Journal of Neurocytology **10**, 101–110 (1981)

The overall morphology of neuromuscular junctions as revealed by scanning electron microscopy

JUNZO DESAKI and YASUO UEHARA

Department of Anatomy, Ehime University, School of Medicine, Shigenobu, Ehime, Japan

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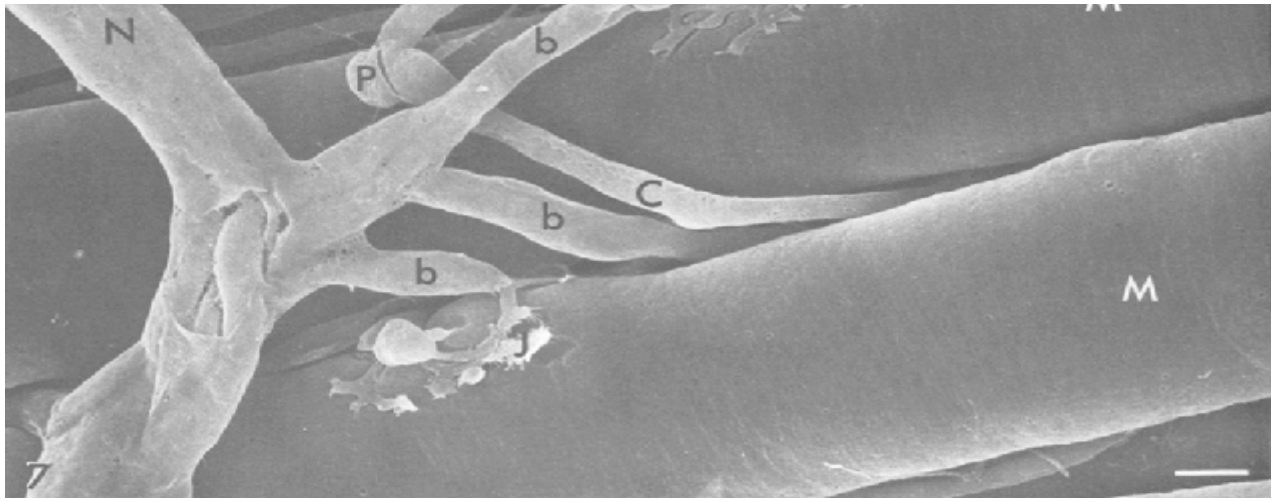


…..putting things into perspective……..

First Transmission Electron Microscope (TEM) created in 1931

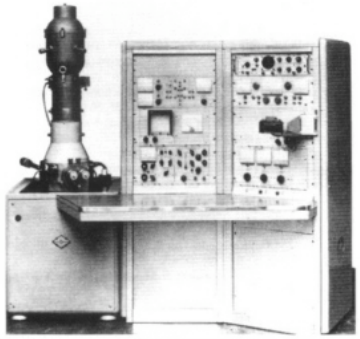


FIG. 14 The prototype of the first Stereoscan SEM, supplied by the Cambridge Instrument Company to the duPont Company, U.S.A. (Stewart and Snelling 1965). Courtesy of Leica Ltd.

First Scanning Electron Microscope (SEM) created in 1942

First commercial instruments arriving around 1965

What had been seen of the structure of the neuromuscular junction at that time?

Couteaux 1960

"Classical light microscopial studies with silver or gold impregnation, and vital staining with methylene blue, have established the general morphology of motor endings and histochemical methods for AchE activity have been applied to study the organisation of junctional clefts"

Peters et al 1976 and Heuser and Reese 1977

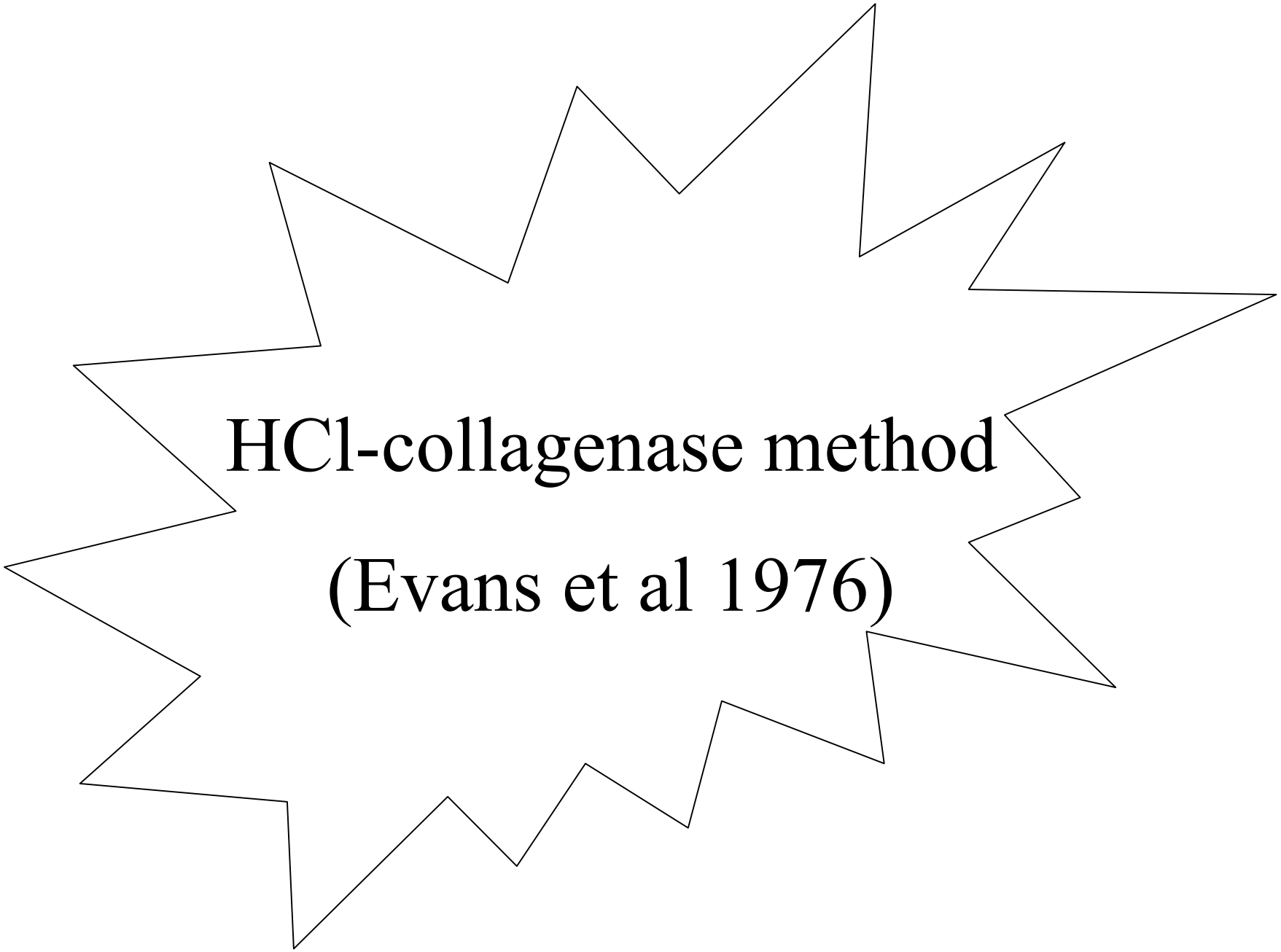
"electron microscopial studies on sections and replicas of freeze-fractured materials have extended our knowledge of NMJs, supplying the structural basis to interpret the process of synaptic vesicle turnover and transmitter mechanism."

However……

There had been no successful imaging of the 3 dimensional organisation of NMJs at the fine structural level.

Due to:

•Connective Tissues


HCl-collagenase method
(Evans et al 1976)

