ELEC ENG 3BB3:

Cellular Bioelectricity

Notes for Lecture 22

Friday, February 28, 2014

10. THE NEUROMUSCULAR JUNCTION

We will look at:

- Structure of the neuromuscular junction
- Evidence for the quantal nature of transmitter release
- Poisson statistics for transmitter release
- The effect of Ca^{++} and Mg^{++} on transmitter release
- Post-junctional response to transmitter

Structure of the neuromuscular junction:

Studying the structure and function of the neuromuscular junction is useful for understanding:

- some *general principles of chemical synapses*, and
- the *specifics of skeletal muscle activation*.

Structure of the neuromuscular junction (cont.):

Motor nerve axons are normally myelinated, except at their terminals where they branch and synapse onto muscle fibers. The set of muscle fibers activated by a single motor neuron is known as a *motor unit*.

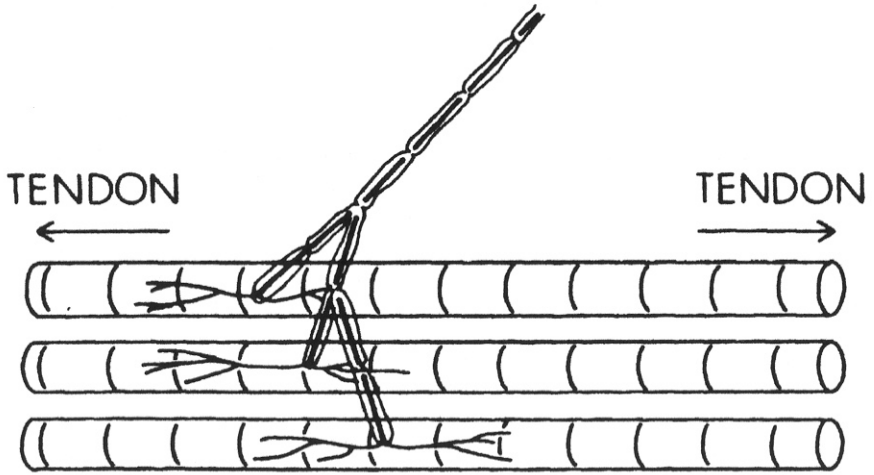


Figure 10.1. Motor nerve showing branching to activate three muscle fibers. The nerve endings are unmyelinated, as shown. (From R. D. Keynes and D. J. Aidley, *Nerve and Muscle*, Cambridge University Press, Cambridge, 1981. Reprinted with the permission of Cambridge University Press.)

Structure of the neuromuscular junction (cont.):

The nerve terminal contacts the muscle fiber at an *end plate*.

The pre- and post-synaptic membranes form a specialized "gutter".

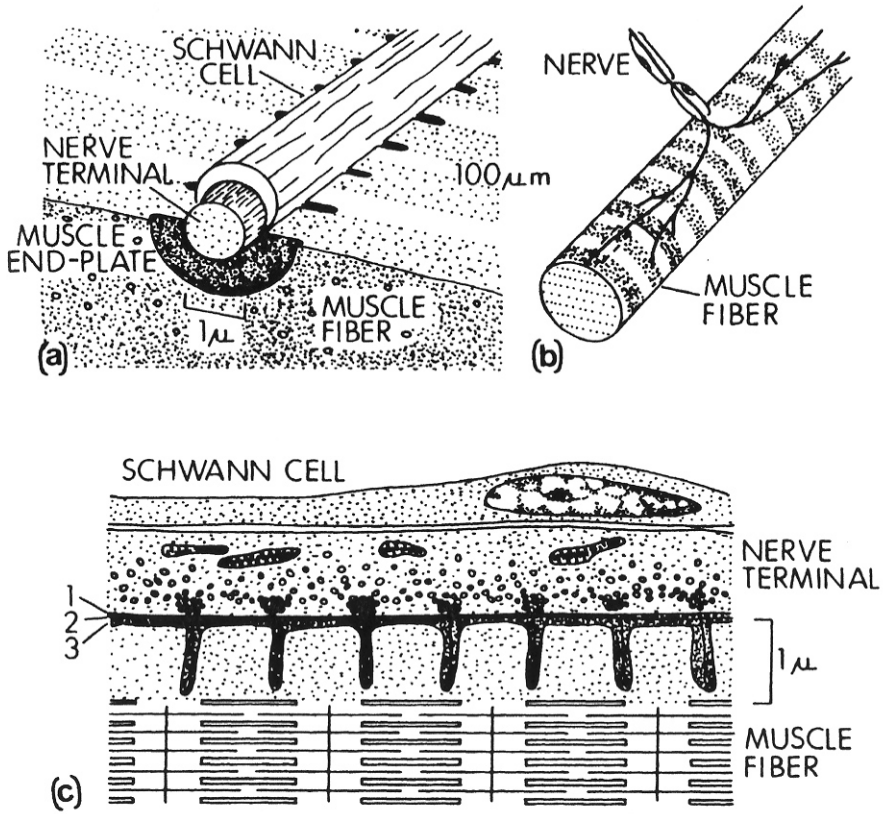


Figure 10.2. Neuromuscular junction of frog. (a) One portion of the junction. (b) General position of endings of motor axon on muscle fiber, showing portion (a) as a small rectangle. (c) Schematic drawing from electron micrographs of a longitudinal section through the muscle fiber. 1, terminal axon membrane; 2, "basement membrane" partitioning the gap between nerve and muscle fiber; 3, folded post-synaptic membrane of muscle fiber. (From B. Katz, *Nerve, Muscle, and Synapse*, McGraw-Hill, New York, 1966. Copyright 1966, McGraw-Hill with permission of the McGraw-Hill companies.)

