MINI-SYMPOSIUM 1 SUMMARY

1. Morphology of NMJs

Aims - To observe the morphology of neuromuscular junctions in 3 different species.

Methods - NMJs were visualised using scanning EM. The HCl-collagenase method was used to remove extracellular matrix, allowing for much clearer images than previous studies.

Conclusions - Good method, effective visualisation. Does the variation in morphology occur between muscles, individuals, or species?

Strengths - Method selected caused the least tissue destruction
- Good results/images

Limitations - No reproduction of results
- It appears that only one animal from each of the species was studied; only one muscle from each of these.
- Different muscles used – no explanation
- Some tissue destruction

Burning Question – Why so variable? Any constant structures?
- Variability not explained
- Required structures include neurone, Schwann cell, muscle fibre, vesicles etc.

2. Intersticularis Connectome

Aims - Reconstruct the entire connectome for one muscle

Method - Transgenic mice used that have 100% fluorescent motor neurones
- Confocal imaging
- Imaging processing and reconstruction
- TED calculations of axon length, calibre, spatial distribution of NMJs

Results - 6 Intersticularis muscles mapped
- Axonal branching of each motor neurone was unique

Strengths - Reconstruction accuracy was confirmed in 3 different ways

Limitations - Scattering evident while imaging deeper structures
- Axonal branches were often close together – identifying individual axons was difficult
- Human monitoring increased the time taken to map axons

Future Research - Studies into the process of axonal pruning
- Different colours used to increase accuracy of axon identification (like brainbow mouse)
- Connectomes in other areas of the nervous system?

Burning Question – What is a minimal wiring model?
- Theory states that an axon will branch in a way that allows it to take a direct route to a muscle fibre - minimizing wiring length and conserving energy.
- ‘Minimal wiring’ does not occur due to axonal competition during development
3. The Architecture of Active Zone Material at the Frog’s NMJ

Aim - ‘To show the arrangements and associations of [active zones] at a model synapse – the frog’s NMJ’

Methods - EM tomography of active sites resulting in computer-constructed 3D images

Results - Images showed ‘beams and ribs’, suggesting that active zone material plays a key part in the docking and possibly priming of vesicles prior to neurotransmitter release

Conclusions - Active zone material may play a key role in docking of vesicles, Ca$^{2+}$-induced exocytosis, spatial organization of the active sites

Future Research - What are the mechanisms involved in AZM making connections with synaptic vesicles?
- How may AZM be involved in vesicle fusion with the presynaptic membrane?
- What are the molecular components of AZM? How do they interact with other proteins?
- What is the significance of some AZM being >15nm away from the presynaptic membrane?
- Are all the beams, ribs and pegs within the synaptic terminal interconnected?

Burning Question – How has EMT changed the way we think about the mech. of docking and release of NT?
- Previously 2D images have shown that active zones are elongated structures on the presynaptic membrane. EMT has allowed for the visualisation of AZM in much more detail (as above).

4. The Spatial Pattern of Exocytosis…

Hypothesis - Neurotransmitter exocytosis occurs preferentially at repeatable locations on the presynaptic membrane called ‘hot spots’ at both mild and titanic stimulation, at body temp.

Methods - Muscle taken from SpH-producing mouse
- Exocytosis stimulated
- Imaging using fluorescence microscopy
- 3 muscles from 3 mice

Conclusions - Mild stimulation – uniform exocytosis
- Tetanic stimulation – appearance of repeatable hotspots
- Ideas about the mechanism of release at hotspots: all disproved
  - Clustering of vesicles
  - Different access to reserve pools of vesicles
  - Clustering of active zones

Strengths - Disproved endocytosis effectively
- Disproved ideas about hotspot activity effectively
- Interesting implications for future work

Weaknesses - Considerable ‘photobleaching’
- Unclear about numbers of experiment repeats

Future Research - Different fluorescent molecule, study release at non-hotspots in more detail, perform experiment at higher temps.

Burning Question – What and where are hotspots? What novel mechanisms of NT release does mild and titanic stim. reveal?
- See above!