THEIR EXPERIMENT

3 different muscle types from 3 different animals

SARTORIUS MUSCLE

from a FROG

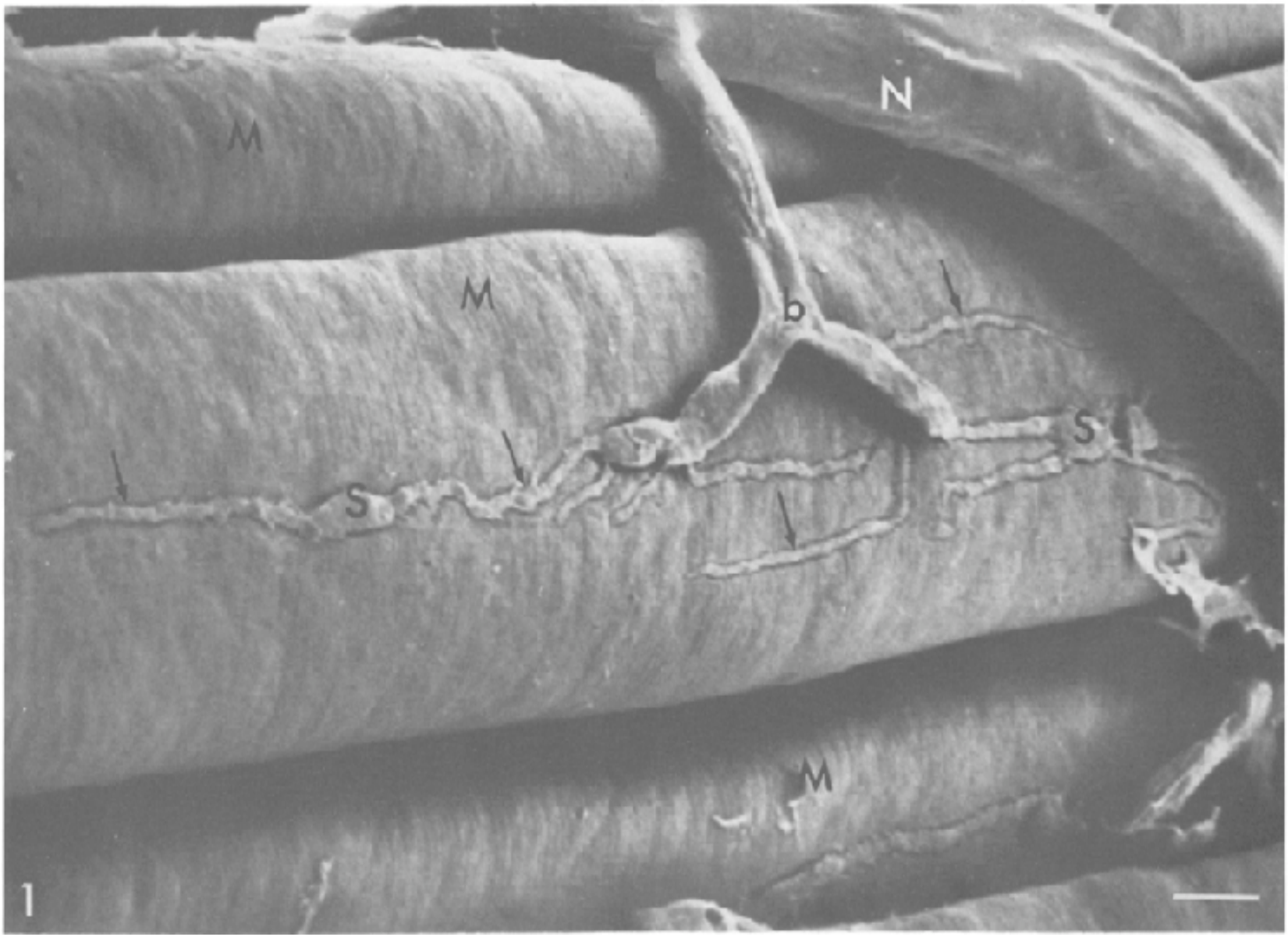
posterior part of the

LATISSIMUS DORSI MUSCLE

from a ZEBRA FINCH

STERNOTHYROID MUSCLE

from a CHINESE HAMSTER



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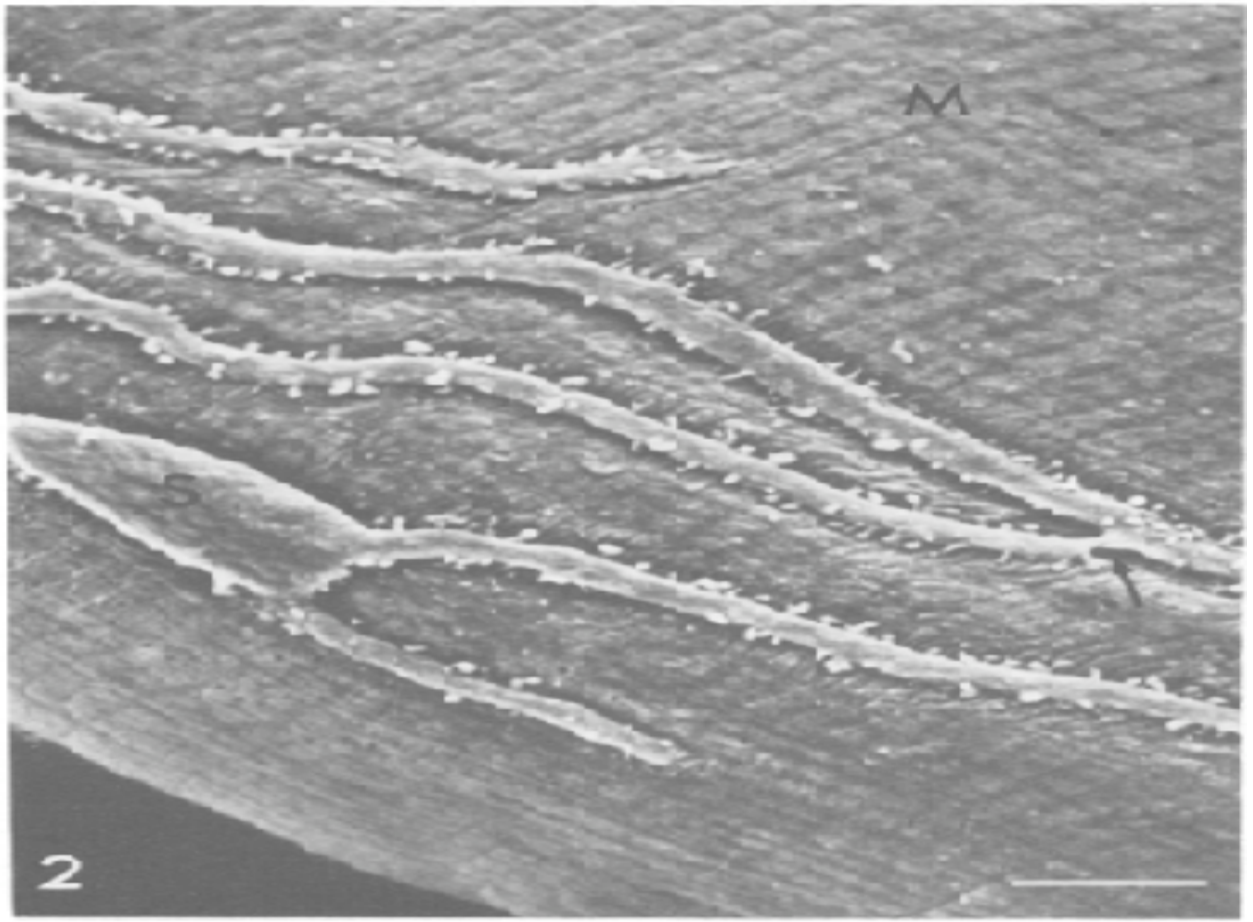
