Activity Dependent Changes At the Developing Neuromuscular Junction

(slides 16, 17 and 18 have been slightly modified for clarity)

MCP Lecture 2-3
9.013/7.68 '04

Neuromuscular Junction Development

1. Muscle fibers twitch spontaneously
2. ACh receptors are widely distributed on surface
3. Multi-terminal innervation
4. Receptors concentrate at the junction (end-plate) via action the actions of Agrin and ARIA (neuregulin).
5. Synapse elimination
6. Conversion of junctional ACh receptors from immature to mature subunit composition. (Decrease in Ca++ entry)
7. Maturation of junctional specializations (junctional folds, junctional proteins, specialized ECM).
8. Increase in receptor stability at the junction.

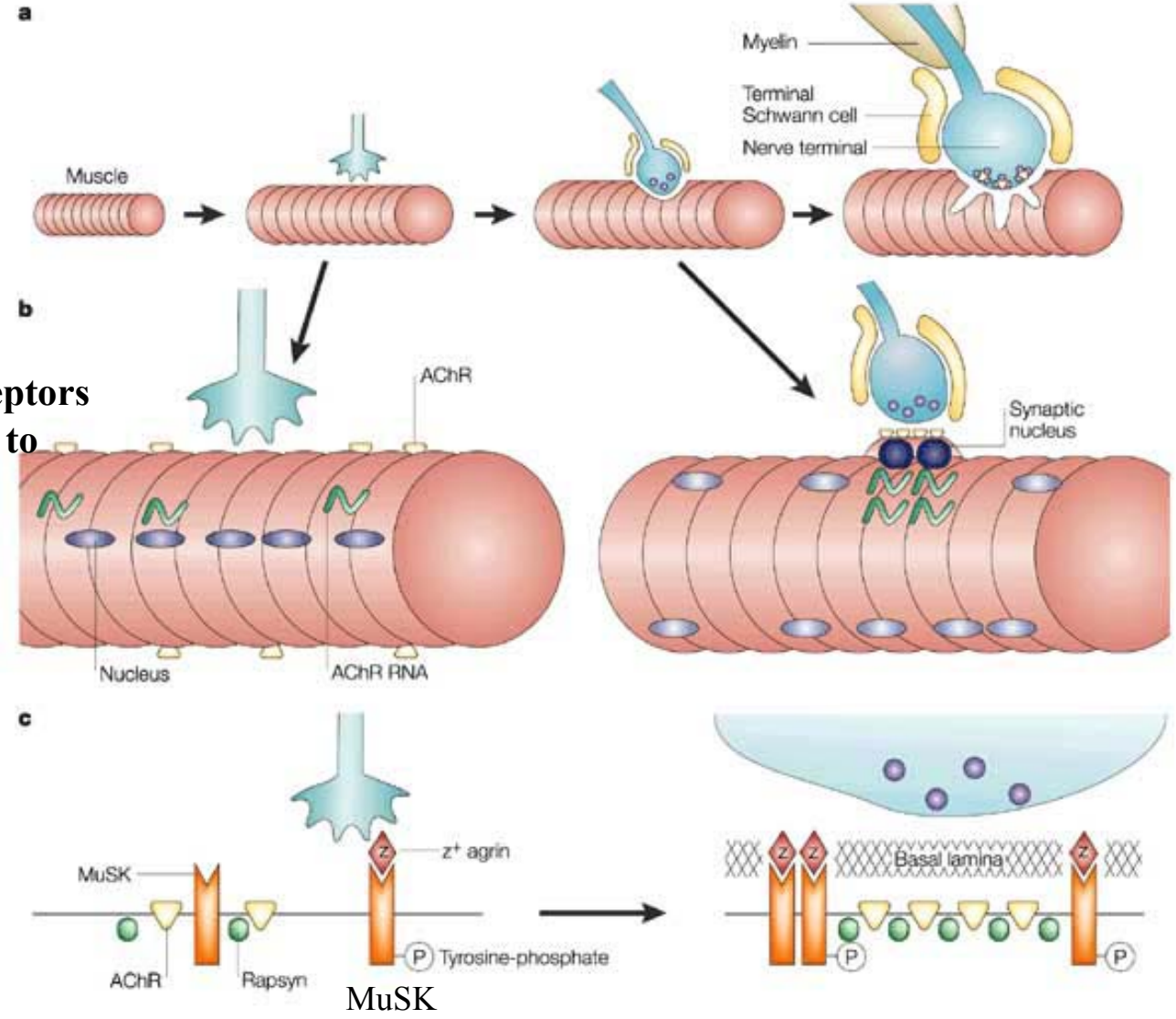
1. Nerve secretes Agrin

2. Agrin stimulatesMuSK

3. MuskK via rapsyn clusters AChRs

4. Aria(neuregulin) via Erb receptors activates subsynaptic nucleus to make more AChRs

5. Synapse specific basal lamina forms to anchor Agrin and adhesion molecules



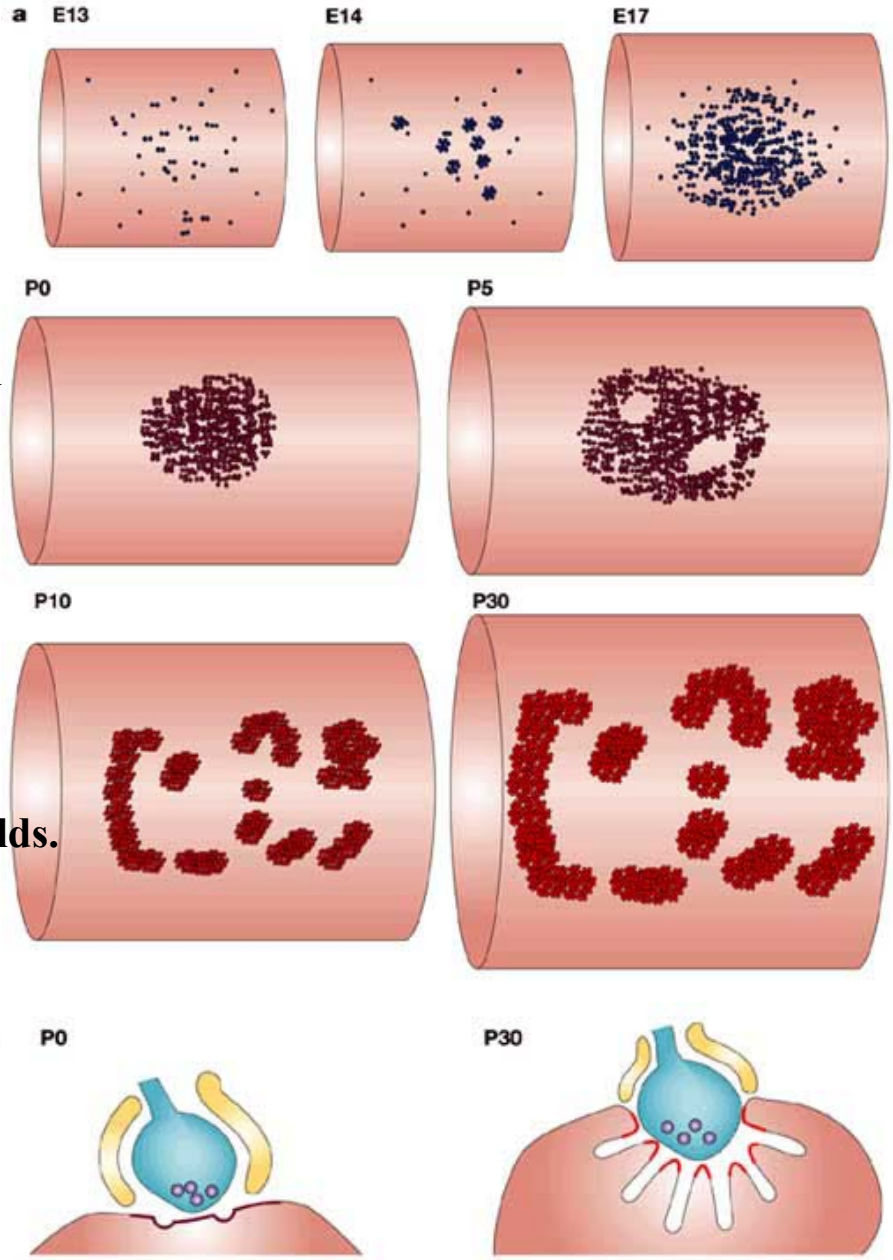
Lichtman & Sanes, Nature Rev. Neuroscience

