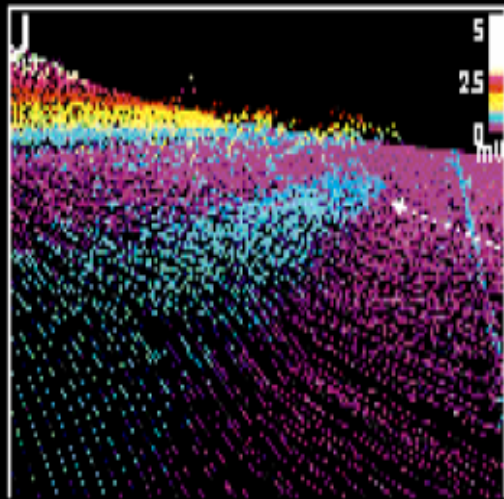
C – traces ranked by amp. largest EPP amp. on top.

D – ranked traces in C represented in height and color.

E – evoked quantal responses arranged in order of amp. Quantal content of axon 1 is 0.23 and quantal content of axon 2 is 1.20.

F- axon 1 shows a gradual decrement in amp. without an obvious cut-off between smallest evoked events and failures.

G – axon 2 shows a distinct cut-off between smallest evoked potentials and failures.



H – axon 1 has a quantal content of 0.31
axon 2 has a quantal content of 0.91.

I – shows a gradual decline for axon 1; the largest EPPs from axon 1 are comparable to the weakest EPPs from axon 2.

J – show a distinct cut-off between smallest potentials and failures for axon 2

K – shows that the larger MEPP falls within the distribution of single quantal responses from axon 2 and that the smaller MEPP falls within the distribution of EPPs from axon 1.

These MEPPs are spontaneous.

MEPP – miniature end-plate potential

Quantal efficacy measured by amplitude of MEPPs

Spontaneous MEPPs were assayed by adjusting the strength of suction electrode to stimulate one input repetitively (10 Hz) to cause an increase in MEPP frequency.

Fig.4, A-C, show a muscle fiber from a 9-day old mouse with one strong and one weak input.

A – response (EPPs) of both the weak and strong inputs.

B – EPP of the larger input; C – EPP of the weaker input.

D – When both inputs were stimulated together repetitively, large (open arrow) and small (filled arrow) MEPPs were seen after each EPP.

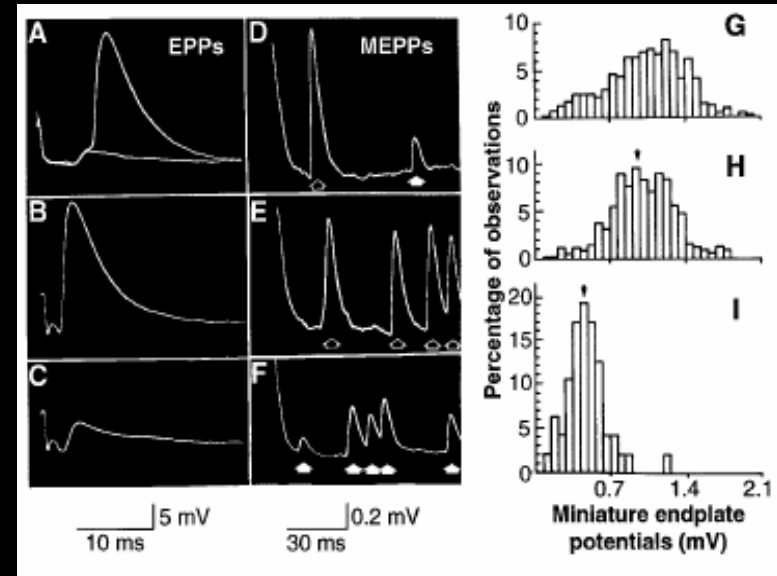
E – Repetitive stimulation of large EPP alone was followed by large MEPPs.

F – Repetitive stimulation of small EPP alone was followed by small MEPP.

G – the distribution of MEPP amplitudes after stimulation of both inputs.

H – distribution of MEPP after stimulation of large input

I – distribution of MEPPs after stimulation of weaker input had a lower mean than (H).



How is quantal efficacy during synapse elimination explained?