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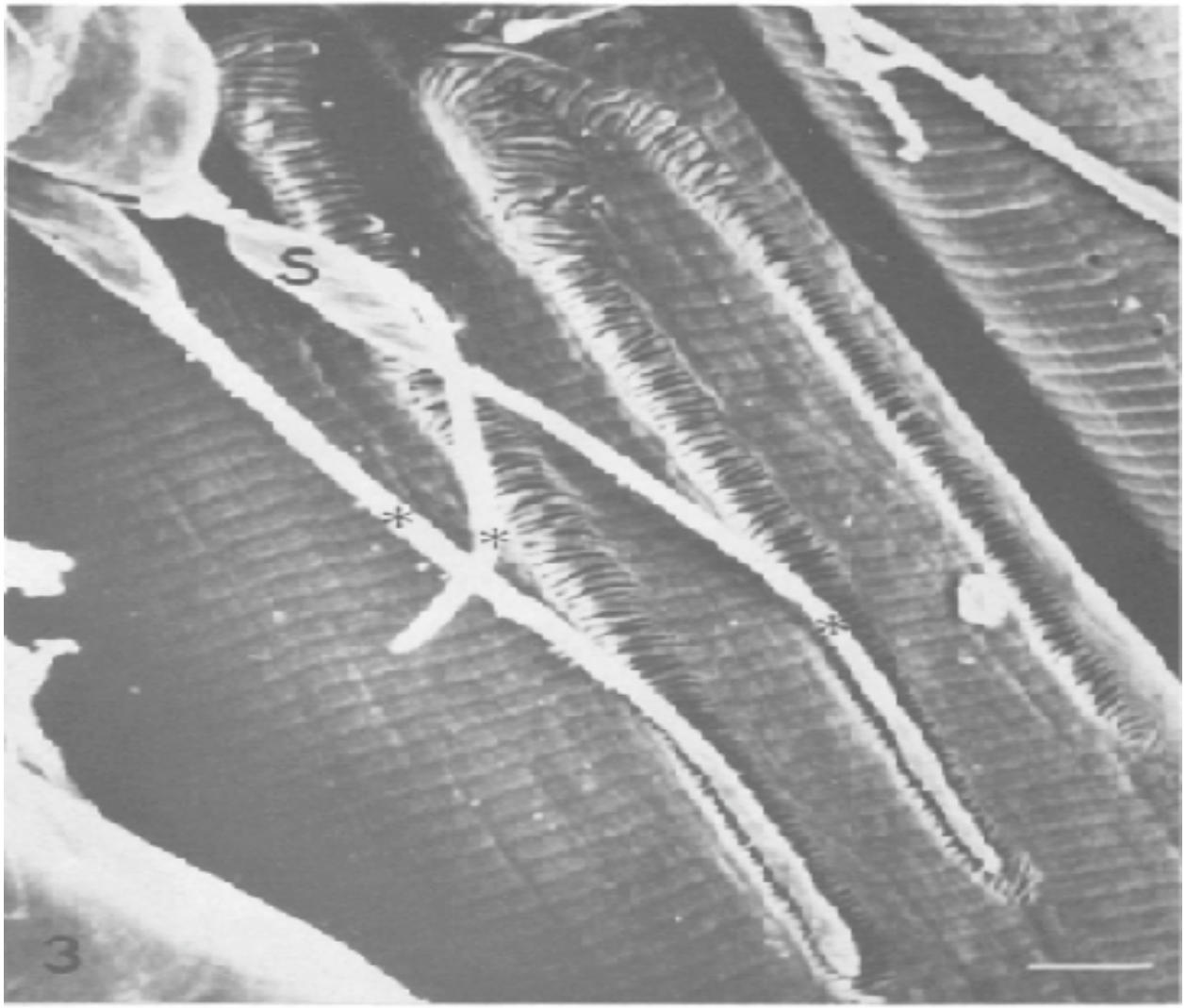
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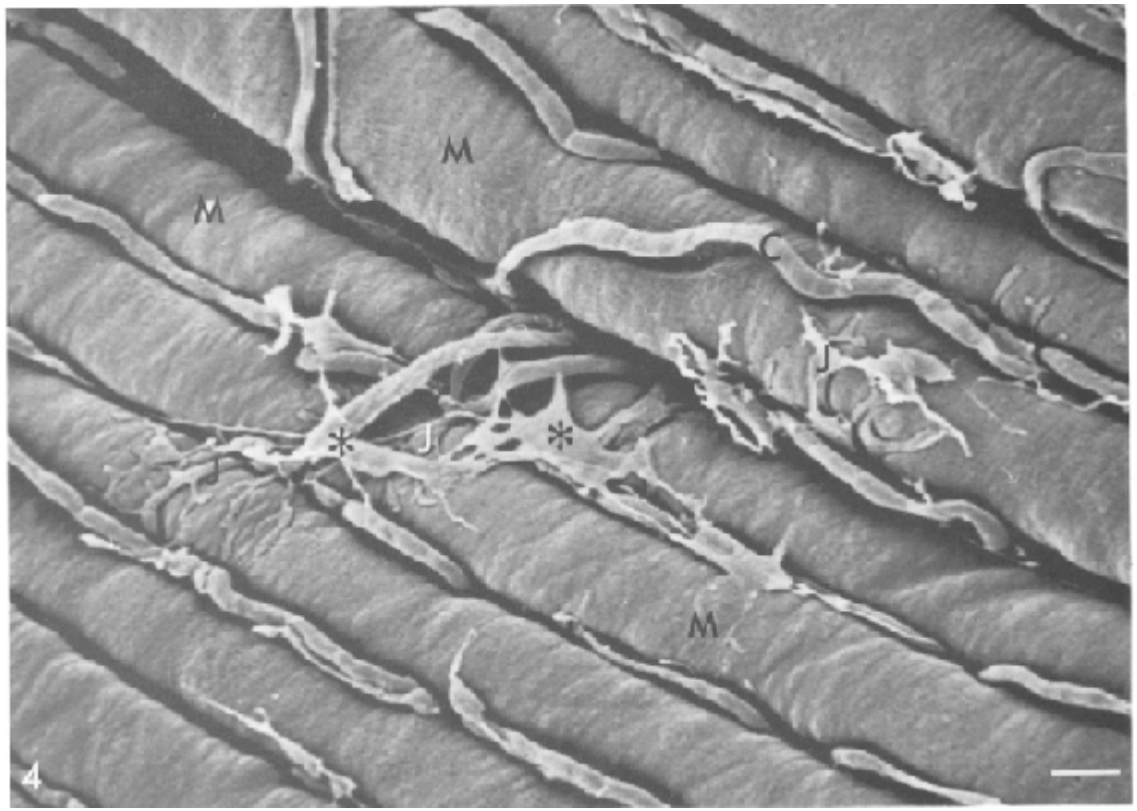
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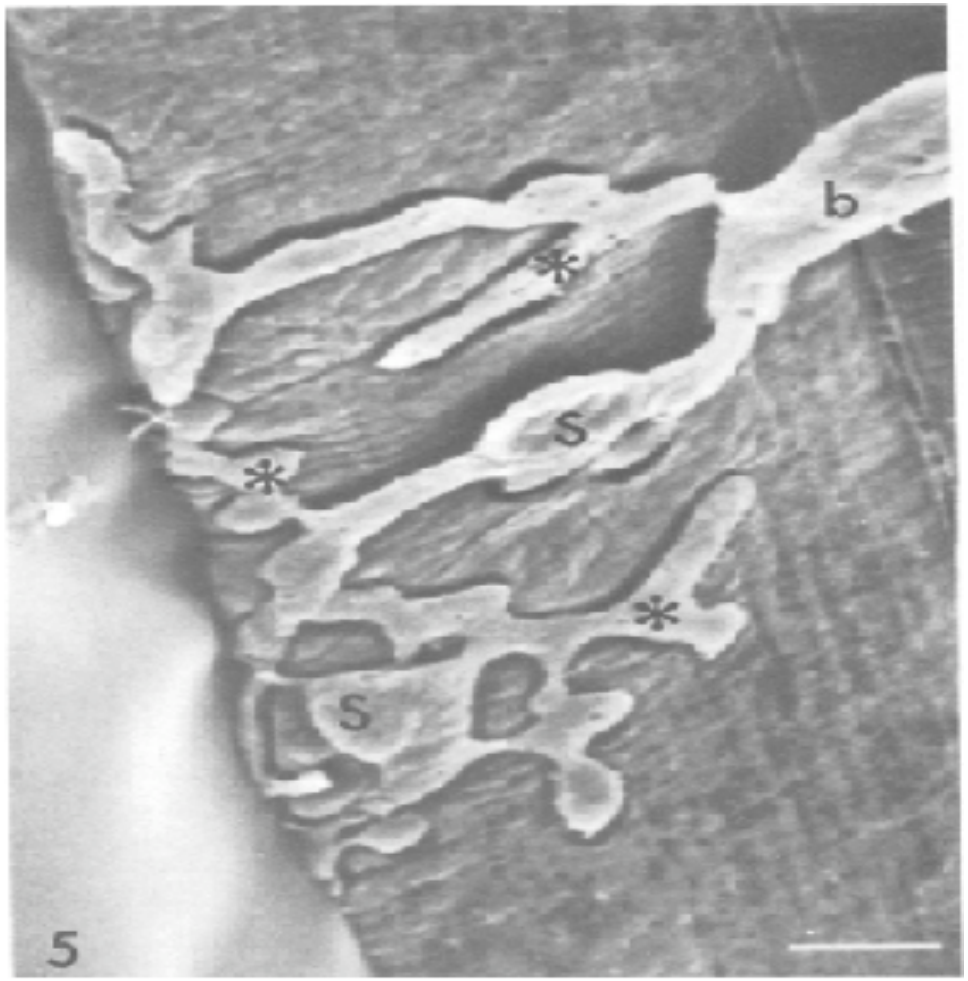