6. NMJ grows as muscle grows

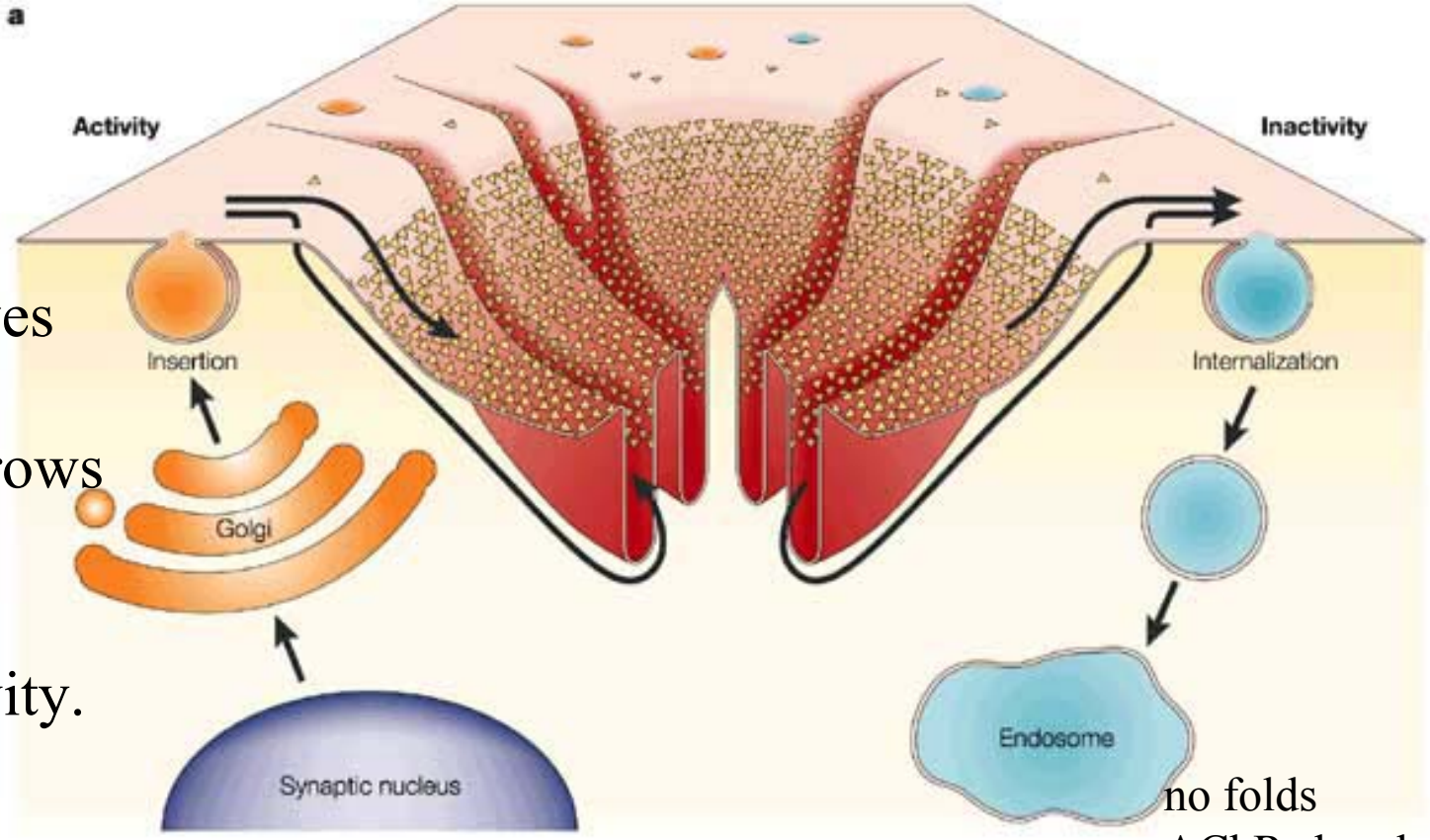
7. AChR density increases

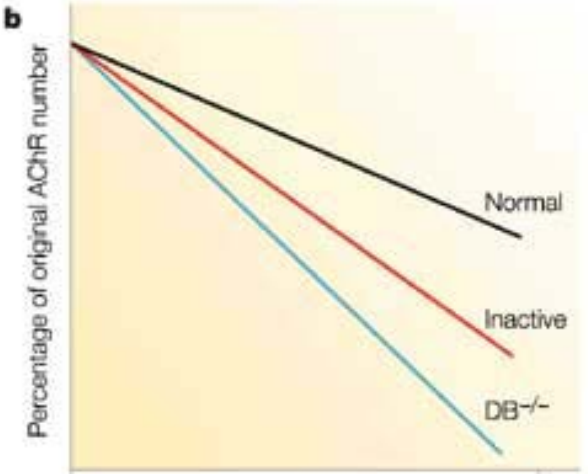
8. Plaques develop holes and begin to develope a pretzel shape. Corresponds to the period of synapse elimination.

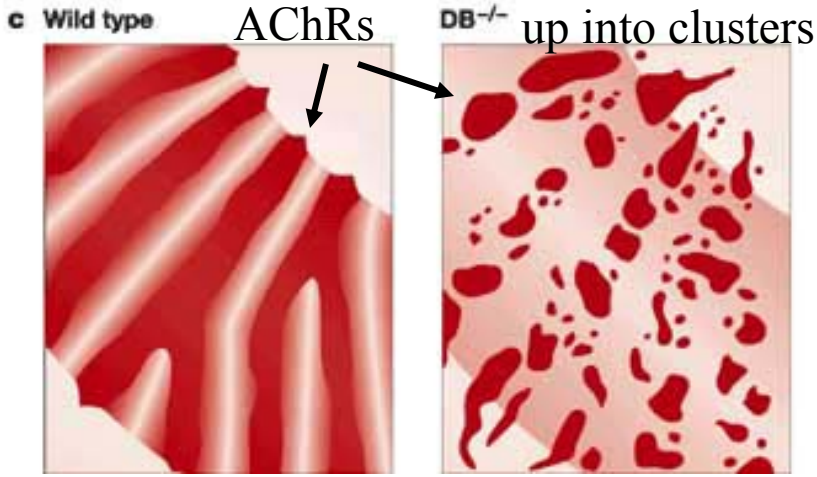
8. Junctional folds form with acetylcholinesterase and voltage dependent Na+ channels concentrated within the folds.



Activity appears to add AChRs to the junctions. Inactivity removes AChRs from the junction. Arrows show processes thought to be affected by activity.



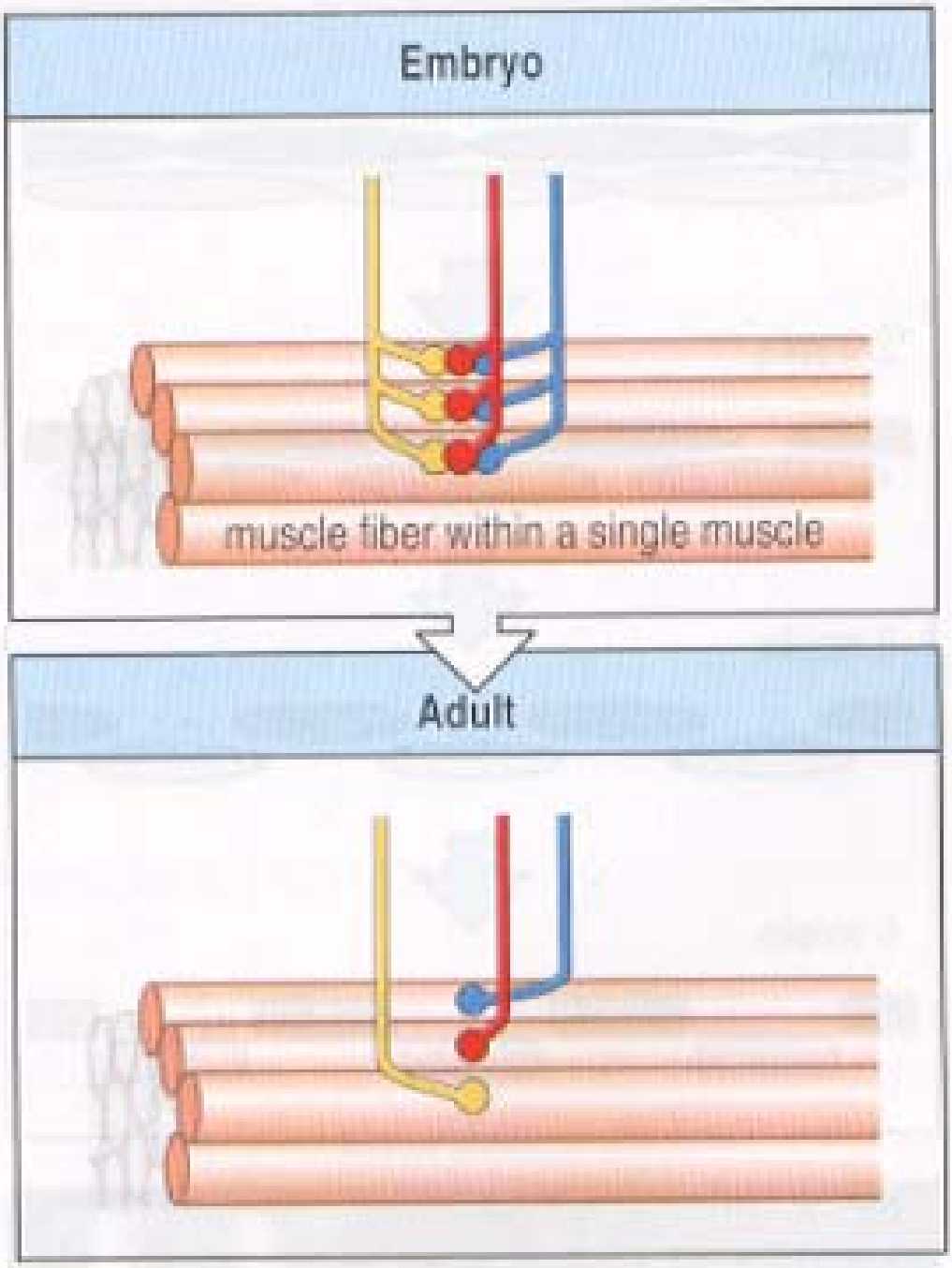




no folds
AChRs break-up into clusters

DB =dystrobrevin

In all fast muscle fibers multiple motoneurons initially innervate each muscle fiber. With activity each muscle fiber retains input from only one motorneuron.