Structure of the neuromuscular junction (cont.):

The pre- and post-synaptic membrane formations are similar to nerve-to-nerve chemical synapses, except for the synaptic gutter.

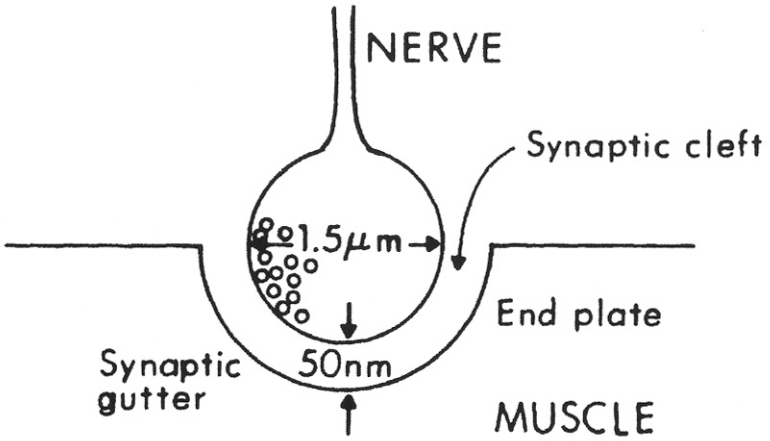


Figure 10.3. Details of the neuromuscular junction at a single nerve terminal.

Structure of the neuromuscular junction (cont.):

Muscle fiber end-plate potentials (EPPs) are equivalent to excitatory postsynaptic potentials (EPSPs) in neurons—note that they are always excitatory.

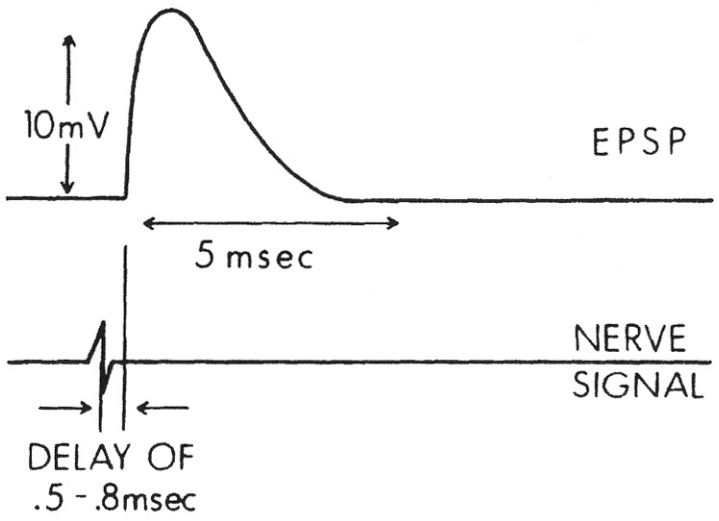


Figure 10.4. The end-plate potential arising from the neural action potential. The EPP is from an intracellular recording while the action potential is recorded separately with extracellular electrodes. The latter is included for timing; its relative amplitude is uncalibrated. [From L. G. Brock *et al.*, The recording of potentials from motoneurons with an intracellular electrode, *J. Physiol.* **117**:431–460 (1952).]

Structure of the neuromuscular junction (cont.):

The neuromuscular junction transmitter is acetylcholine (ACh).

A model of the ACh receptor is shown to the right.

Channel opening requires binding of ACh with both α subunits.