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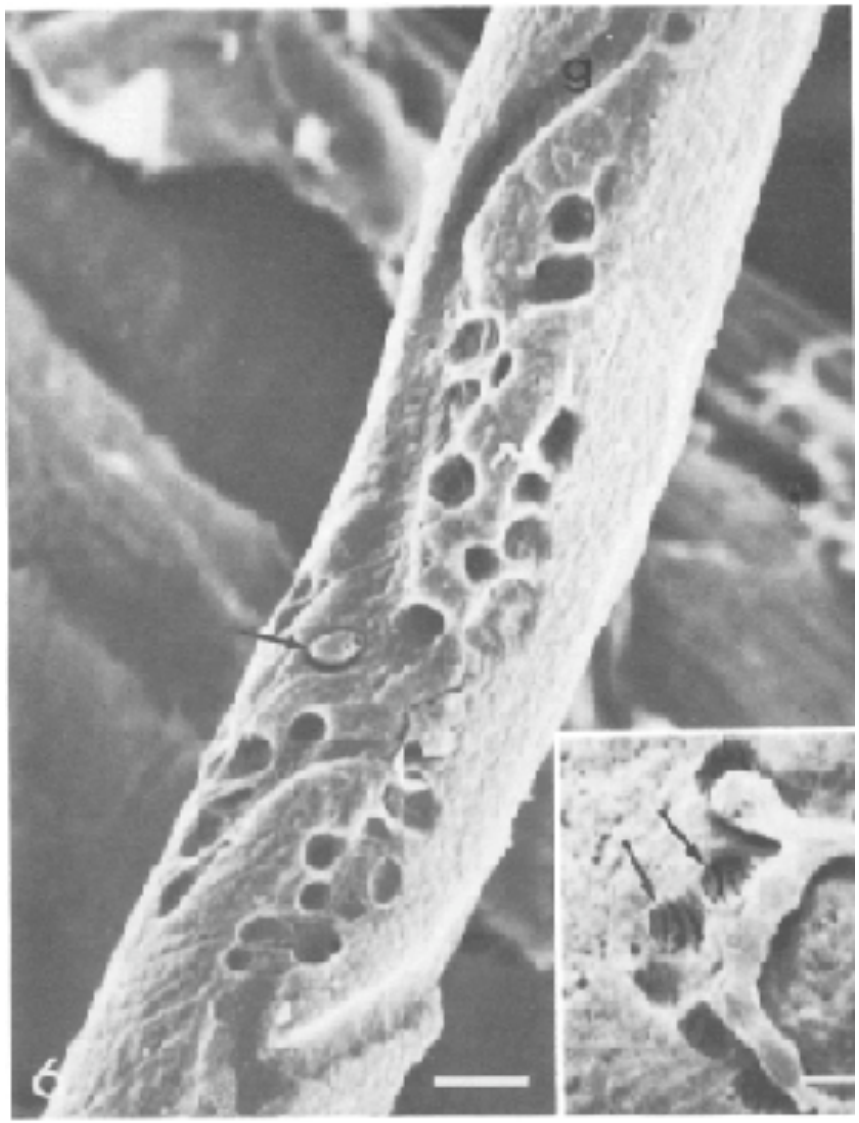
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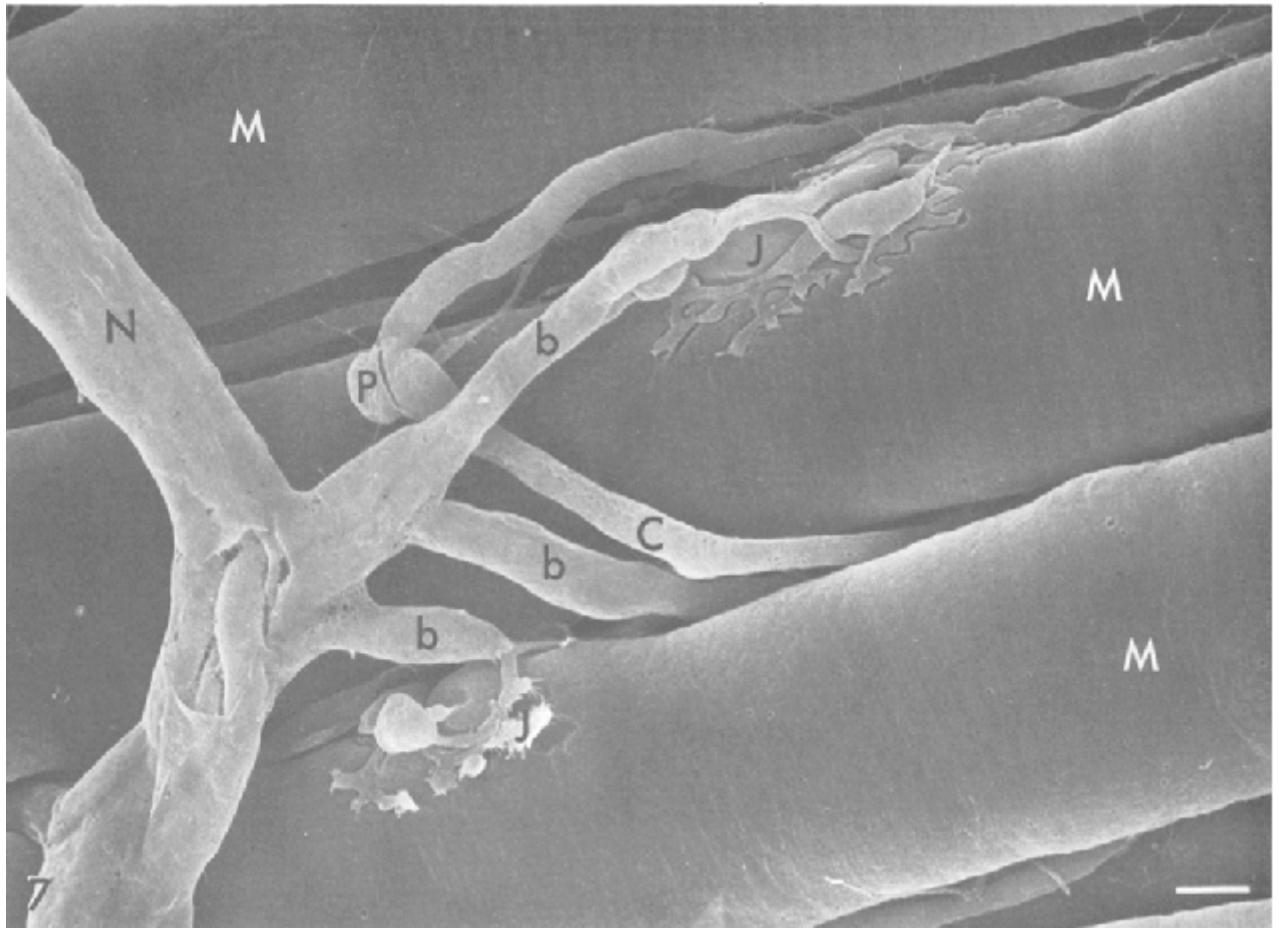
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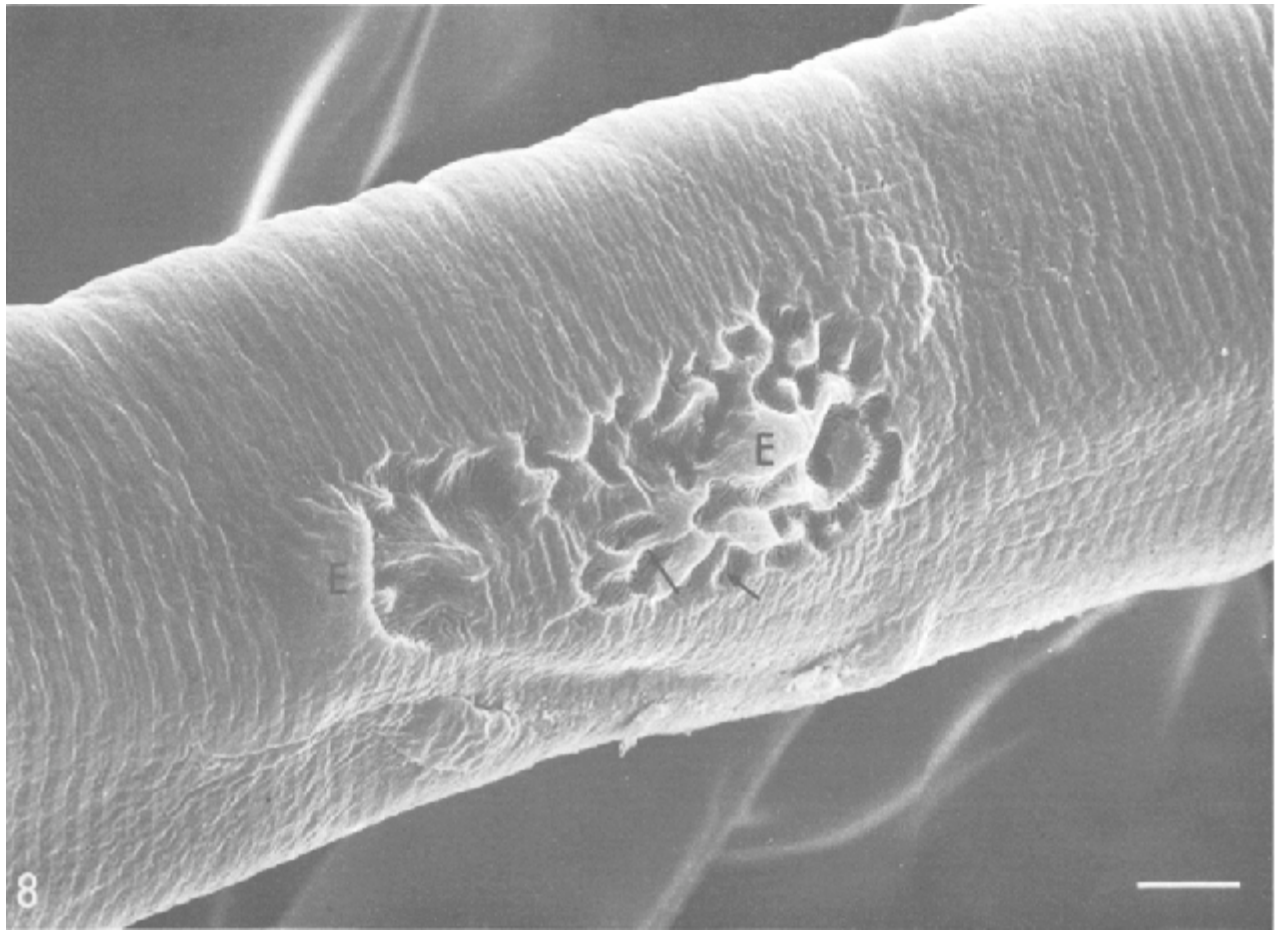
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Conclusions/Discussion