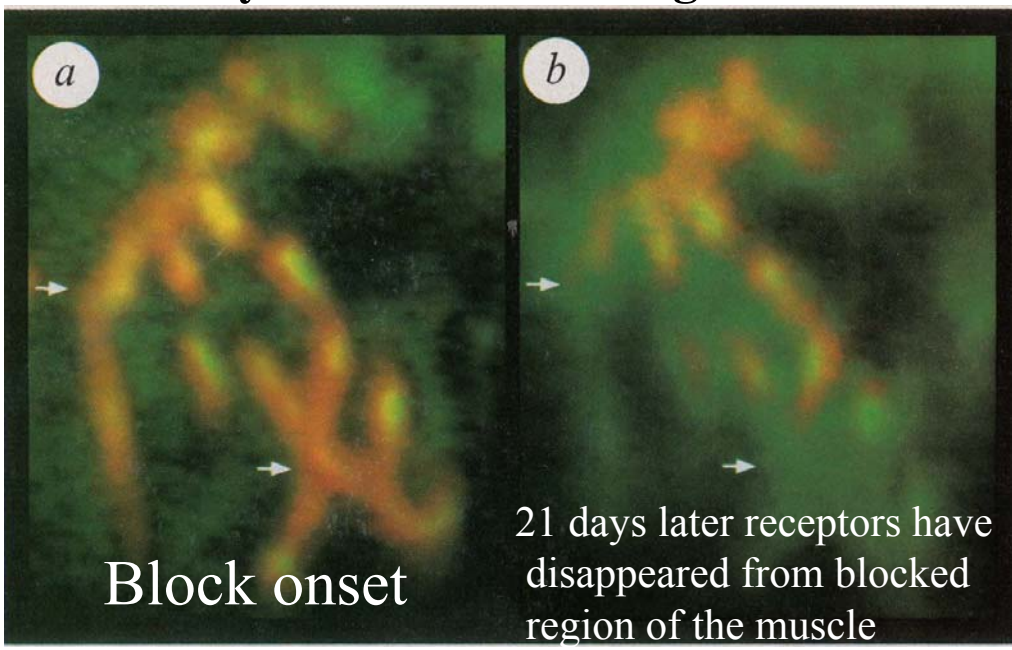
If the entire NMJ is silenced by high concentrations of bungarotoxin (an irreversible inhibitor of AChRs) there is very little change in polyneuronally innervated junctions.

If part of a junction is silenced by local application of bungarotoxin the nearest terminal withdraws from that region.

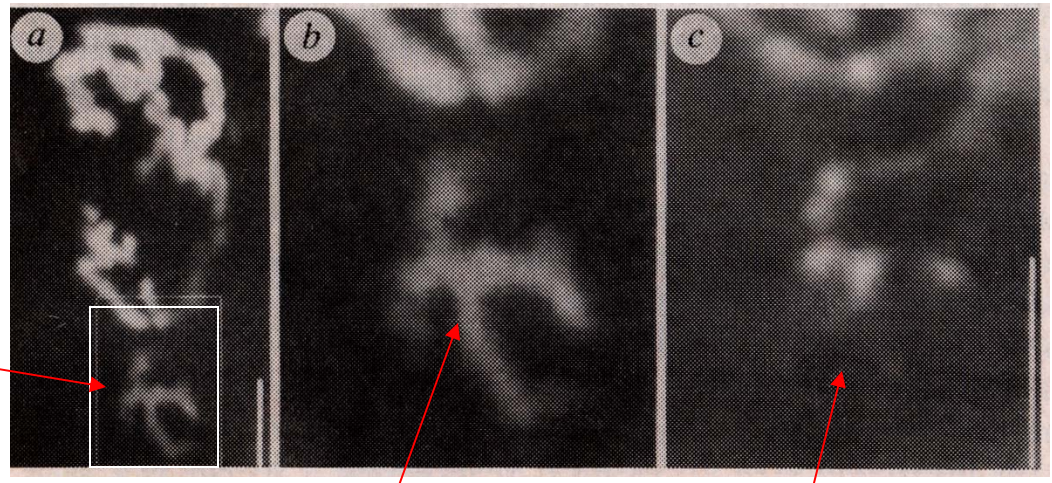
Presynaptic terminal withdraws from region of the EP muscle membrane where some of the AChRs have been irreversibly blocked with bungarotoxin

Postsynaptic
Bungarotoxin= red

Presynaptic
Green =motoneuron terminal mitochondria



Region of junction with ACh receptors blocked 17 days earlier



Faintly stained ACh receptors

Withdrawal of nerve terminal in same region

From Balice-Gordon & Lictman 1994, Nature 372:519.

REMEMBER CRITICAL OBSERVATION:

When the entire end-plate is silenced (massive dose of Bungarotoxin), multiple terminals remain.

Suggests competition among synapses for survival.

Competition Model:

Activation produces a long range signal destructive to presynaptic release mechanism. Also a short-range signal that is protective.

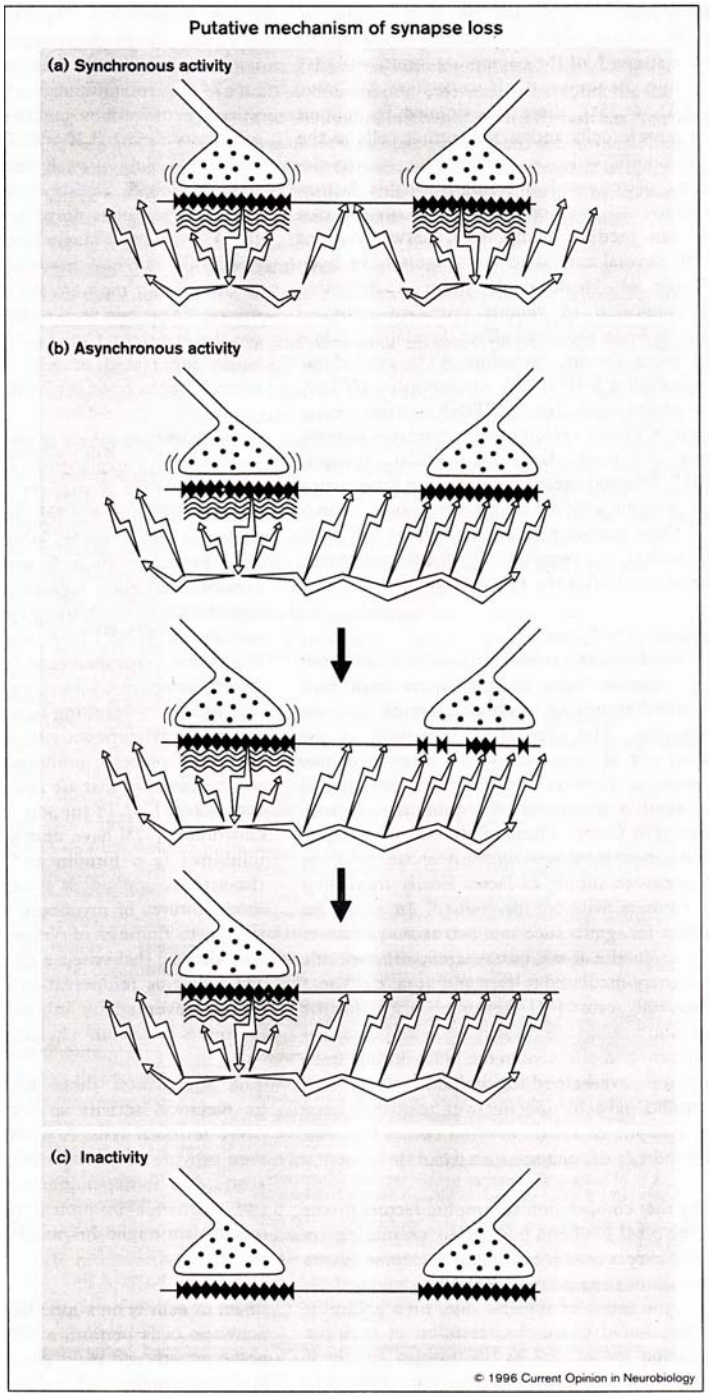
1. Inactive input begins to decrease its release

2. Decreased release results in decreased receptor expression.

3.With decreased receptor expression get decreased receptor activation etc.

