The Neuromuscular Junction: Structure and Function

"*Every breath you take, every move you make*……" The first line of this famous song, written by Sting, has nothing to do with neuromuscular junctions (NMJ's); but it underscores the fact that we cannot live without them. When NMJ's fail physiologically, pathologically or when they are blocked pharmacologically, muscles may become paralysed. When neuromuscular paralysis affects respiratory muscles, then patients can no longer breathe for themselves and death may rapidly ensue.

Neuromuscular junctions are the last synaptic outpost in the "final common path" that leads, via the axons of motor neurones, out of the central nervous system. These axons terminate in skeletal muscle. This puts NMJ's among the most accessible of all synapses to study using morphological and physiological techniques. As a result, a great deal has been learnt about physiology and morphology of synapses in general, from studies that initially focused specifically on the NMJ. However, NMJ's have structural and physiological specializations that are not found at other synapses, and these make NMJ's important objects for study in their own right. Many drugs have been developed that control or modulate synaptic transmission at NMJ. We will consider the pharmacology of neuromuscular junctions in the next lecture. This lecture is concerned with what NMJ's look like and how their structure relates to their function.

Morphology

1. Neuromuscular junctions were first observed by light microscopy using silver staining techniques in the mid-19th Century. Nowadays, immunostaining for synapse-specific proteins, or expression of fluorescent proteins in transgenic mice are the preferred methods for visualizing NMJ's. However, our perception of the general form of motor nerve endings has remained essentially unaltered since they were first described. In both vertebrate and invertebrate muscle, collateral nerve branches expand a few micrometres away from the muscle fibre, and form complex small arbors (**motor nerve terminals**) on **motor endplates** of the muscle fibres. In mammals, motor nerve terminals are only about 30 µm long and cover an area of about 400 µm^2. This corresponds to less than 0.1% of the total surface area of a muscle fibre.
2. Electron microscopy of the NMJ was first reported in the 1950's. EM studies established that the motor nerve terminals contain spherical **synaptic vesicles**, each about 30 nm in diameter; numerous mitochondria; and "**active zones**", where synaptic vesicles are aggregated. On the muscle side of the synapse – a 50 nm gap – the motor endplate membrane is thrown into complex and deep primary and secondary **junctional folds**.
3. Immunocytochemistry and observations made using the light microscope, and electron microscopy, have established two other cell types closely associated with the NMJ: terminal **Schwann cells**, which form tight caps over the motor nerve terminal; and cells of a more loosely-associated connective-tissue type, recently named **kranocytes**.
4. Between the plasma membranes of the motor nerve terminal and the motor endplate, i.e. within the synaptic cleft, is a sticky layer of extracellular matrix called the synaptic **basal lamina**. It also contains important molecules necessary for proper synaptic function.

Physiology

5. Like all chemical synapses, NMJ's utilise a neurotransmitter - acetylcholine (ACh) - as their medium of communication. The main criteria that must be met and demonstrated to prove the identity and function of a chemical neurotransmitter are its **synthesis, storage, release, action and inactivation**.
6. Experiments performed by Otto Loewi in the 1930's, on isolated perfused frog hearts, established that ACh was released when stimuli were applied to the vagus nerve supplying the heart. Sir Henry Dale and colleagues then went on to show that ACh is also released from motor nerve endings in frog and cat skeletal (voluntary) muscle and that this effect could be blocked by the antagonist curare (d-tubocurarine: see next lecture).
7. Acetylcholine is **synthesized** in the cytoplasm of the motor nerve terminals by the enzyme choline acetyltransferase (ChAT), from choline and acetyl-CoA.
8. Acetylcholine is **stored** in high concentration in synaptic vesicles: about 5,000 molecules of ACh per 30 nm vesicle. The ACh is pumped into the vesicles by a transporter that

utilizes energy from ATP and co-transports protons (acid, H^+). The interior of a synaptic vesicle therefore has an acidic pH.