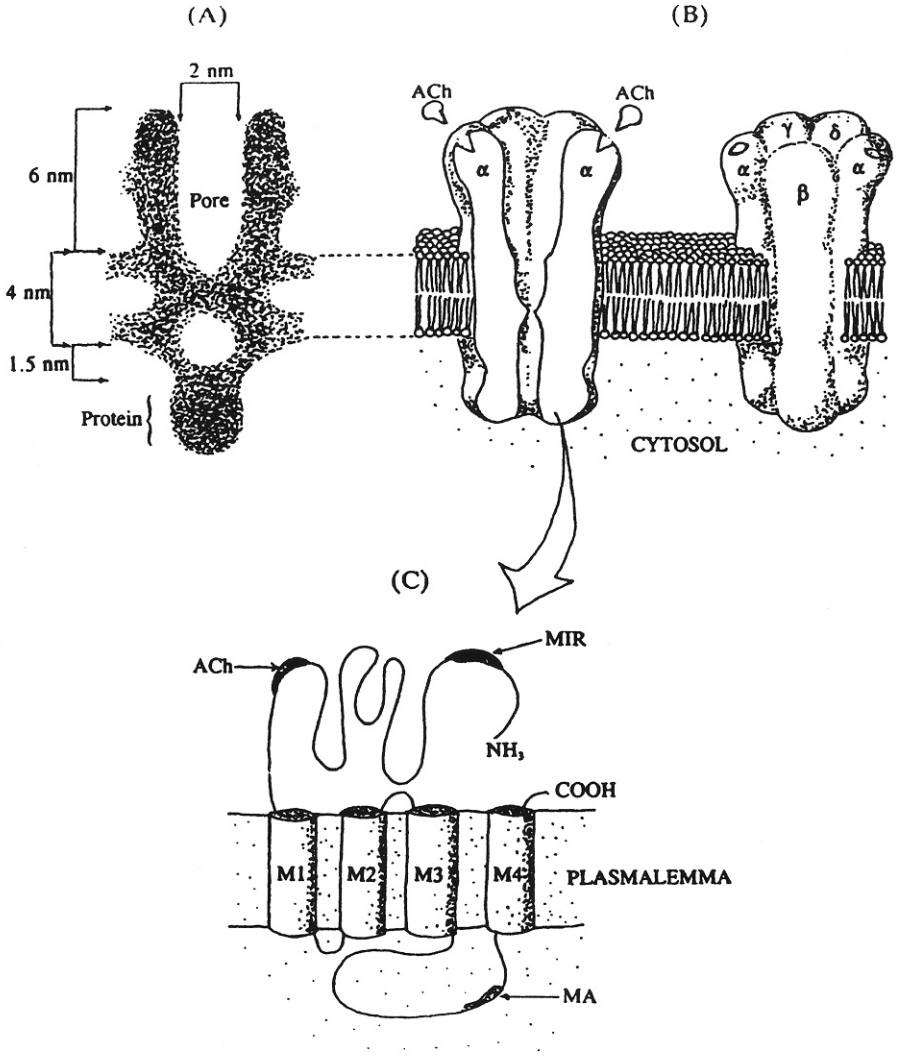


Figure 10.5. Details of the ACh receptor. ACh binding to both α units is necessary for gate opening. Also shown is the amino-acid sequence of the α subunit and their membrane crossings. (A) A three-dimensional reconstruction of the channels adapted from C. Toyoshima and N. Unwin, Ion channel of acetylcholine receptor reconstructed from images of post-synaptic membranes, *Nature* **336**:247–250 (1982). (B) AChR model consisting of the 5 subunits described in the text. The internal pore admits Na^+ and K^+ ions, following binding of ACh to each of the α subunits. (C) Molecular structure of one of the two α subunits which has four membrane spanning regions. (Adapted from D. Beeson and E. Barnard, Acetylcholine receptors at neuromuscular junctions, in A. Vincent and D. Wray, *Neuromuscular Transmission: Basic and Applied*, Manchester University Press, 1970. Reprinted with permission from A. J. McComas, Skeletal muscle: Form and function, *Human Kinetics*, Champaign, IL, 1996.]

Evidence for the quantal nature of transmitter release:

- In the absence of stimulation of the presynaptic nerve, *miniature end-plate potentials* (MEPPs) of around 0.5 mV are observed.
- EPPs produced by presynaptic nerve stimulation always have amplitudes that are integer multiples of the MEPP amplitude

) transmitter release is quantal

Poisson statistics for transmitter release:

Suppose there are n presynaptic release sites and that the probability of release at any site is p, where p depends on the presynaptic transmembrane potential and the concentration of Ca^{++}, Mg^{++} and other ions.

The probability of exactly x of n releases is given by the *binomial distribution*:

$$f(x) = \frac{n!}{x!\,(n-x)!} p^x q^{n-x}\,, \qquad (10.1)$$

where q = 1 – p.

Poisson statistics for transmitter release (cont.):

In the case where n ! 1, p ¿ 1 and x ¿ n, then the binomial distribution is well approximated by the *Poisson distribution*:

$$f(x) = \frac{\mathrm{e}^{-m} m^x}{x!}, \qquad (10.6)$$

where m = np is the *mean release rate*.

Note that the *variance* in the number of events (e.g., synaptic releases) for a Poisson process is *equal to the mean*.

The effect of Ca^{++} and Mg^{++} on transmitter release:

Decreasing the concentration of extracellular Ca^{++} or elevating the concentration of Mg^{++} has the effect of reducing p.

The reason is that the pre-junctional nerve terminal has many voltage-gated Ca^{++} channels, which facililates Ca^{++} entry near the ACh release sites.

It is actually binding of intracellular Ca^{++} to proteins in the release site that triggers exocytosis and neurotransmitter release. Mg^{++} can block the Ca^{++} channel, which reduces neurotransmitter release.

Post-junctional response to transmitter:

The postsynaptic membrane has an ACh receptor density of around $10^4/\mu m^2$, which is an order of magnitude greater than the density of sodium and potassium channels in squid axon.

To simulate the effects of transmitter binding to the post-synaptic receptor, a parallel-conductance model can be created where a mixed potassium, sodium and chloride channel is opened when ACh binds to the receptor.