4. Result = removal of the terminal

When both inputs are silenced there is no competition. Multipe inputs remain.



Definitions:

Motor Pool= in the CNS all the motoneurons that innervate the same muscle on one side of the body.

Motor Unit= One motoneuron and the subset of muscle fibers within a muscle that it causes to contract.

Questions:

If you backfilled motoneurons from a muscle nerve with a retrograde label, what would you visualize in the CNS?

The cell bodies and dendrites of the motor pool for that muscle.

If you were intracellular in a motoneuron in a preparation where the ventral roots were still attached to muscles and you stimulated a soleus muscle motor neuron, what would you expect to see contract in the periphery?

The motor unit . The subset of muscle fibers in the soleus muscle innervated by the motoneuron you were stimulating

Muscle fibers (mutinucleate muscle cells) can be either fast twitch: rapid onset short contraction or
Tonic or Slow: onset prolonged contraction prolonged contraction
Fast muscle fibers have only one motorneuron innervating them. This is not true in babies.

Mouse soleus is composed of only fast muscle fibers innervated by ~ 20 motorneuron. The soleus has 20 motor units.

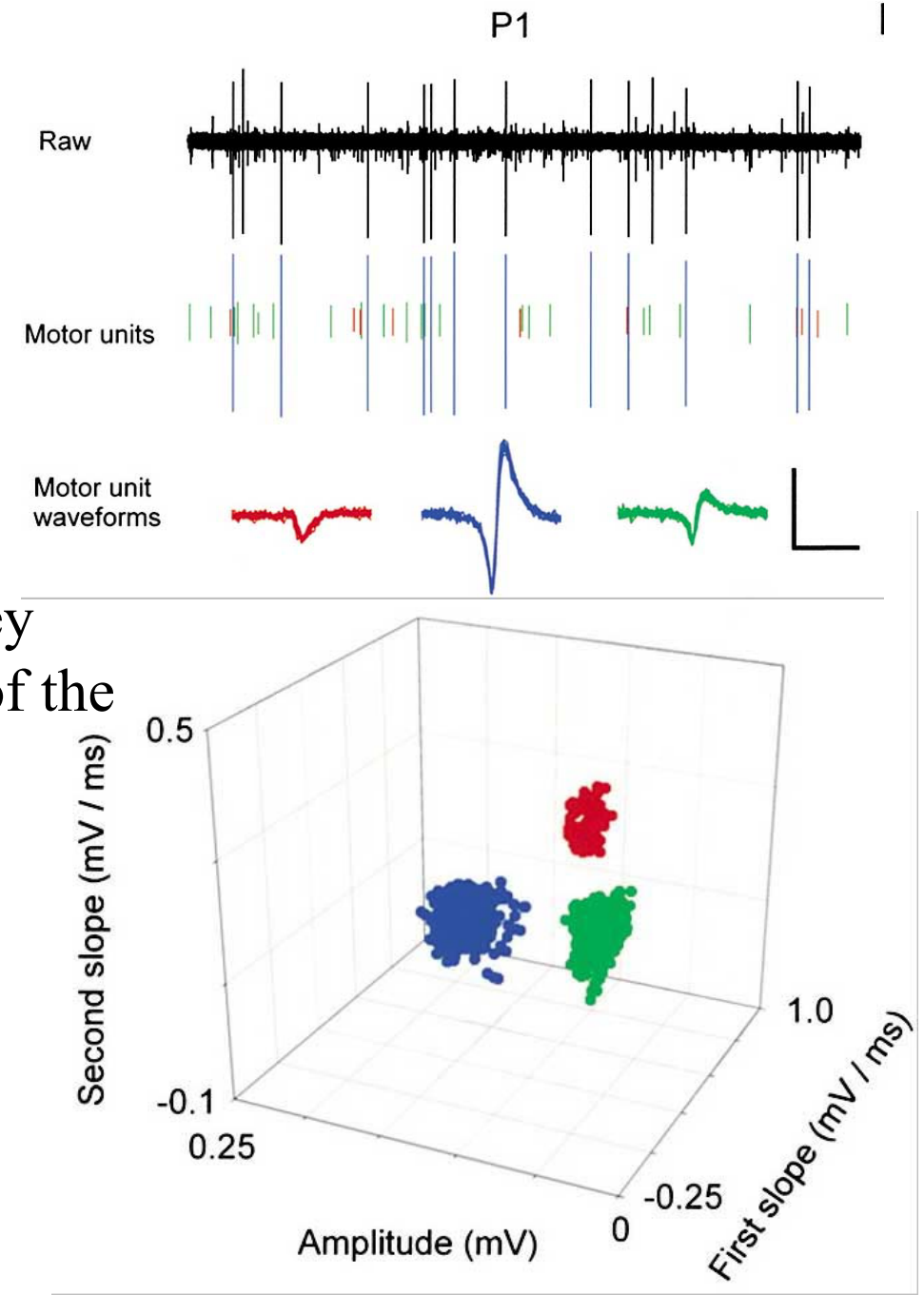
Electromyography uses an extracellular electrode inserted into the muscle to record the compound action potential whenever a single motor neuron and its innervated muscle fibers contract. This is an EMG.

EMGs were recorded from awake behaving baby mice.

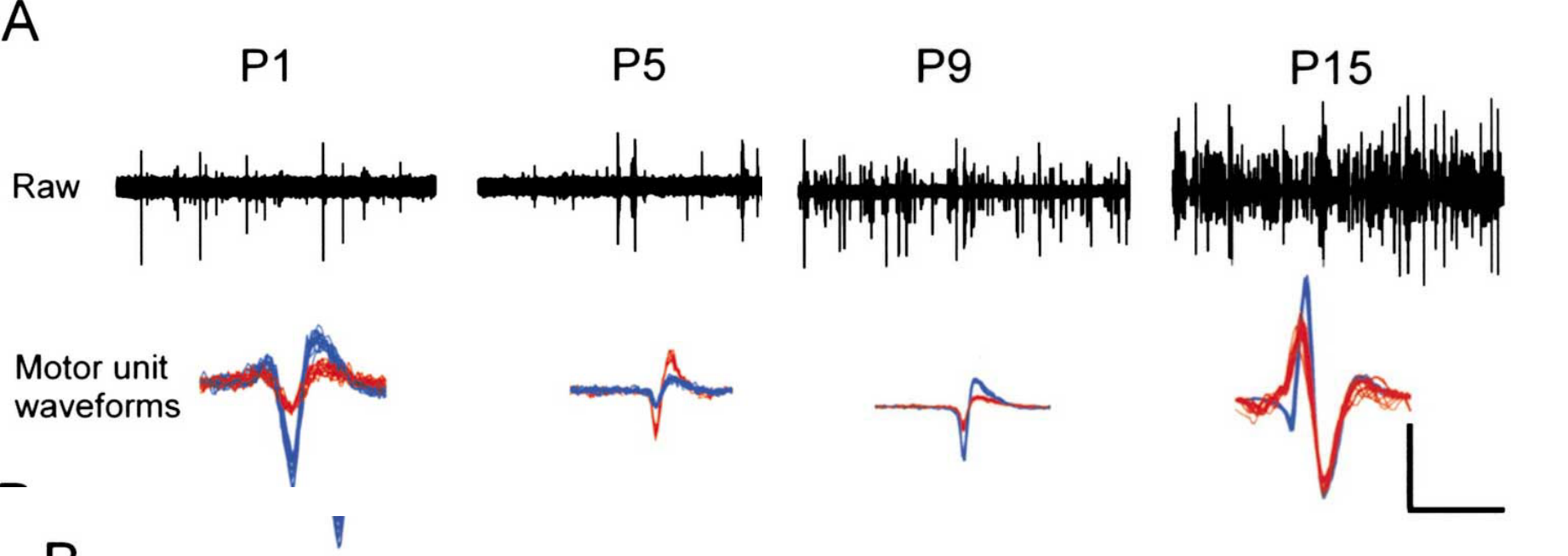
Earlier observations reveal:
P0-P4 80-100%of muscle fibers are multiply innervated
P5-P6 ~ 70% of muscle fibers are multiply innervated
P8-P9 ~ 50% of muscle fibers are multiply innervated
P14-P15 ~100% of muscle fibers are multiply innervated

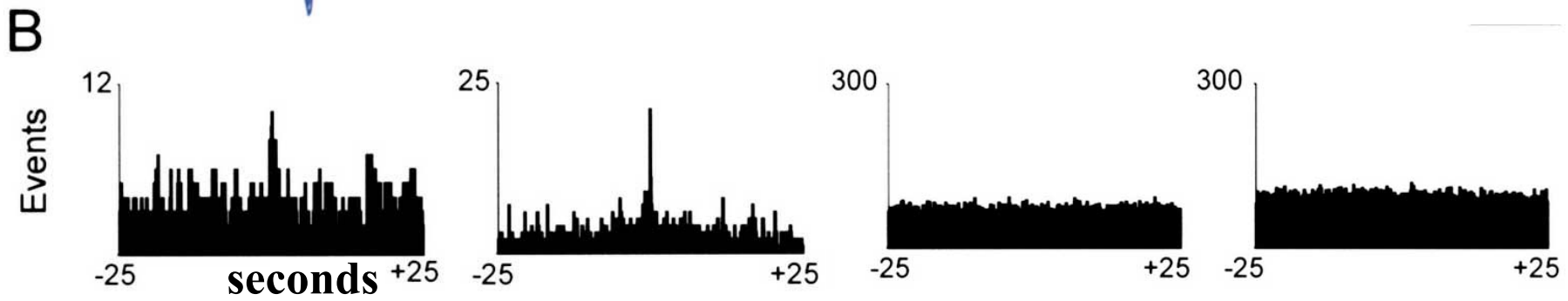
In vivo recordings from single motor units.
At birth 2 to 6 motoneurons innervate soleus muscle fibers.