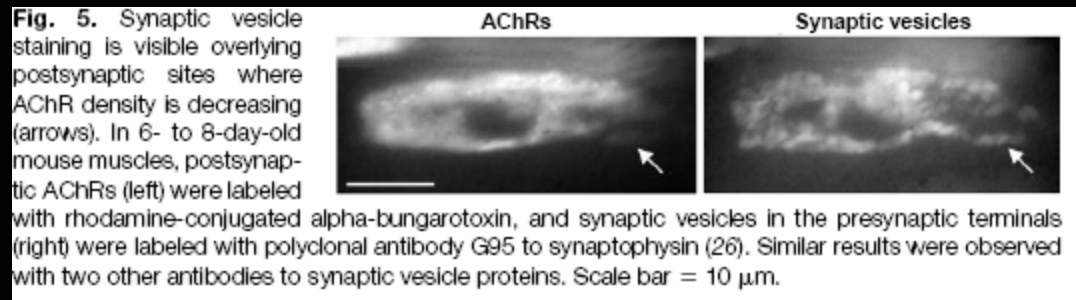
Small MEPPs are also associated with weaker inputs in multiply innervated cells.

To determine whether changes in quantal efficacy could be explained by a reduction in the amount of acetylcholine molecules per vesicle, repetitive stimulation under the presence of hemicholinium-3 (depletes synaptic vesicles of acetylcholine) were done.

Stimulation in the presence of hemicholinium-3 and high magnesium decreased the amplitude of evoked quanta to a size similar to that observed for the small quantal event in multiply innervated muscles fibers, and the median rise times of the small events were as fast or faster than before.

Since the small events observed during synapse elimination are not mimicked by using hemicholinium – 3 they turned to decreases in the density of AChRs in the areas of the postsynaptic membrane.

Given that a low density of AChRs could account for small quantal responses, area with low density of receptor density were identified in NMJs and overlying nerve terminals were studied for signs of synaptic vesicle at these sites.

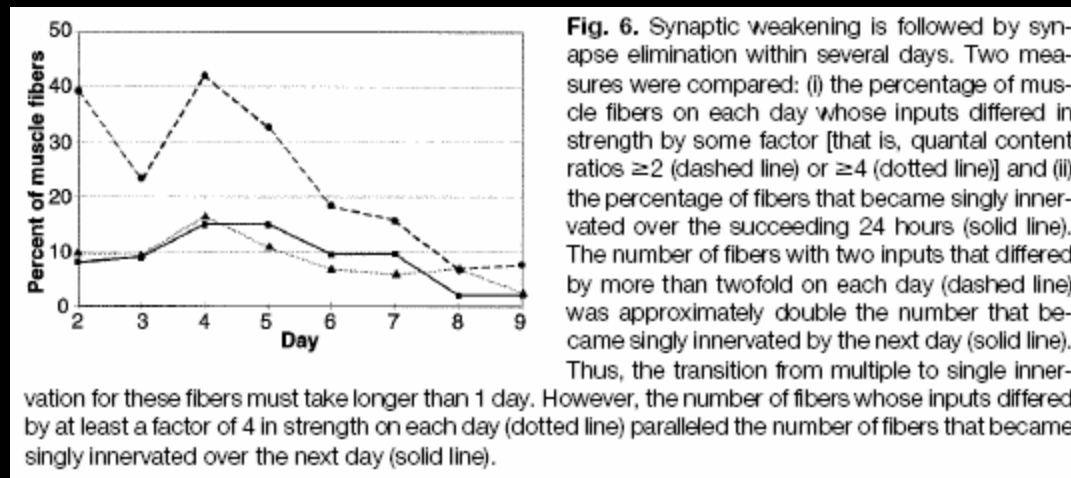


Synaptic vesicles proteins were found overlying some areas of decreased receptor density (above).

This supports the idea that small quantal responses observed are explained by a reduction in postsynaptic sensitivity.

Synaptophysin is an integral membrane protein in synaptic vesicles.

Once an input is four times stronger than the other, axon withdrawal of the weaker input would appear imminent



Conclusions

The rapid nature of the changes in synaptic strength may be related to the activity dependence of synaptic competition.

The ability of an axon to induce elimination of its competitor is related to its synaptic efficacy.

The skewing observed in the experiments in quantal content and efficacy would be expected to cause further changes in synaptic strength by inducing the elimination of synaptic sites and progressively tipping the competitive balance in favor of one axon over another.