Post-junctional response to transmitter (cont.):

For the frog neuromuscular junction:

- g_{Cl} ¼ 0
- g_{Na}/g_K ¼ 1.29

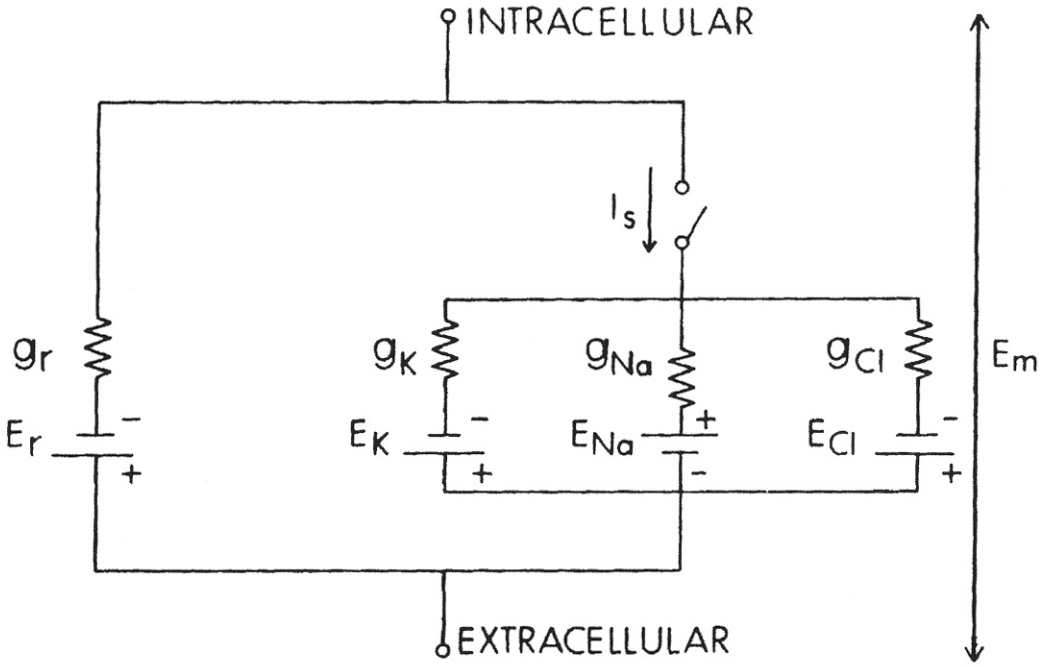


Figure 10.6. Parallel-conductance model of post-synaptic membrane that is influenced by transmitter, and remaining cell membrane (in resting state) [1]. The switch closes at the point of arrival of ACh when the circuit describes the instant of maximum conductance. [From D. Junge, *Nerve and Muscle*, 2nd edn., Sinauer Associates, Sunderland, MA 1981. Based on A. Takeuchi and J. Takeuchi, On the permeability of end-plate membrane during the action of transmitter, *J. Physiol.* **154**:52–67 (1960).]

Post-junctional response to transmitter (cont.):

The synaptic membrane model can be simplified to a single ionic current with conductance g_s and reversal potential E_s. Since $E_s > E_r$, the synapse is excitatory.