- Desaki and Uehara have managed to produce the first images displaying some of the finer structural features of the NMJ.
- The morphology differs between the 3 species, especially with regards the junctional folds, synapse depressions and projections of the sarcolemma.
- Revised the HCl-collagenase method.

The architecture of active zone material at the frog's neuromuscular junction

Hadlow ML, Ress D, Stoschek A, Marshall RM and McMahan UJ
(Stanford University) *Nature Vol 409 p479 Jan 2001*

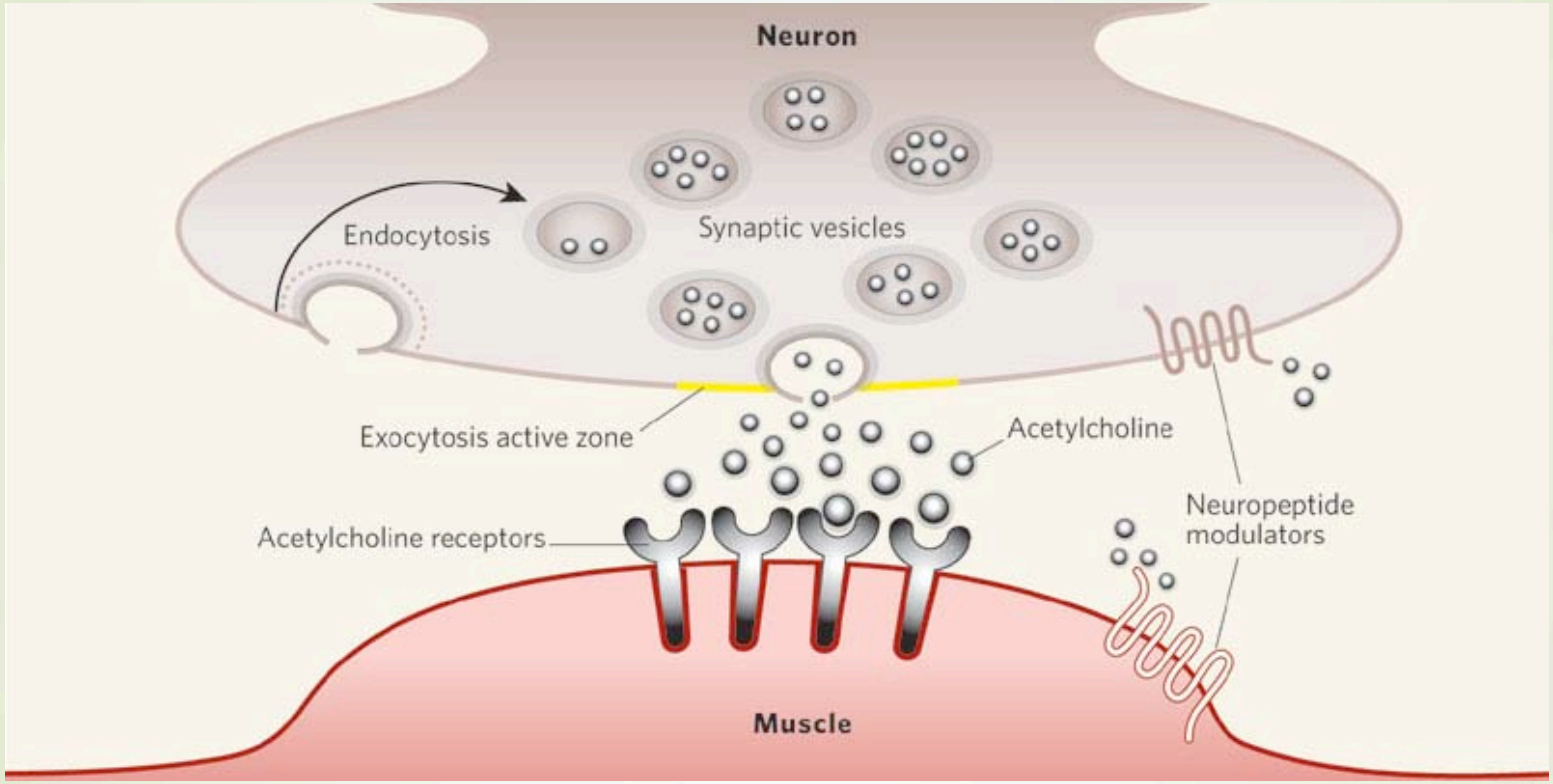
AIM

- To use electron microscope tomography
- Show the arrangement and associations of the active zone material (AZM)
- Using a frog neuromuscular junction as a model synapse

Eventual burning question….

- What is the exact mechanism for vesicle docking and Ca induced ‘exocytosis’ of neurotransmitter at the NMJ?

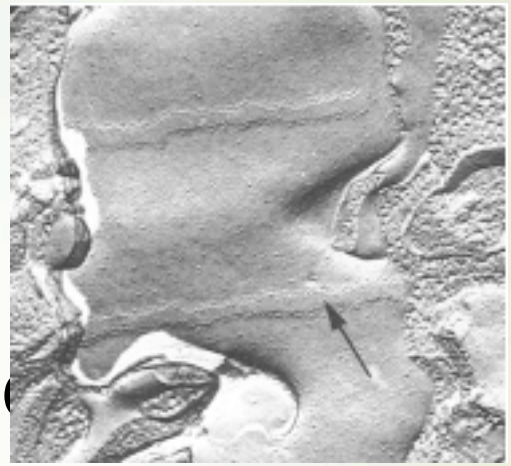
…what did we know already?



Bargmann *Nature* ***436, 473-474 (2005)***

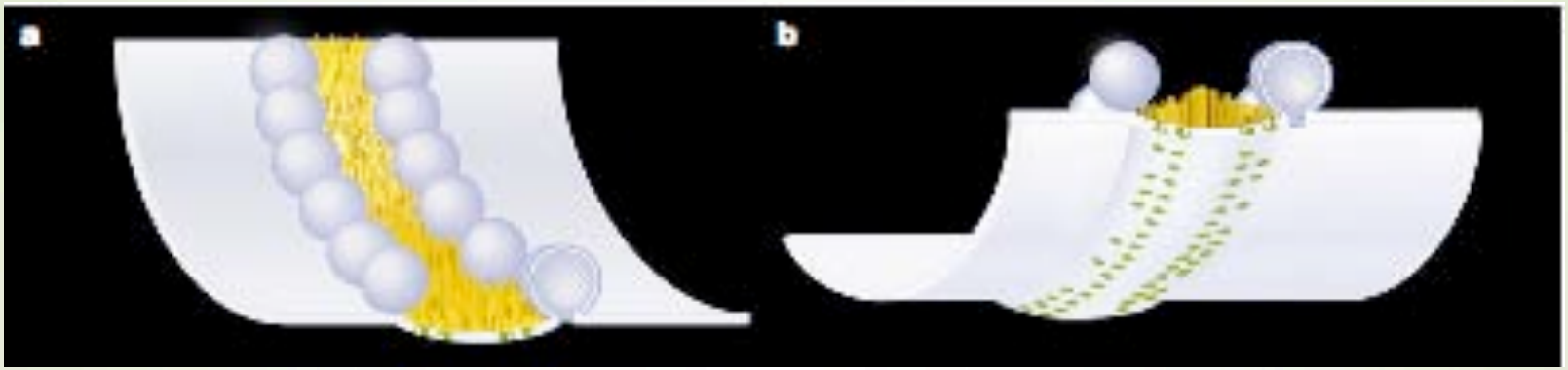
Active Zone

- ‘Ridge’ shaped
- Docked vesicles either si
  of ridge
- Protein aggregates:
  ‘Presynaptic dense
  projections’ = AZM



Heuser JE et al 1974

Macromolecules

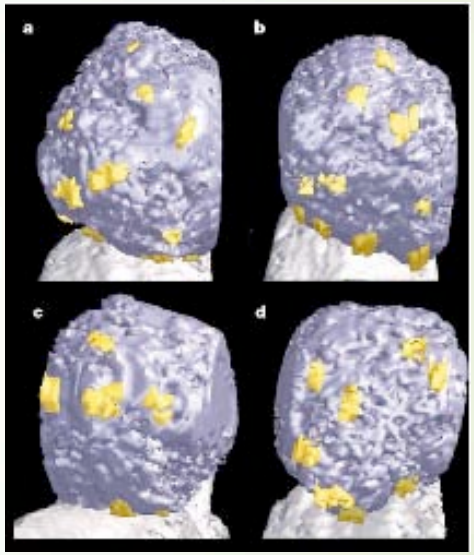


- Macromolecules in-line with docked vesicles inside the cell.
- Thought to include Ca channels

  (e.g. Robitaille R, Adler EM, Charlton MP)

What were the findings?