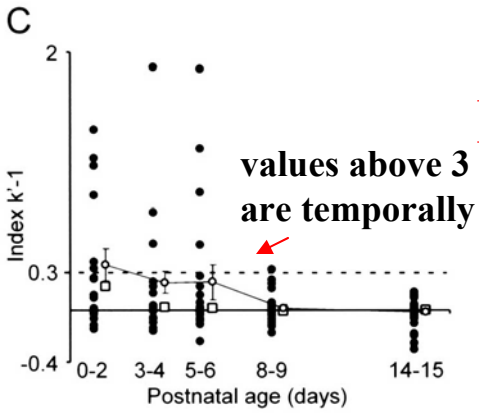
Distinctions between different innervating motor neurons and the fibers they Activate are made on the basis of the shape of extracellularly recorded compund action potentials.



From: Personius & Balice-Gordon (2001) Neuron 31:395-408.

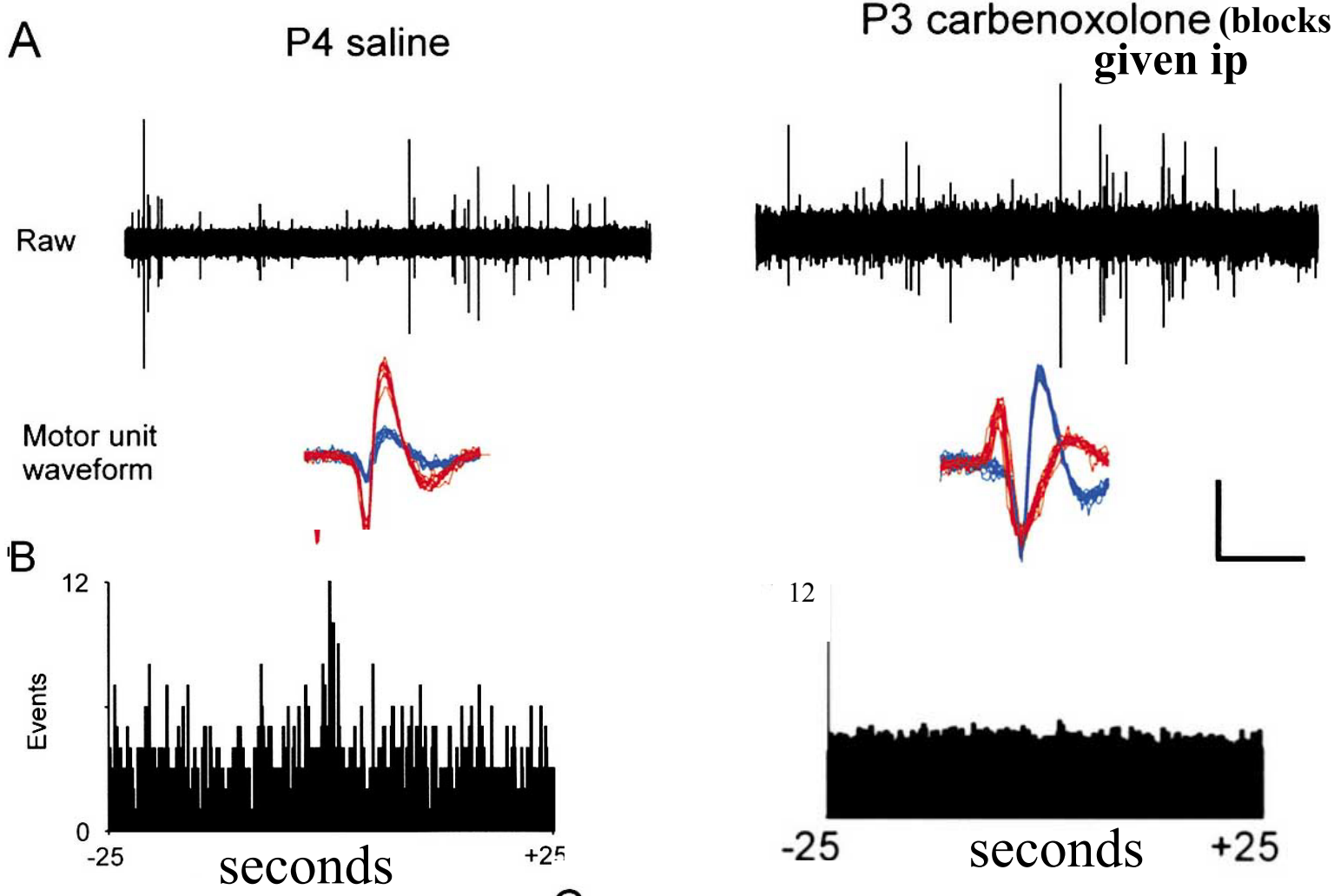




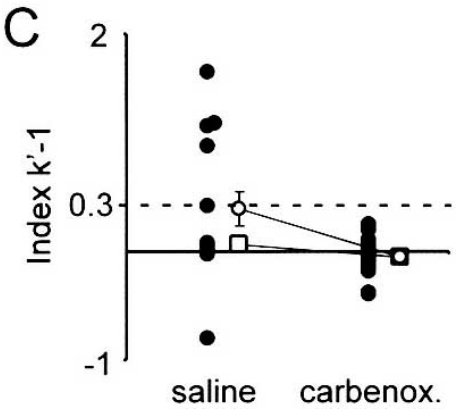


Highly Correlated Motor Unit Firing Is Is Detected Only In Early Polyinnervated Muscles

Strength of correlation given by the correlation index *k*'-1 = calculated from the total number of extra counts within the peak region above that expected by chance.



Blocking gap junctions in the spinal cord blocks motor unit correlations in the muscle.



Open circle = mean
Open square = median

Suggests that correlated activity among motoneurons of the same motor pool may allow multiterminal innervation during early development. However, once gap junctions begin to disappear each motoneuron's activity will be slightly different and competition begins.

What is the molecular basis of this competition?

The standard experiment is to find 2 inputs to the same muscle cell. Stimulate one input vigorously,while recording the end-plate current (or potential) of the the stimulated and the second input. The **non-stimulated** input decreases in amplitude **(post-synaptic depression).**