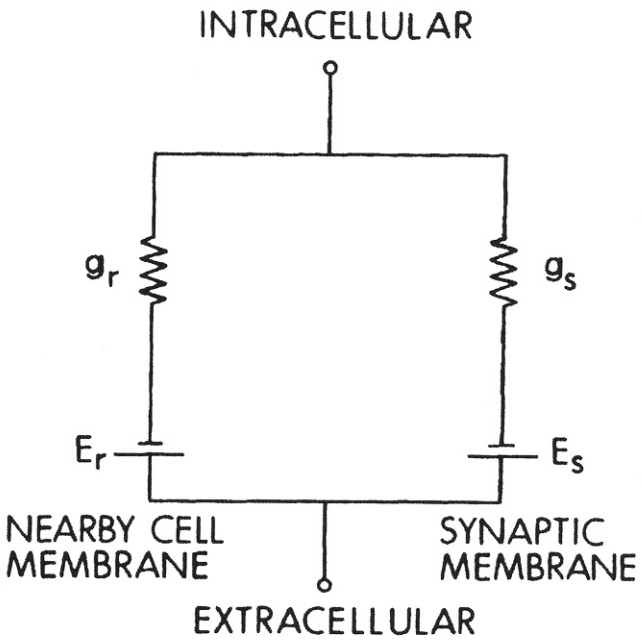


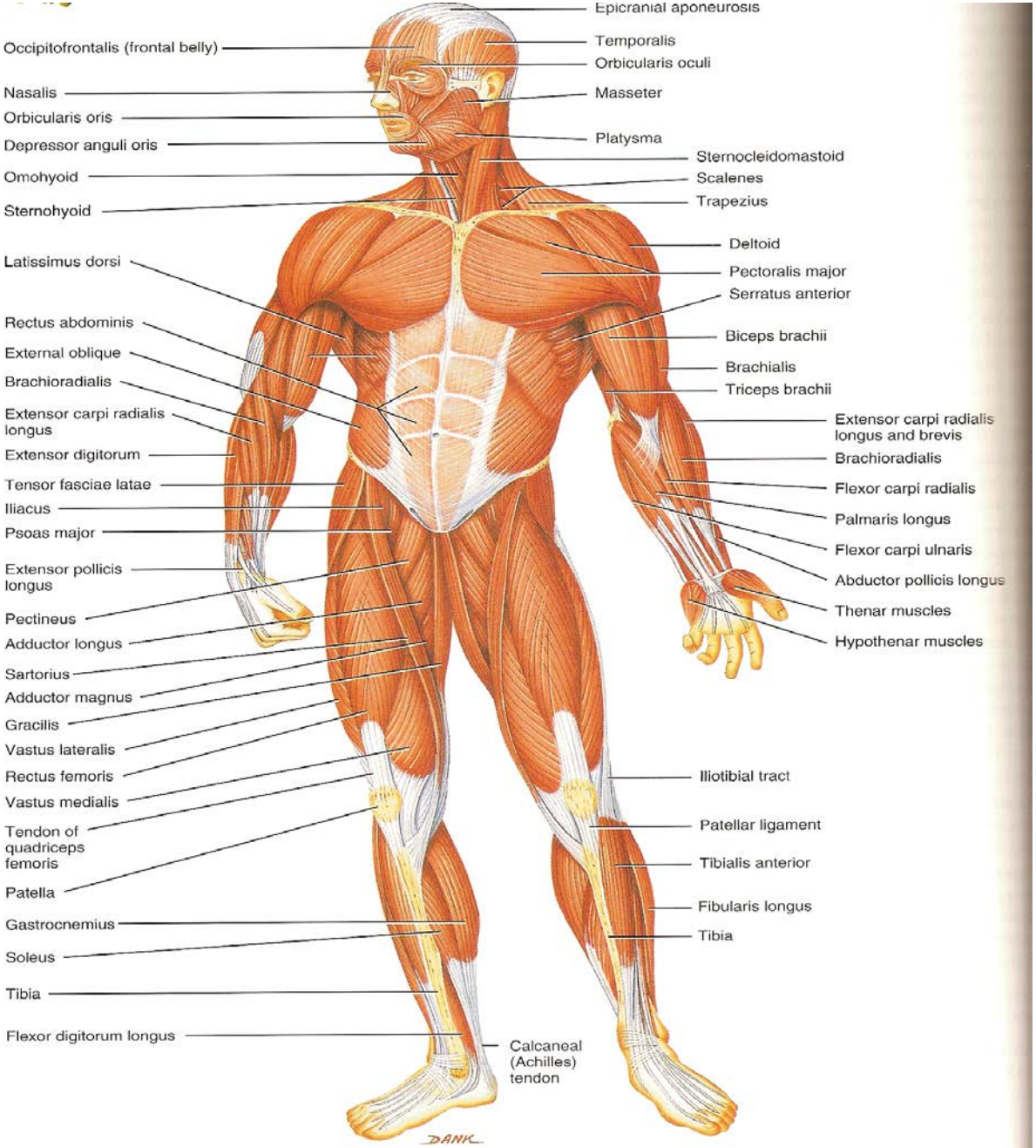
Figure 10.7. Simplified electrical model of post-synaptic junction and adjoining cell membrane following release of transmitter and activation of synaptic channels. (From D. Junge, *Nerve and Muscle Excitation*, 2nd edn., Sinauer Associates, Sunderland, MA, 1981.]

11. SKELETAL MUSCLE

We will look at:

