NMJ in Health and Disease Mini Symposium I

Paper1: The interscutularis muscle connectome

Connectomes are connectional maps of neural circuits. They are essential for understanding its structure and function and only in Caenorhabditis elegans has this been obtained fully. This paper, in an attempt at solving mammalian circuits, reconstructs the connectomes of six interscutularis muscles (a muscle that extends from the base of the ear to the middle of the skull) from adult transgenic mice expressing YFP-16 yellow fluorescent proteins in all motor axons. This muscle was studied because it is very small and thin, and each muscle fibre is innervated by only one motor neuron. Every motor unit was catalogued by confocal microscopy and semi-automated 3D reconstruction tools. Producing a connectome shows the complete inventory of connectional information in one sample. The results demonstrated four organizational principles of neuromuscular circuits. First, the motor unit size distribution correlated with previous results from physiological recordings of twitch tensions, showing an anatomical correlate for Henneman’s size principle, that most motor units generate small twitch tensions and few generate large ones. Second, there showed a strong relationship between axonal caliber, arbor length and motor unit size. That as motor unit size increase, the required incremental in axonal arbor length is reduced. It also showed that the axonal branching structure of each motor neuron was unique, even compared to corresponding neurons in left-right pairs of the same animal, there was no less variation than in ipsi or contralateral pairs from different animals. Fourth it found that the wiring was longer than necessary, suggesting that mammalian wiring may rely more on activity-dependent reorganizations for each neural circuit to settle on a particular wiring diagram. This paper determines that in mammals, muscle function is implemented with varied wiring diagrams that share global features but have highly varied anatomical forms, however they exhibited no appreciable functional differences between them. This may be a common theme in the entire nervous system, but due to its complexity has yet to be studied in its entirety.

Paper2: Identity, developmental restriction and reactivity of extralaminar cells capping mammalian neuromuscular junctions.

NMJs are interfaces between motor axon terminals and motor endplates of skeletal muscle fibres. Each endplate is innervated by a single motor axon terminal. To ensure high fidelity of conduction of action potentials (APs) from motor axon terminals, myelinating Schwann cells cover intramuscular axon collaterals, and non-myelinating Schwann cells (contained in a continuation of the synaptic basal lamina) “cap” the nerve terminal itself. Therefore perisynaptic terminal Schwann cells, motor neuron terminals and skeletal muscle fibres were thought to be the 3 major cell types that make up the neuromuscular junction. This paper studies a fourth cell type in mice and rats using a new type of cytoskeletal antibody (2166); after stumbling upon them when originally looking for oligodendrocytes using immunofluorescent staining. No oligodendrocytes were found, however instead a subpopulation of cells that formed caps over the NMJs (termed Kranocytes) were revealed. Triangularis sterni muscles (TS muscle) of postnatal mice aged 1-28 days were immunostained, with 2166-positive cells being gradually restricted to NMJs.
The localization of these NMJ-capping cells led to questions about their possible functions in development and plasticity of NMJs and were studied in muscle atrophy by either denervation, paralysis or disease. It was shown that NMJ-capping cells reacted and took the lead in forming bridges between NMJs following either denervation or paralysis in adults ahead of terminal Schwann cells. They also dispersed from NMJs in a disease model of muscle atrophy with no paralysis or denervation. One explanation is that the signalling mechanism derives from the muscle fibres rather than other cell types at the NMJs. Whilst one of the functions of NMJ-capping cells is to repair damaged neuromuscular connections, data was found to suggest that it does not require or depend on tenascin-C, an early molecular marker associated with axonal sprouting and regeneration.

Paper3: Macromolecular connections of active zone material to docked synaptic vesicles and presynaptic membrane at neuromuscular junctions of mouse.

The active zones at the presynaptic plasma membrane of axon terminals consist of three prominent structures: aggregates of cytoplasmic macromolecules called active zone material (AZM), synaptic vesicles docked on the membrane next to the AZM and aggregates of macromolecules including calcium channels in the membrane. The role of docked vesicles and calcium channels are well understood. From depolarisation of the cell to exocytosis of the material contained in the vesicles to act on the post synaptic cell. However the function of AZM has only recently been studied. The hypothesis is that the AZM is a multifunctional organelle: that helps dock vesicles onto the membrane, anchors the calcium channels in the membrane at a particular distance to each other, and is involved with fusion of the vesicles with the membrane. The paper aims to assess whether the AZM found in the mouse levator auris muscle has a comparable relationship to the active zone to what has been found in the frog model. Electron tomography (ET) was used to view the macromolecules composing AZM in a 3D structure. What was shown were two bands of AZM, with a double row of nearly parallel membrane macromolecules attached to the presynaptic membrane, situated directly opposite junctional folds in the postsynaptic membrane of the muscle fibre. Vesicle fusion sites were found between the two rows of macromolecules, with two primary docked vesicles (PDVs) per active zone and up to three secondary docked vesicles at the ends of the AZM bands. Two AZM bands were found in the mouse compared to the one band in the frog, which could be up to ten times in length, however had macromolecular components corresponding to the beams, ribs, and pegs in the frogs’ AZM band. The findings also support the first two aspects of the hypothesis, and is likely that they play a role in the operation of fusing the vesicles to the membrane, although it has yet to be proved.

Paper4: A compensatory subpopulation of motor neurons in a mouse model of amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons leading to neuron death and denervation atrophy of the muscle. Death from respiratory failure usually occurs within 5 years of diagnosis, and current therapeutic treatment with riluzole only extends life expectancy by three months. Previous data revealed that some motor axon branches attempt to compensate for loss of innervation resulting in enlarged axonal arbors. Some axonal branches however die back, and thus the question was asked whether the two types of behaviour of compensatory growth or die
back occur in different branches of single neurons or whether entire motor units are of one type or the other. Neuromuscular junctions in double transgenic mice expressing mutant SOD1 and YFP were imaged by confocal imaging of axonal arbors. At some junctions the postsynaptic sites were completely denervated, at others, postsynaptic sites were partially occupied by nerve terminals that can be subdivided into fragmented, or continuous thin axons. The conclusions drawn from this were that the lack of changes in the thin axons suggests reinnervation rather than degeneration, that the process of denervation occurs rapidly and observations suggest that presynaptic axons degenerate prior to degeneration in either muscle finre or Schwann cell. The main conclusion is that in genetically valid mouse models of ALS, individual motor pools contain two distinct types of motor neurons. The “Losers”, which contain fragmented axons and the “Compensators” that contain thin reinnervating branches.