In culture simplify this situation by using a pipette filled with ACh

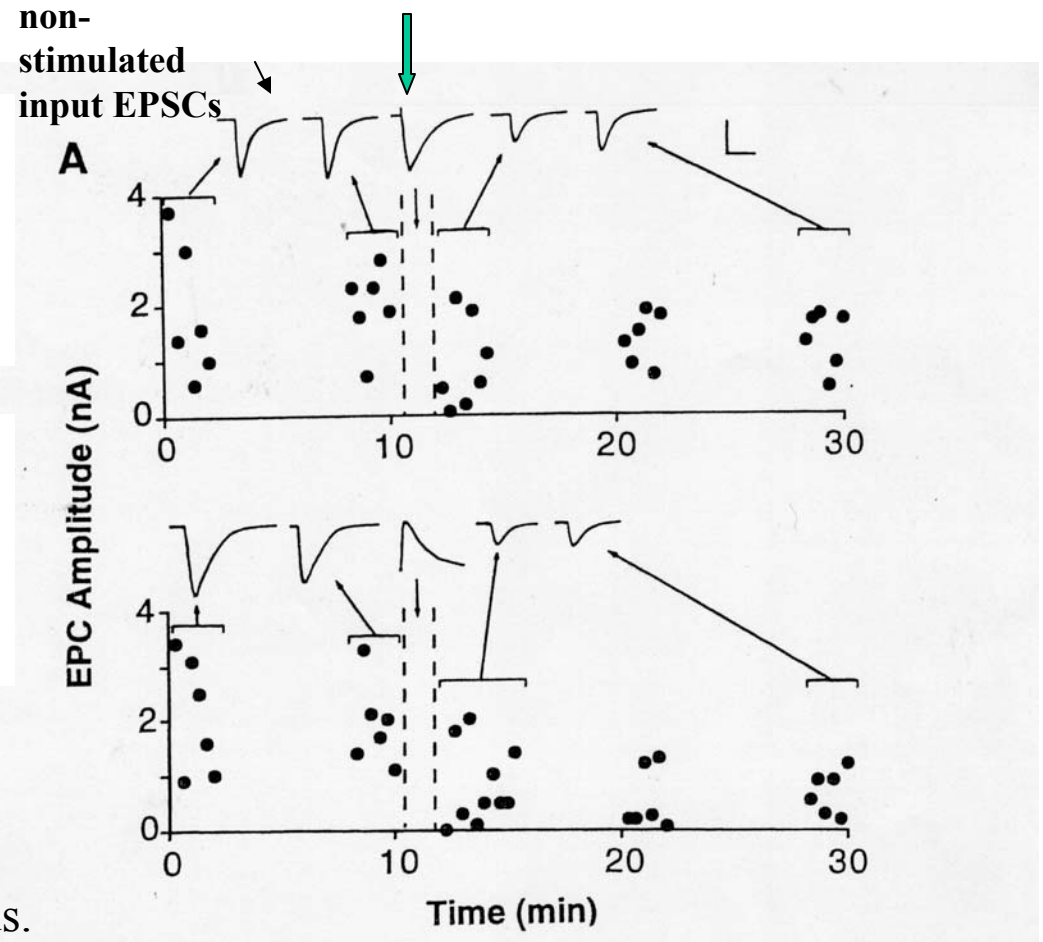
From: Dan & Poo 1992, Science 256:1570

ACh applied at 2/sec for 50 seconds

Fig. 2

Muscle held in voltage clamp

Muscle held in current clamp during stimulation



But, must allow postsynaptic Ca++ change! If BAPTA is in the muscle cell, there is no change in current or potential to the test stimulus.

How Do You Determine Where Synaptic Plasticity Is Implemented Pre- or Post-synaptic?

(Bekkers & Stevens,1990, Nature 346: 724)

Three variables of quantal synaptic transmission from one pre-synaptic cell to one post-synaptic cell.

Physiological event or state	*variable*
1. Number of vesicle release sites present. Likely to be number of docked vesicles. Total number of available quanta.	**N**
2. Probability of release of 1 quanta of transmitter. Probability of release at 1 vesicle release site.	***p***
3. Size of the quantal response. Post-synaptic change as a result of the release of 1 vesicle. Generally estimated as the the average size of the smallest mini peak in frequency versus amplitude plot of minis.	***q*** ***a***
5. Quantal content of any response	$m = aNp$

blue = generally measured variables

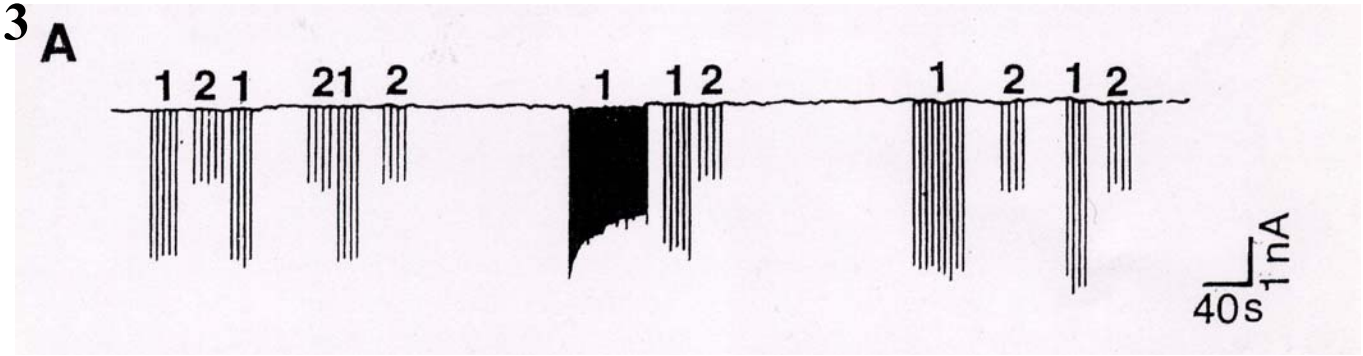
Is the Change Pre- or Postsynaptic?

Answer: Appears to be presynaptic.

Take an uninnervated myotube and use two **ACh pipettes** at positions 1 & 2

This eliminates any real nerve terminal, Consequently any change has got to be post-synaptic.
Now alter the frequency of stimulation at one position.
Get NO CHANGE at 2.

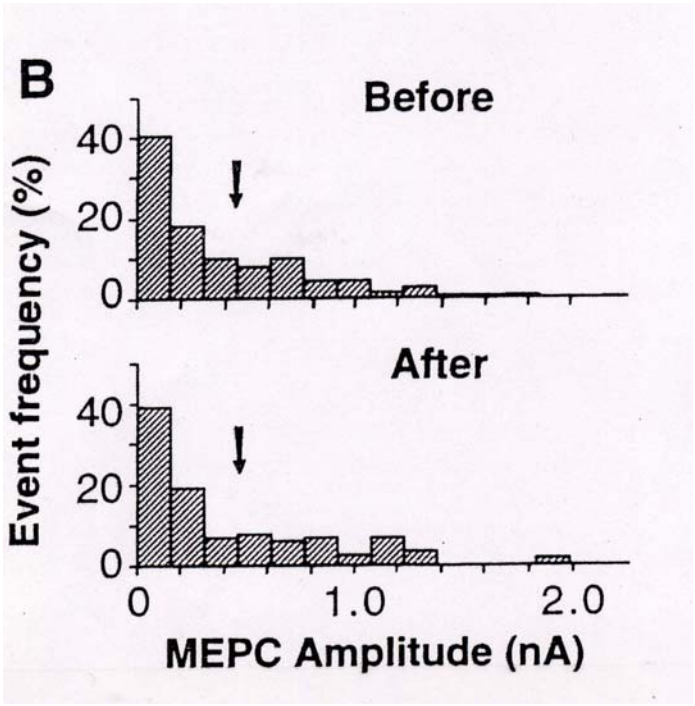
Fig. 3



From: Dan & Poo, 1992 Science,256:
pp.1570

2nd Experiment: Look at miniature currents (with TTX) from a real terminals before and after stimulation

Fig. 3



Looking at post-synaptic current
From the "depressed" input before
And after ACh pipette stimulation
Then put on TTX to block evoked responses. Look at miniature synaptic events.
NO CHANGE IN MEAN
(after versus before)

Definitions (again)

q = a quantum = 1 synaptic vesicle of transmitter
m = quantal content
Quantal content of an evoked EPSC = aNp
Where:
a="average" quantal size;
N=total number of available quanta;
p= release probability
(believed to be very small)
(see Bekkers & Stevens 1990, Nature,346:724)

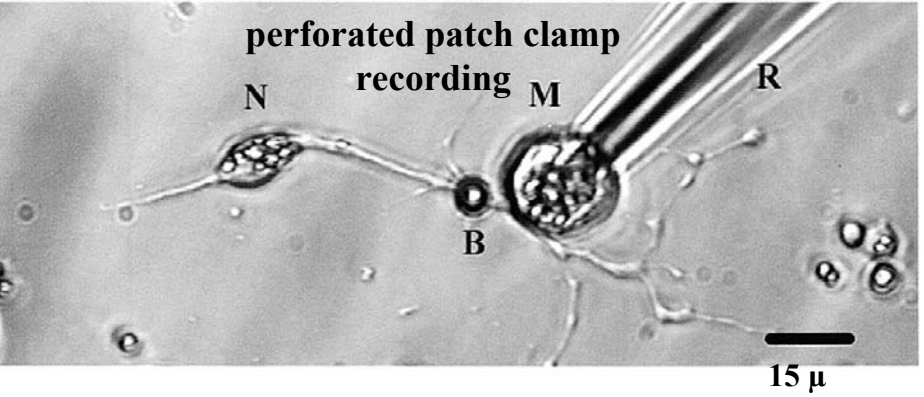
Quantal Analysis:

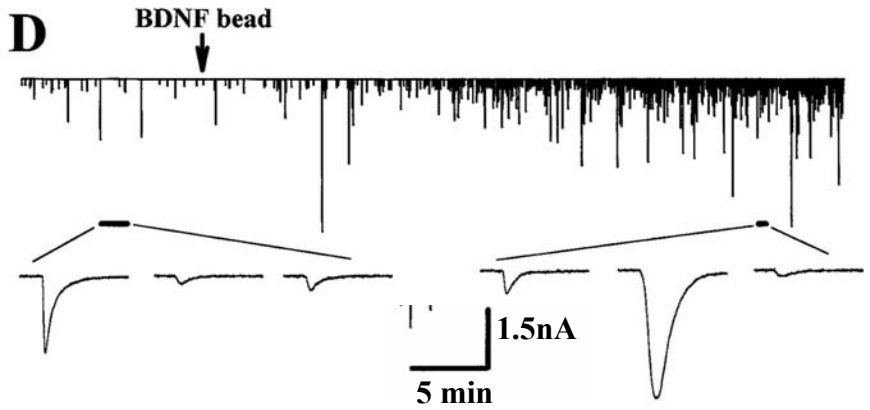
Mini's are due to the spontaneous release of 1 packet (1 vesicle) of transmitter.
Minis are believed to be independent of the mechanisms of evoked release (an assumption tested many times, generally but not always true).