- Muscle structure
- Excitation-contraction
- EMG measurement and interpretation

Skeletal Muscles



3.2 Muscle biomechanics

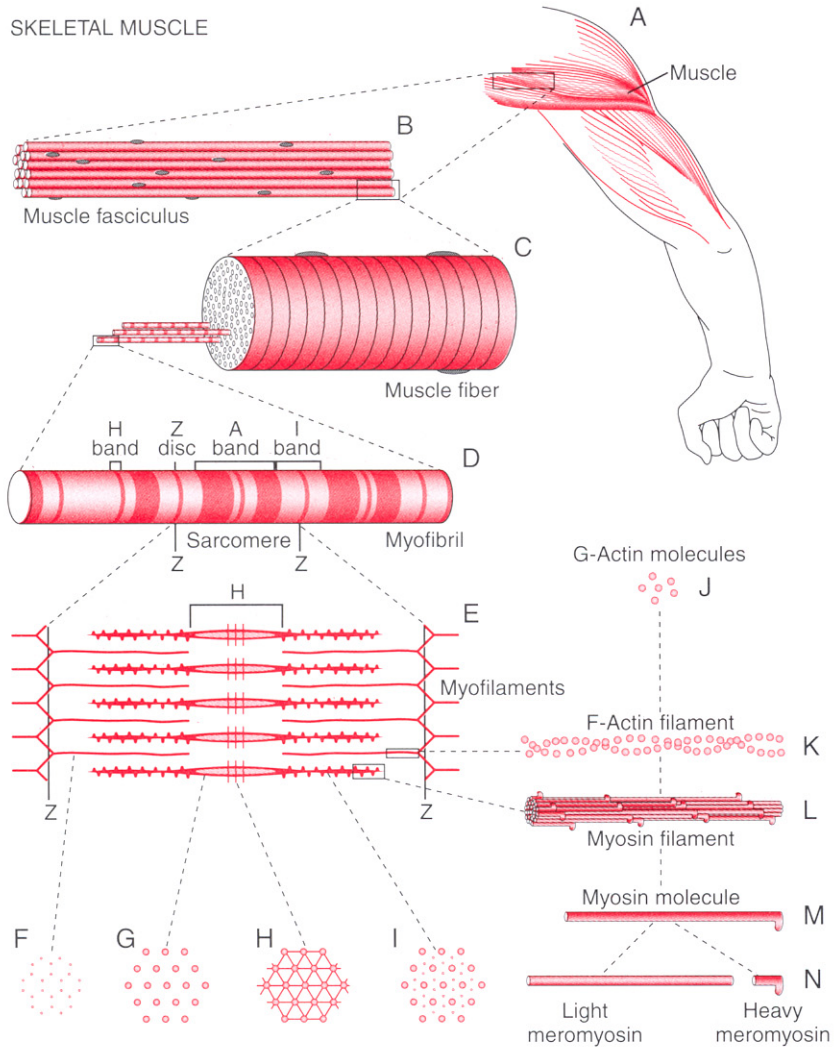


FIGURE 6–1

Organization of skeletal muscle, from the gross to the molecular level. *F, G, H,* and *I* are cross sections at the levels indicated. (Drawing by Sylvia Colard Keene. Modified from Fawcett DW: Bloom and Fawcett: A Textbook of Histology. Philadelphia: WB Saunders Co, 1986.)

Organization:

- skeletal muscle is made up of *muscle fibers*
- each fiber is a single cell
- the contraction of a fiber is achieved by the *motor proteins actin & myosin*

(from Guyton and Hall, 10th Edition)

Muscle structure:

- skeletal muscle is made up of *muscle fibers*
- each fiber is a single cell
- the contraction of a fiber is achieved by the *motor proteins actin & myosin* which form *fibrils*

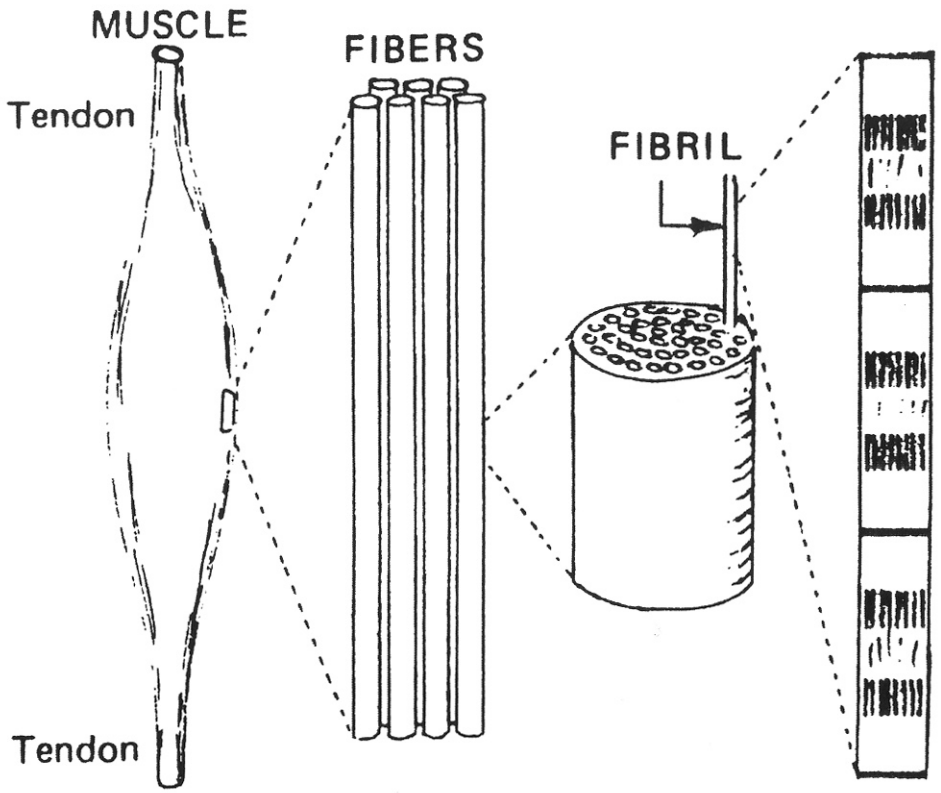


Figure 11.1. The structure of a whole muscle and its components. The cross striations are visible under light microscopy. [From R. D. Keynes and D. L. Aidley, *Nerve and Muscle*, Cambridge University Press, Cambridge, 1981. Based on K. Schmidt-Nielsen, *Animal Physiology*, Cambridge University Press, Cambridge, 1979. Reprinted with the permission of Cambridge University Press.]