9. Experiments by Sir Bernard Katz and colleagues in the 1950's-1970's established that acetylcholine is **released** in "quantal packets". Subsequent studies established that this release occurs by **exocytosis** from synaptic vesicles when they fuse at the active zones. The current model is that fusion occurs at active zones when "SNARE" proteins on the vesicle membrane (v-SNARE) and terminal membrane (t-SNARE) become intertwined.
10. After exocytosis, vesicle membrane is recycled, and synaptic vesicles are reformed. There is still some debate about whether this can occur immediatey and rapidly ("kiss-and-run"), or by a slower process requiring a cage of clathrin molecules to form around the collapsed vesicle, then its retrieval mediated by vesicular neck-tightening functions of the protein dynamin. Current evidence suggests that the slow, clathrin-mediated endocytosis and vesicle recycling predominate at NMJ's.
11. Molecules of ACh diffuse across the synaptic cleft and produce their **action** by binding to ACh receptors. These proteins are concentrated at high density (>$10^5/\mu m^2$) on the crests of the junctional folds. Each receptor comprises five subunits surrounding a central pore ($2\alpha, 1\beta, 1\delta, 1\varepsilon$ subunits). The binding of two ACh molecules to the α-subunits opens the ion gate on the channel, allowing cations (predominantly Na^+ and K^+ ions) to flow, in opposite directions down their transmembrane concentration gradients. The net result, however, for each open channel is a tiny pulse of inward current, producing less than a microvolt of endplate membrane depolarization.
12. At rest, spontaneous fusion of synaptic vesicles occurs randomly at a slow rate, about, once per second. Each fused vesicle releases thousands of molecules of ACh within less than1 ms of formation of the fusion pore. The result is the summed activation of thousands of ACh receptors and a small, 1 mV sub-threshold depolarization of the muscle fibre called the **miniature endplate potential (MEPP).** MEPP's are not normally large enough to cause an action potential or a muscle twitch, however.
13. When an action potential from the nerve axon is propagated into the nerve terminal, voltage-gated Ca^{2+} ion channels in the synaptic membrane open, allowing an influx of Ca^{2+} ions. The intracellular concentration of Ca^{2+} rises from about 10^{-7} to about 10^{-5}M. Since the rate of exocytosis occurs in proportion to the 4^{th} power of the Ca^{2+}concentration. ($m=k[Ca^{2+}]^4$), this rise in intracellular Ca^{2+} profoundly increases the probability of synaptic vesicle fusion, triggering exocytosis of about 50 vesicles. The resulting depolarization is called the **endplate potential** (EPP). It resembles the depolarization caused by MEPP's in time course, but EPP's are much larger: up to about 40 mV in amplitude. The EPP is therefore normally "supra-threshold", meaning it normally triggers an action potential in the muscle fibre (which then contracts as a consequence of excitation-contraction coupling mechanisms in the muscle fibre itself).
14. Neuromuscular junctions normally operate with a large margin for error called the '**safety factor'** for neuromuscular transmission. Only about 15 quanta (vesicles) are required to depolarize the muscle membrane to threshold for firing an action potential. However, about 50 quanta are actually released, amounting to more than three times that required to trigger an action potential in the muscle fibre: the safety factor is about threefold.
15. Repetitive excitation of an NMJ, for example during sustained voluntary movement, produces an initial increase (**facilitation**) followed by a progressive decrease (**depression**) in the size of the EPP. Thus, the safety-factor may decline during repetitive excitation, but normally transmitter release remains well above threshold for firing a muscle action potential. (Thus, muscle fatigue during sustained exertion - which we are all familiar with - is not caused by failure of neuromuscular transmission; but rather to other properties of muscle, such as build-up of lactate).
16. **Inactivation** of transmission at the NMJ is effected by the enzyme acetylcholinesterase (AChE). This enzyme is contained in the synaptic basal lamina. It breaks down ACh extremely rapidly, to choline and acetate. Choline is transported back into the motor nerve terminal, where it is used as a substrate in the resynthesis of ACh.

Further Reading

Bear et al . (2007) Neuroscience: exploring the brain. 3rd edn. Chapters 5 & 6

More advanced:

Byrne, JH & Roberts, JL (2009) From Molecules to Networks. 2nd edn. Chapter 8

Nicholls, JG et al (2012) From Neuron to Brain. 5th edn. Chapters 11,13