- On average 7-12 AZM components touched four vesicles (3 per vesicle)



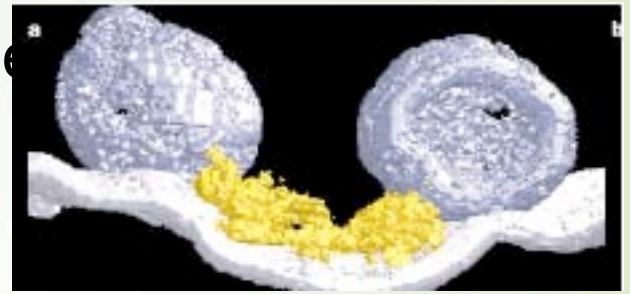
- Most with one connection per component

- Facing the AZM

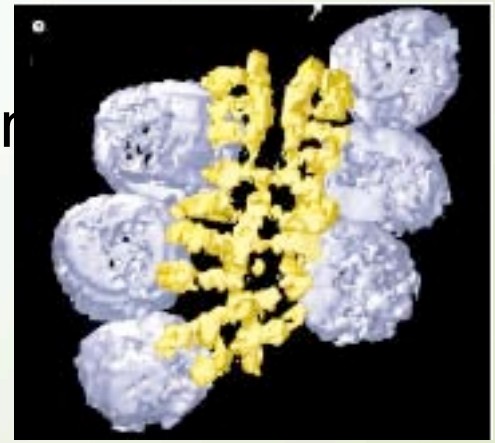
(?? Cytoplasmic structures found to be in contact with the vesicles that weren't facing into the AZM were not identified and were not regarded as part of the AZM)

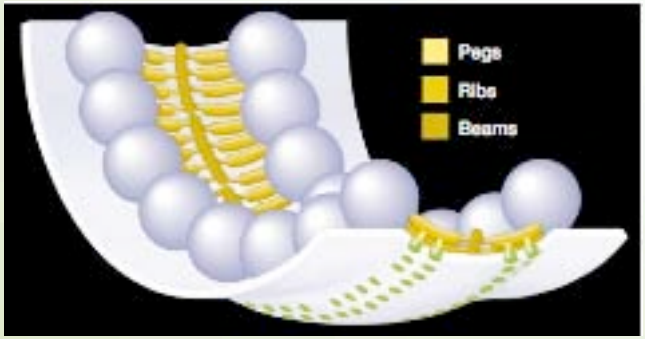
On 3d rendering…

- AZM widely distributed on the slope of the active zone ridge



- Components were interconnecting
- Formed a beam / ribs / pegs formation (7nm gap for pegs)





Correlation?

- Is there a correlation between:

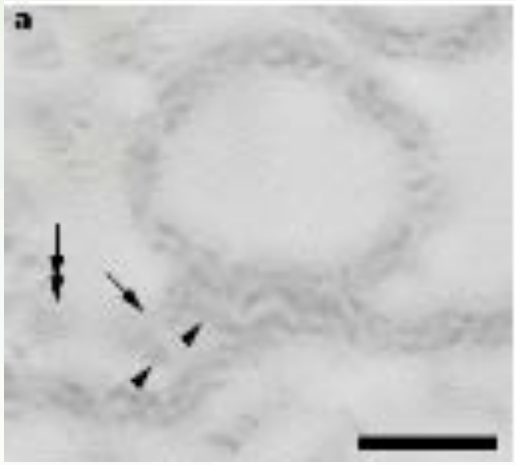
MACROMOLECULES

and
PEGS?

Yes!

- Thirteen docked vesicles were associated with forty ribs
- Compared mean centre-centre spacing of 1,100 vesicles (17.2 ± 3.6 nm) and mean midline-midline spacing of 45 ribs (16.1 ± 3.4 nm) (? Further test to show correlation of localised variations around the mean)
- As the only proteins in the ridge having a similar distribution to pegs were the macromolecules, the conclusion was that ribs are connected by means of pegs to macromolecules in the presynaptic membrane.

Further discussion….



- Pegs and ribs may be directly involved in vesicle docking during synaptic transmission

- As a significant portion of a calcium channel extends into the cytoplasm, they could potentially make up part or all of the ‘pegs.’

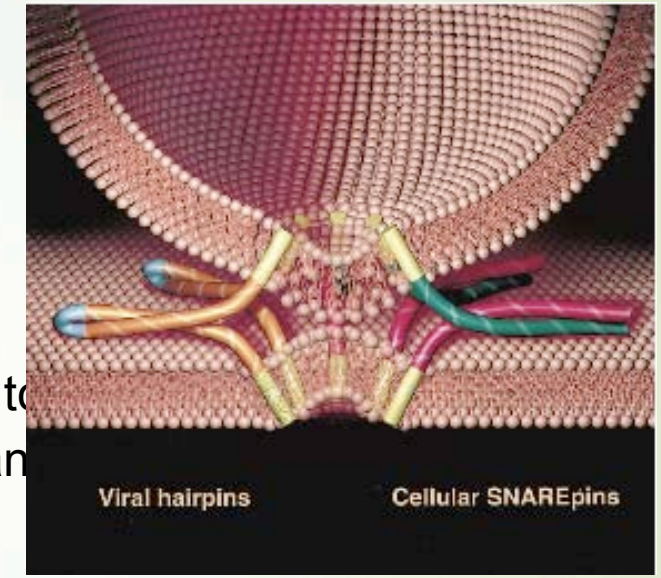
Meaning?

- If Macromolecules = Ca channels
- Direct link between Ca binding and vesicle fusion

What happened to SNARE proteins?

- Described in 1993
  Sollner, T et al. SNAP receptors implicated in vesicle targeting and fusion. Nature 362, 318–324 1993

- V & T Snare complexes that have been shown t
  needed fully intact milliseconds before membran
  fusion



Weber et al 1998

- SNAREs and synaptotagmin (vesicle protein) may by linked to calcium channels to execute calcium induced exocytosis.

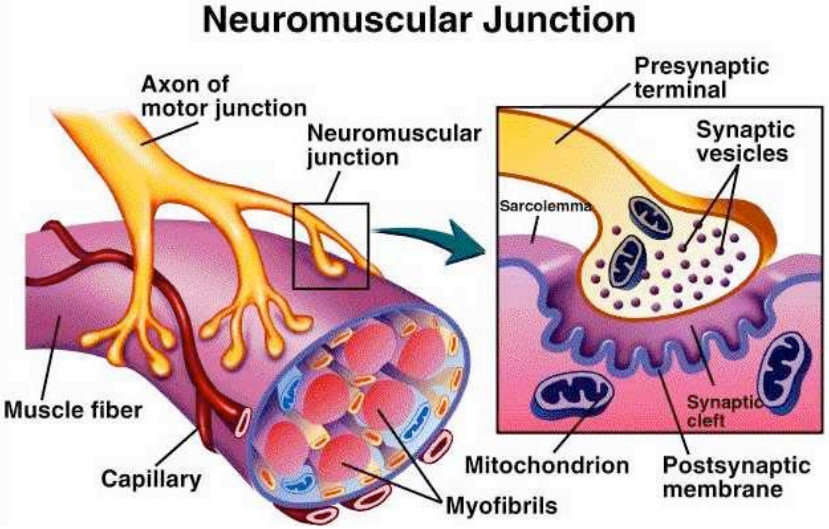
- For this to happen, syntaxin (SNARE) and synaptotagmin would have to pass through AZM ribs. Are ribs the component of AZM that not only docks but fuses also vesicles with the cell membrance also?

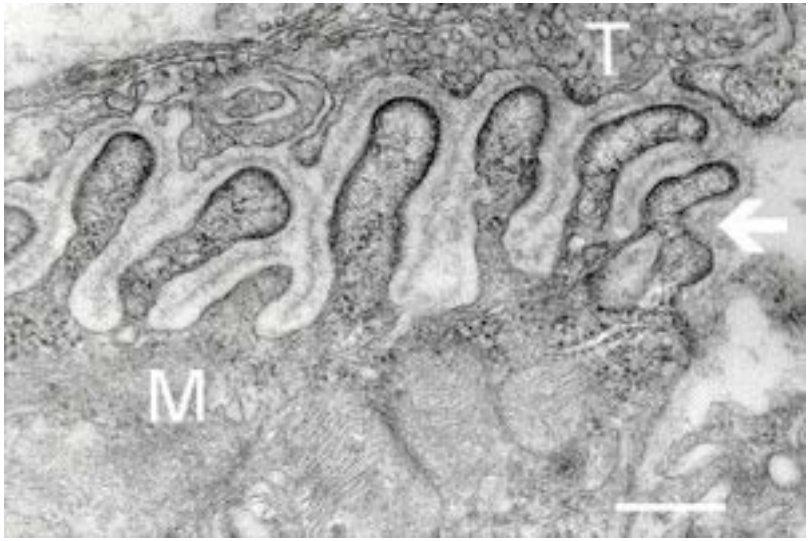
Structure and Function of NMJ's
Paper 3

The contribution of postsynaptic folds to the safety factor for neuromuscular transmission in rat fast- and slow- twitch muscles.