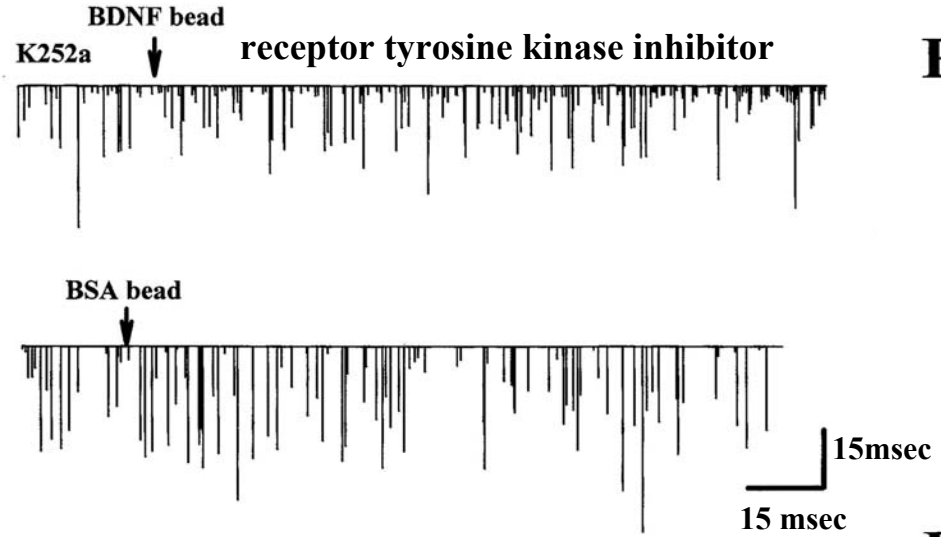
This experiment found:
No change in mini-size (estimates a)
but a decrease in quantal content
of EPSCs (a)(Np) which
strongly suggests the change is in Np
Both N & p are pre-synaptic parameters.
Thus the results suggest
that the change is pre-synaptic.

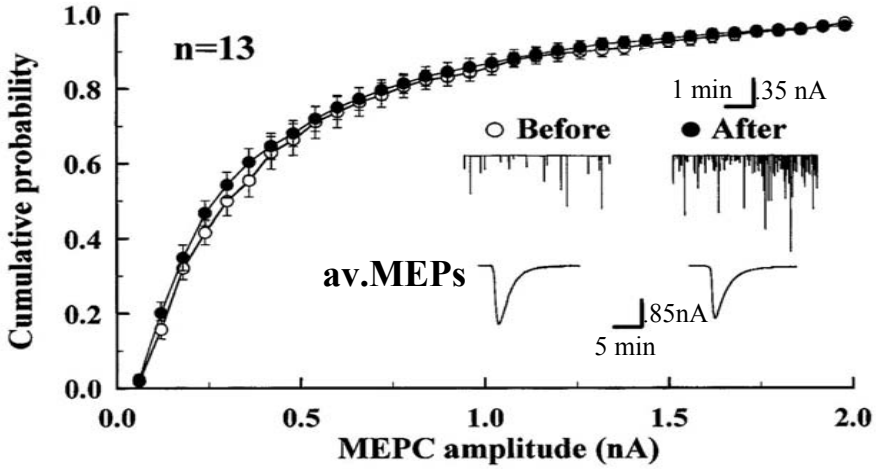
BDNF-coated bead within 0.25μ of endplate increases the frequency not the amplitude of MEP currents

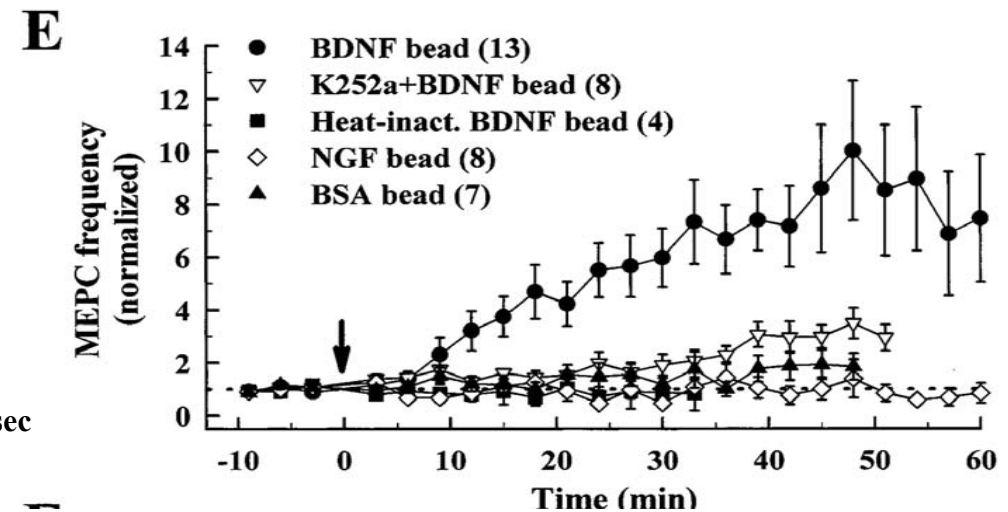
(Zhang & Poo, 2002)





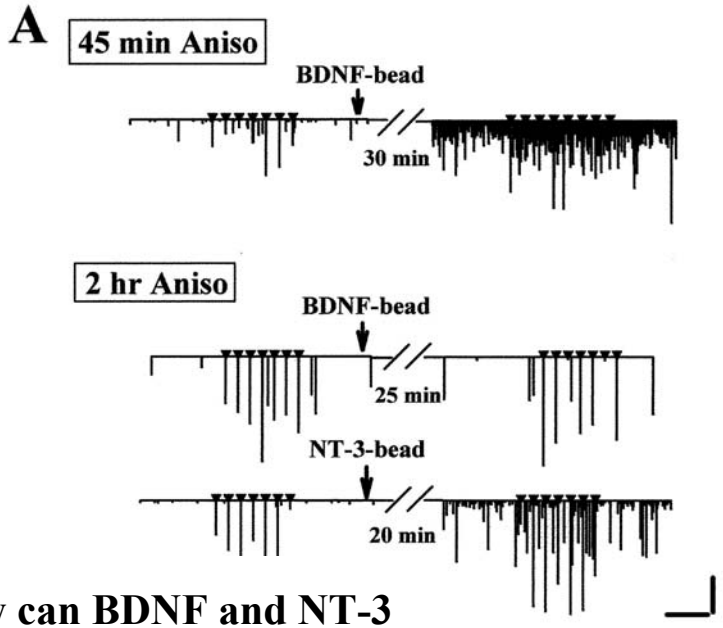


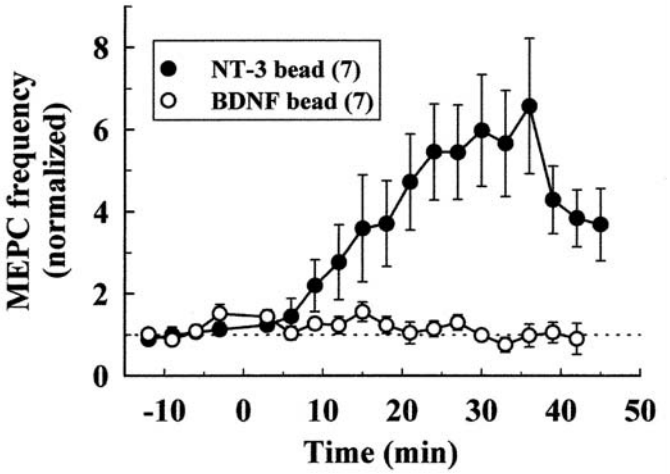


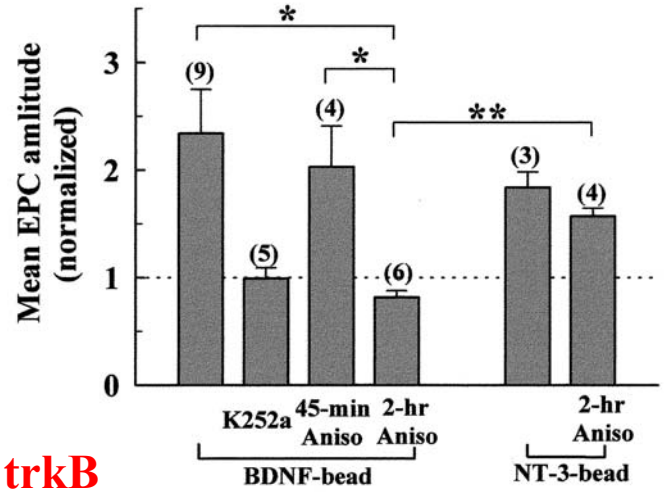


Synaptic Potentiation Induced by BDNF Beads Requires Protein Synthesis

1. Covalent attachment of neurotrophins to beads restricts activity to the presynaptic process.
2. Cells are incubated in anisomycin for either 45 min or 2hrs.
3. NT-3 also produces an increase in MEP frequency.