Muscle structure (cont.):

Each fibril is surrounded by:

- a *sarcoplasmic reticulum* (SR), which stores Ca^{2+} for triggering muscle fiber contraction, and
- the *transverse tubules system* (TTS), which ensures that action potentials propagate deep into the fiber.

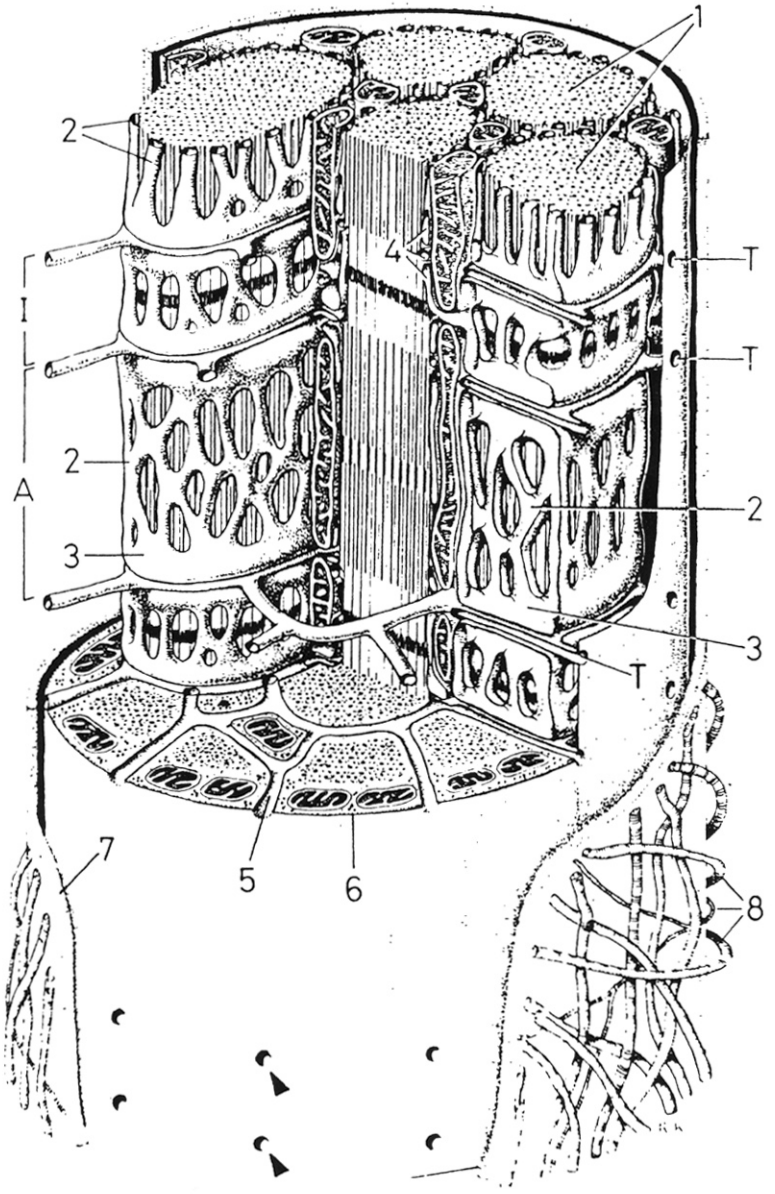


Figure 11.2. A magnified view of the structure of a single muscle fiber with a cutaway view of the myofibrillar structure. Each fibril is surrounded by a sarcoplasmic reticulum (SR) and by the transverse tubules system (TTS) which opens to the exterior of the fiber. [From R. V. Krstić, *Ultrastructure of the Mammalian Cell*, Springer-Verlag, Berlin, Heidelberg, New York, 1970 with permission.]

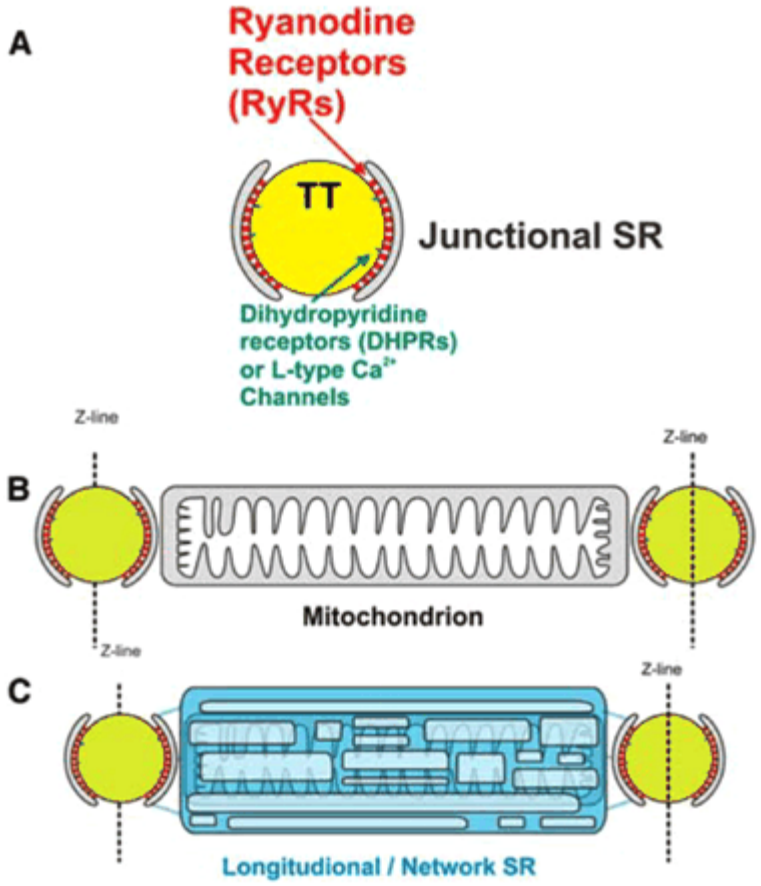
Excitation-contraction:

Steps in muscle fiber contraction

1. Motor neuron action potential
2. Action potential propagation along motor axon (myelinated fiber)
3. Transmission of *acetylcholine* (ACh) at neuromuscular junctions (synapses)
4. Action potential generation in muscle fiber
5. Release of Ca^{2+} from *sarcoplasmic reticulum* initiates attractive forces between actin & myosin filaments, causing them to slide alongside each other) muscle contraction
6. Return of Ca^{2+} to sarcoplasmic reticulum, ending muscle contraction

Excitation-contraction (cont.):

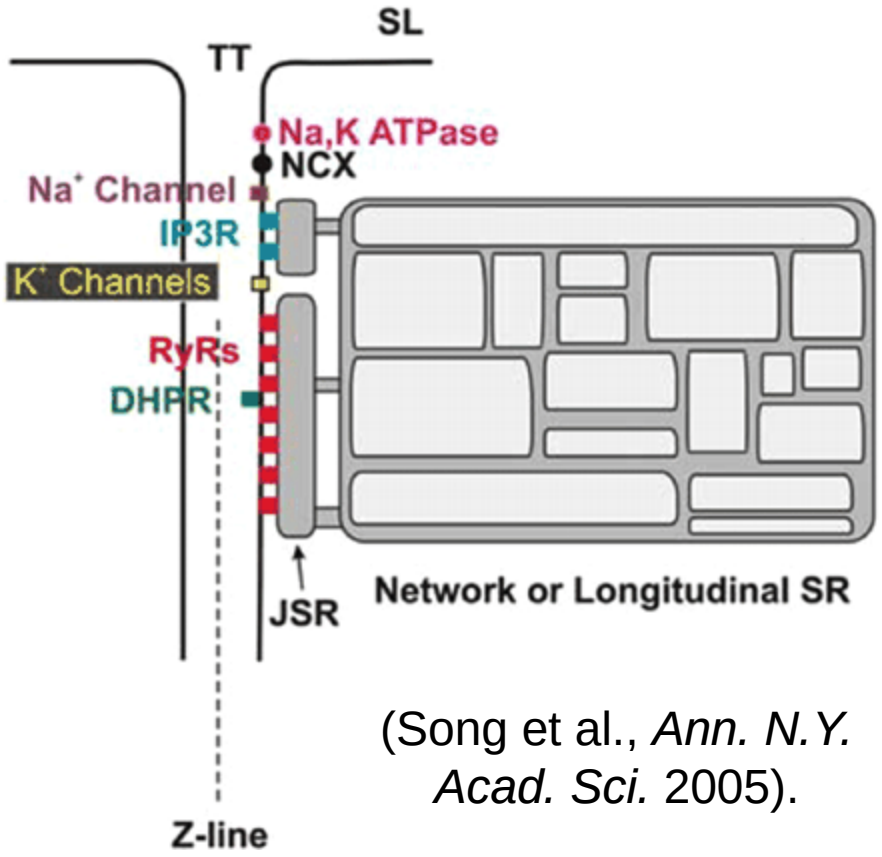
The TTS and SR are crucial for synchronized contraction of all fibrils in skeletal myocytes and mammalian cardiac myocytes.



(Song et al., *Ann. N.Y. Acad. Sci.* 2005).

Excitation-contraction (cont.):

Organization of key channel and transporter proteins and SR structures, including the junctional SR (JSR), near the TT —



(Song et al., *Ann. N.Y. Acad. Sci.* 2005).

Excitation-contraction (cont.):

The release of Ca^{2+} to trigger contraction of fibrils is facilitated by two different types of Ca^{2+} channels:

1. dihydropyridine receptors (DHPRs) or L-type Ca^{2+} channels, and
2. ryanodine receptors (RyRs).

Excitation-contraction (cont.):

- L-type Ca^{2+} channels are voltage-gated and appear on the TT and sarcolemmal (SL) membrane. These are opened by Na^{+} action potentials, allowing Ca^{2+} to flow into the intracellular space near the JSR.
- Reception of Ca^{2+} by RyRs opens up Ca^{2+} channels in the JSR membrane allowing release of Ca^{2+} from the SR. This leads to a positive feedback loop of Ca^{2+} release, triggering fibril contraction.