S.J. Wood & C.R. Slater (1997)

Background


Neuromuscular Junction
Axon of motor junction
Neuromuscular junction
Presynaptic terminal
Synaptic vesicles
Sarcolemma
Muscle fiber
Capillary
Myofibrils
Mitochondrion
Postsynaptic membrane
Synaptic cleft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Introduction 1

Determine Safety Factor as defined by…

Number of quanta released : Number of quanta required to reach threshold

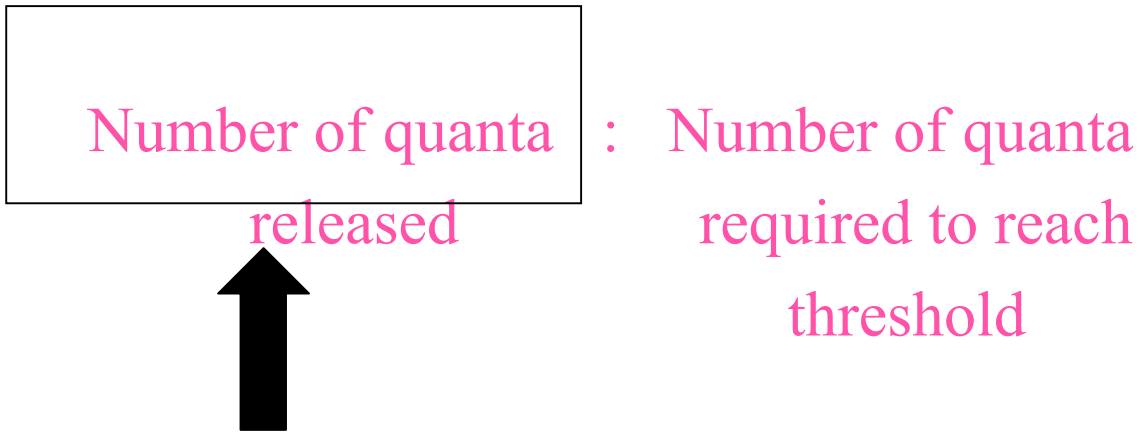
… as this variable is the most likely to vary during normal motor activity.

Introduction 2

Assess the contribution of the post-synaptic folds to the safety factor at the NMJ.

Method 1

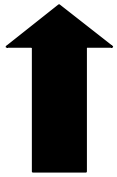
Method 2



- Block the muscle fibre A.P. with μ-conotoxin (preferentially blocks muscle VGSC's)
- Use a two-microelectrode voltage clamp on the post-synaptic membrane to record the EPC
- This gives a measurement of the amount of ACh released pre-synaptically.

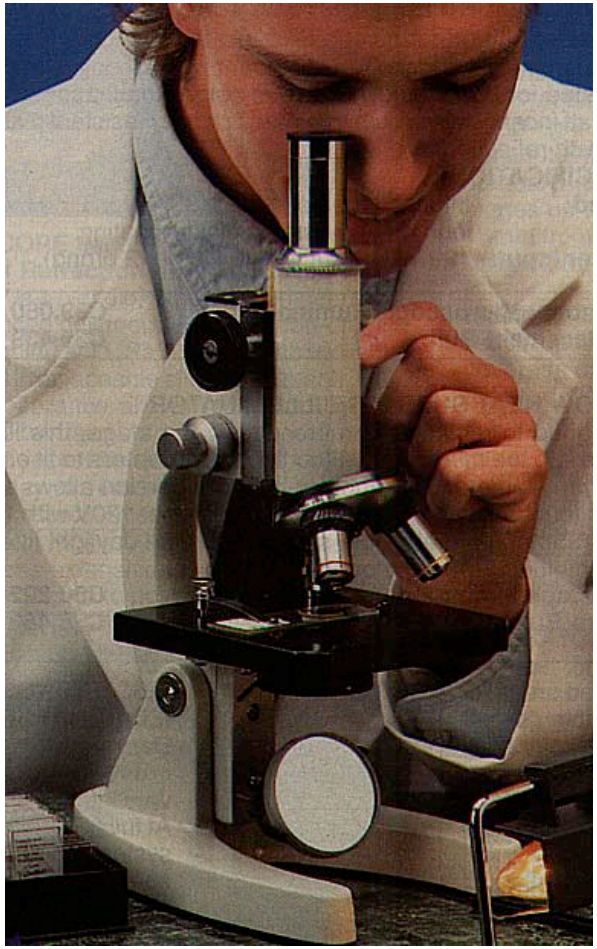
Method 3

Number of quanta released : Number of quanta required to reach threshold

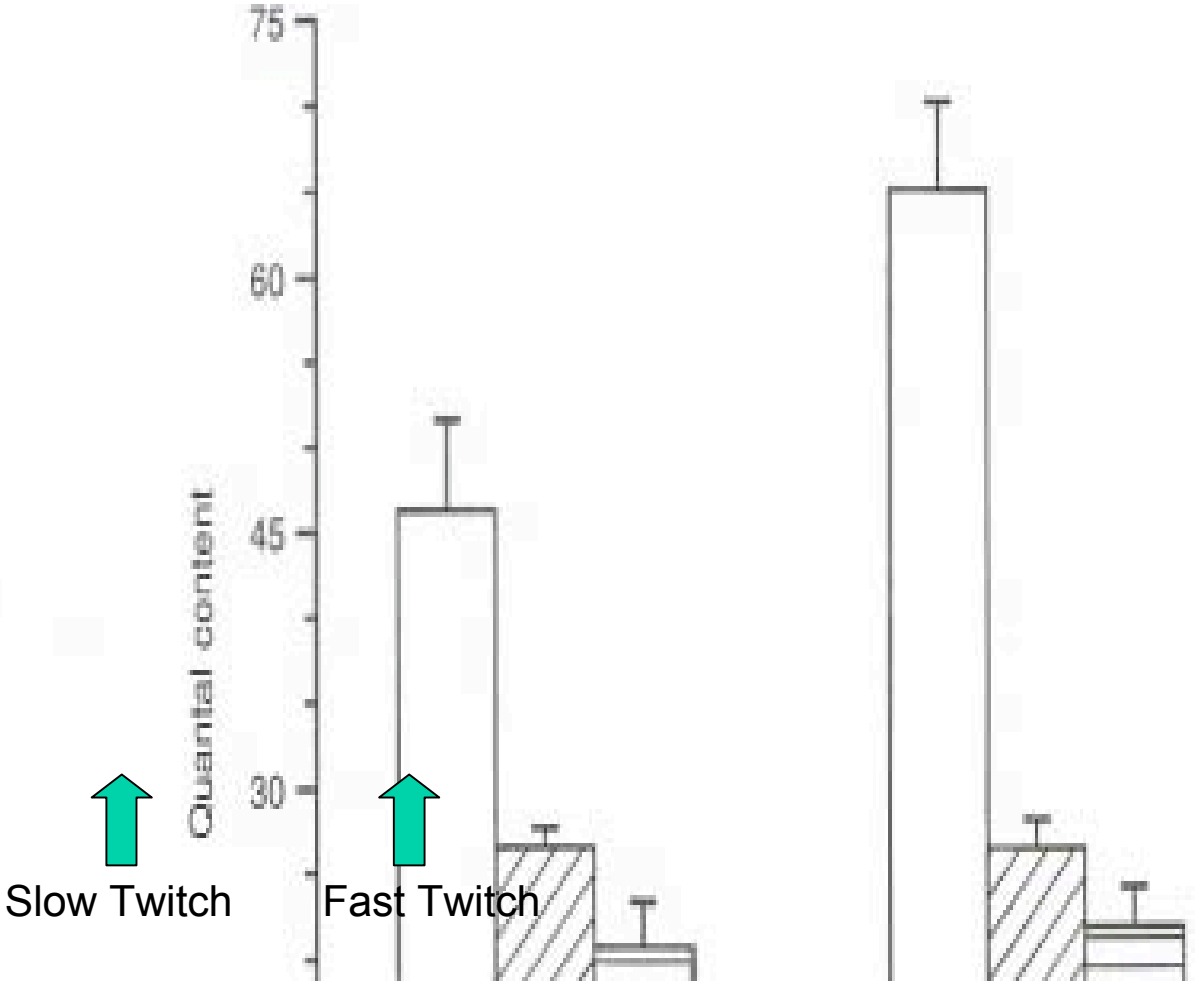


- Incubate nerve- muscle preparations in *d*-tubocurarine (nAChR antagonist) to block transmission in most fibres.
- On the few fibres that fire A.P.'s, measure the EPC using voltage-clamp recording to estimate the number of quanta required to reach threshold.
- Record threshold levels extrajunctionally (using injected current impulses as the stimulus) to allow a comparison between specialised and non-specialised post-synaptic membranes.

Method 4



Results 1



Results 2

Table 4. Safety factor for neuromuscular transmission

	Charge				Voltage			
	$m_Q/m'_{Q_{INJ,thr}}$		$m_Q/m_{Q_{thr}}$		$m_V/m'_{V_{INJ,thr}}$		$m_V/m_{V_{thr}}$	
	J	XJ	N	Ratio N/XJ	J	XJ	N	Ratio N/XJ
Soleus	2·23	1·74	3·48	2·00	1·93	1·47	3·14	2·14
EDL	2·95	2·46	5·01	2·04	1·72	1·61	3·42	2·12

Results 3

- Post-synaptic folding represents a 5-fold ↑ in amount membrane at NMJ c.f. outside junction
- NO difference in the folding spacing or amount of membrane per fold between muscle types
- Ratio of NMJ area : fibre size also same in both muscles
- However soleus (slow) muscle fibres and NMJ's= 50% bigger than EDL (fast)
- This smaller synaptic area in combination with a 40% higher quantal content in EDL= >2x as much ACh is released per unit area of synaptic contact

Conclusions

- Post-synaptic specialisations contribute to the safety factor by a factor of 2.