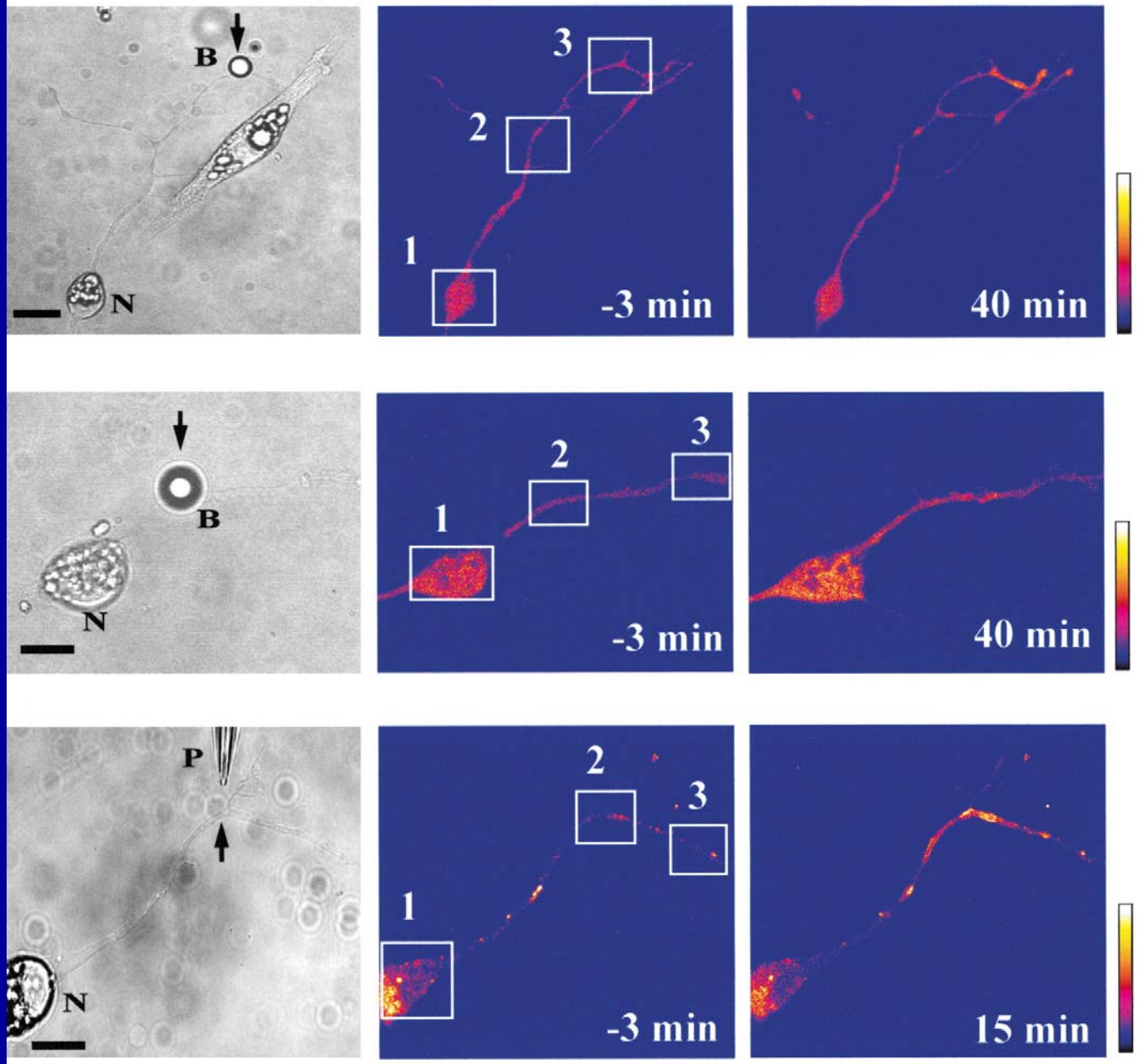
How can BDNF and NT-3 be differentially sensitive to translation inhibitors?

BDNF acts through trkB

NT-3 acts through trkC

BDNF beads induce a local increase in [Ca++]i. A puff of BDNF to an axon will induce a local Ca++ change.

A puff of BDNF to an axon will induce a local Ca++ change.



Cells loaded with Ca++ indicator through a sharp electrode earlier

BEFORE

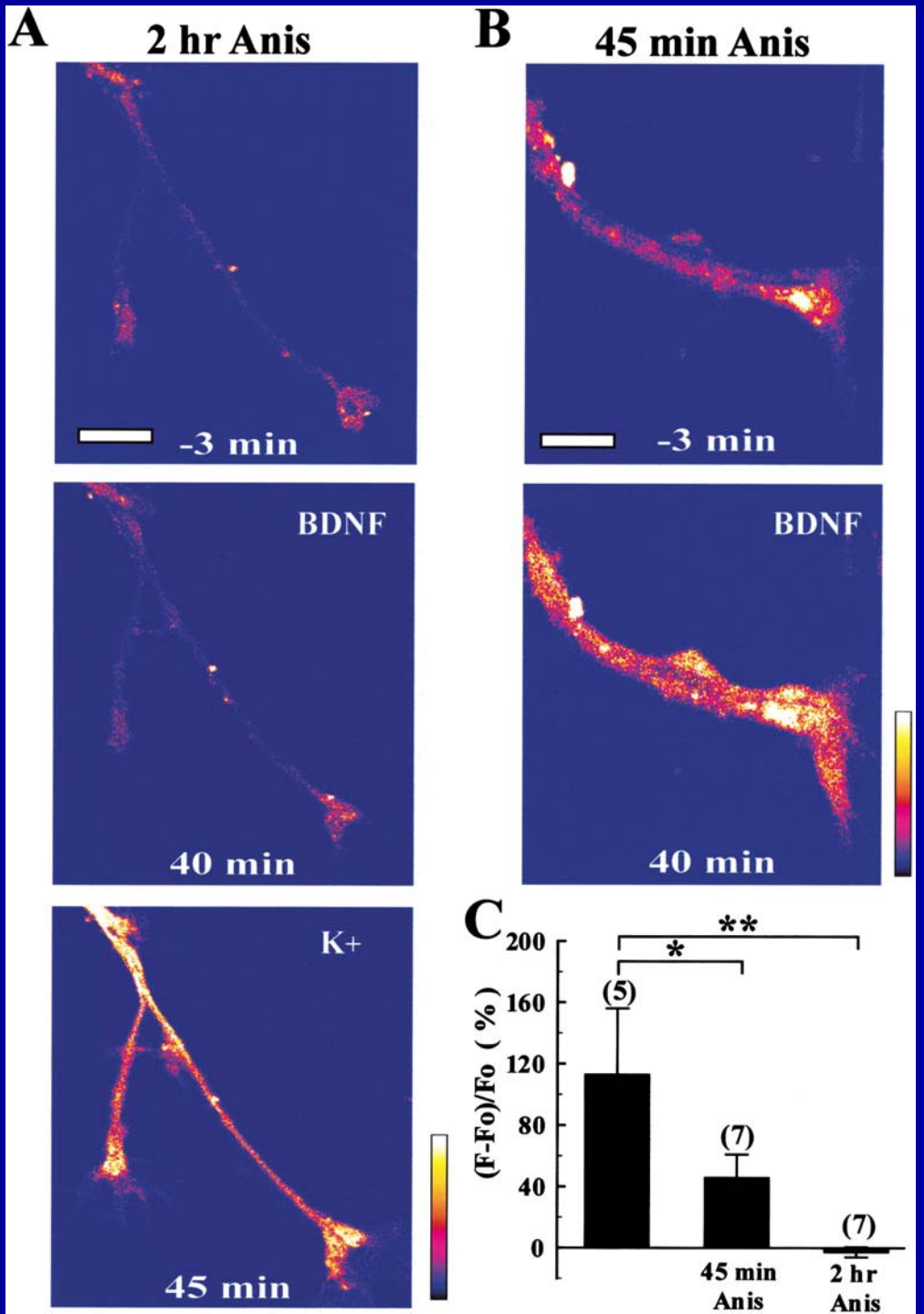
After

BDNF beads induce the local increase in [Ca++]i. This is blocked by 2 hrs in anisomyosin but not by 45 min in anisomysin.

Anisomycin does not block Ca++ channels when activated by membrane depolarization induced by K+

How can you test that the protein translation is in the axon itself ?

Cut the axon off from the cell body.



Suggests that a neurotrophin or neurotrophins may be involved in the “protective” response. As we will see there is evidence that neurotrophins may be released upon activation of a neuron.