- These specialisations include junctional folds, which increase the amount of membrane (and therefore VGSC's contained within it) at the NMJ by a factor of 5.

- Fast-twitch muscles have a higher safety factor than slow-twitch muscles. This is most probably the result of a pre-synaptic difference (fast-twitch release 40% more Ach per nerve impulse), than post-synaptic specialisations, which contribute to the safety factor equally between muscle types.

What Next?

- Other post-synaptic specialisations contributing to the safety factor at the NMJ?
-

c.f.

How do the junctional folds amplify the EPP?

What features of the NMJ are conserved between species and muscle types? Why?

How does variability in NMJ structure contribute to effective neurotransmission?

Sexual Differentiation of Identified Motor Terminals in *Drosophila* Larvae

Lnenicka G. A, Theriault, K, Monroe, R.

Background, Aims and Hypothesis

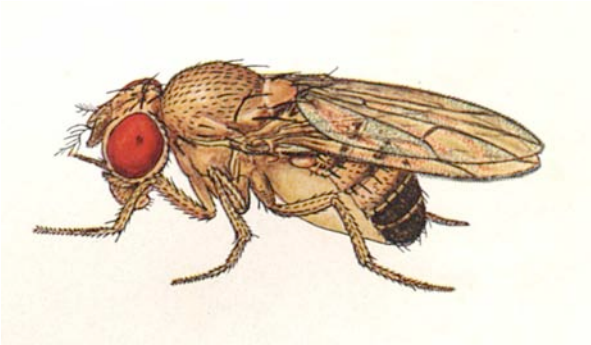
- **Well documented evidence for sexual differentiation in the nervous system.**

Aim

- **The aim of the study was to investigate whether or not there were morphological differences in the physiology of synapses in the CNS.**

Hypothesis

- **They hypothesised that females show a larger synaptic response than males.**



Materials and methods

- **The group used synapses found on the body wall musculature of larvae for the study.**
- **Wild type third-instar larvae were used.**
- **They were kept an appropriate media at 25^{o}C.**
- **Late (wandering) third-instar larvae were used for most of the experiments.**
- **They were dissected in saline and pinned flat.**

Neuromuscular Physiology

- **Segmental nerve was stimulated using a suction electrode and response recorded using a micro electrode.**

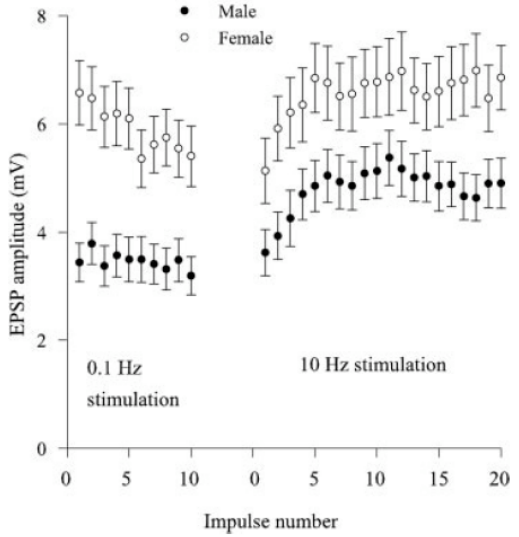
Motor Terminal Morphology

- **The motor terminals were stained and morphological measurements taken**
- **Statistical comparisons made in the study were done using Mann-Whitney U tests**

Results and Conclusions(1)

Investigation into the size of EPSPs produced by the motor terminal innervating muscle fibre 5.

- **EPSP produced by the motor terminal innervating muscle fibre 5 in segments 3 and 4 were larger in females.**
- **No differences were seen between the neuromuscular synapses on fibre 6 between the sexes.**

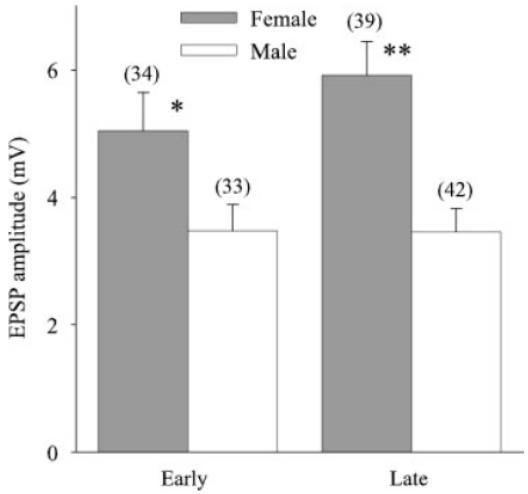


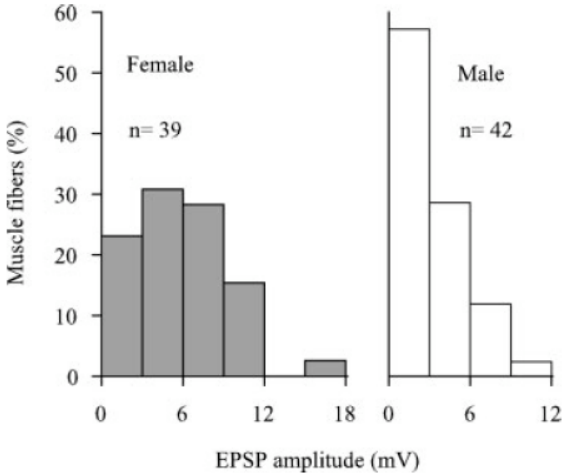
Sexual Differentiation of EPSPs Produced in Muscle Fibre 5

Results and Conclusions (2)

Investigation into whether or not sexual differentiation was seen throughout the duration of the third instar

- **Female larvae at the early third instar stage showed larger EPSPs than males**





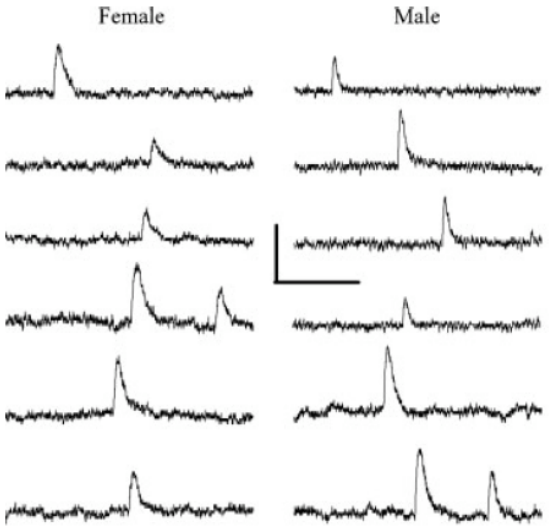
•Differences between the sexes were smaller for early instars but there was no significant difference between early and late instars for either sex.

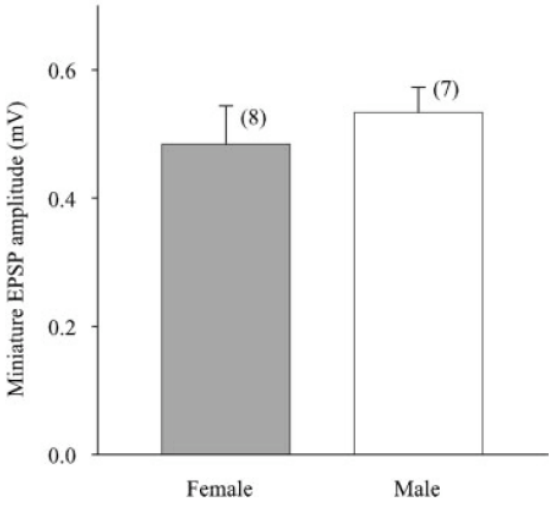
Sexual Differentiation of Neuromuscular Synapses on Muscle Fibre 5 Is Seen In Both "Early" and "Late" Third-Instar Larvae.

Results and Conclusions(3)

Investigation into the reason for the size differences seen between the EPSPs of males and females

- **No significant difference was seen between the amplitude of muscle fibre 5 miniature EPSPs between the sexes.**
- **Therefore conclude that female motor terminals release more neurotransmitter than male ones.**





Female Motor Termainals Release More Transmitter than Male Motor Terminals

Results and Conclusions(4)

Investigation into whether or not greater transmitter release from nerve terminals could be the result of larger terminals in females compared to males.

- **No difference was observed.**
- **Concluded that the differences seen were not due to differences in the size of the axon terminal**

Motor Terminal Size Is Not Sexually Differentiated

To sum up…

- Female terminals produced a larger synaptic response than males in three of the four body-wall muscles that were understudy
- The greater synaptic response it thought to be due to greater release of transmitter from female than male synaptic terminals
- The difference between the sexes is present throughout the third instar
- The difference in transmitter release are not due to differences in the size of the motor terminal