Experiments on frog neuromuscular system
Outline

• theoretical background
  – neuromuscular junction
  – electromechanical coupling
• animal experiments
• experimental procedures
• testing basic principles of skeletal muscle
Skeletal muscle

• skeletal muscle insures movement and fixation at the level of joints (articulations)
• contraction of muscle fibers is only possible through the activation of motor neurons
• organized in motor units (single $\alpha$-motor neuron and all of the corresponding muscle fibers it innervates)
Motor units

(A) SINGLE MOTOR UNIT

Neuromuscular junctions

Motor neuron

(B) TWO MOTOR UNITS

Motor neurons
Neuromuscular junction

- Depolarization of the motor neuron will create action potential that propagates through the axon.

- AP will open Ca\(^{2+}\) channels in the presynaptic bouton that will trigger the release of acetylcholine.

- Ach depolarizes postsynaptic membrane acting on nicotinic receptors, if threshold is reached a postsynaptic AP will form.
Electromechanical coupling

• postsynaptic AP (through influx of cations) triggers the opening of Ca$^{2+}$ channels and an increase of Ca$^{2+}$ concentration within the cell

• Ca$^{2+}$ will bind to troponine C changing its structure and thus allosterically modulating the tropomyosin

• this will allow myosin to bind to the newly uncovered binding sites on the actin

• the release of ADP and inorganic phosphate are tightly coupled to the power stroke that will pull the Z-bands towards each other, thus shortening the sarcomere
Relaxation of muscle

- ATP binds myosin, allowing it to release actin (a lack of ATP makes this step impossible, resulting in the rigor state)
- the myosin then hydrolyzes the ATP and uses the energy to move into the "cocked back" conformation
- repeat these steps as long as ATP is available and calcium is present
- calcium is actively pumped back into the sarcoplasmic reticulum, when it is no longer present on the thin filament, the tropomyosin changes conformation back to its previous state so as to block the binding sites again
Outline

• theoretical background
  – neuromuscular junction
  – electromechanical coupling
• animal experiments
• experimental procedures
• testing basic principles of skeletal muscle
Definition: physical pain is an unpleasant feeling that typically consists of negative affect and aversion, and has location, duration, intensity and a distinctive quality (e.g., burning, stabbing)
• Definitions:
  – condition of having sensation (including the feeling of pain) blocked or temporarily taken away
  – reversible lack of awareness
    • local (including spinal)
    • general
  – pharmacologically induced reversible state of amnesia, analgesia, loss of consciousness, loss of skeletal muscle reflexes and decreased stress response
Animal experimentation - ANESTHESIA METHODS

• pharmacological
  – local anesthetics: membrane stabilizing drugs (i.e. Na\(^+\) channel blockers - Lidocaine)
  – general anesthetics: reversible loss of consciousness

• other (only for animal research)
  – decapitation (guillotine, scissors)
    • when using frogs destroying spinal cord as well
Animal experimentation –
Types of experiments

• in vivo: “within the living” (experimentation using a whole, living organism )
• in situ: “in the place” (examine the phenomenon, organ exactly in place where it occurs, i.e. without moving it to some special medium)
• in vitro: “within the glass” (outside of the living organism, in a controlled environment, such as in a test tube or Petri dish)
Outline

• theoretical background
  – neuromuscular junction
  – electromechanical coupling
• animal experiments
• experimental procedures
• testing basic principles of skeletal muscle
Frog neuromuscular system in vivo

- anesthesia through decapitation and destroying the spinal cord
- animal laying on abdomen
- incision on calf skin, barking of skin exposing the gastrocnemius muscle; section the tendon of Achilles at the point of insertion
- incision on thigh skin, exposing sciatic nerve
- stimulation of nerve induces contraction of muscle
Outline

• theoretical background
  – neuromuscular junction
  – electromechanical coupling
• animal experiments
• experimental procedures
• testing basic principles of skeletal muscle
Muscle twitch

• contraction-relaxation curve
  – latency (10 msec) – electromechanical coupling
  – contraction (40 msec)
  – relaxation (50 msec)
Excitability threshold

- variable among muscle fibers
Spatial recruitment

- multiple fiber summation: as more and larger motor units are activated (i.e., more muscle fibers are contracted in the same time), the force of muscle contraction becomes progressively stronger.
Superposition

- if two stimuli are applied within a short interval (shorter than the duration of the muscle twitch) there is an overlap of the two contraction-relaxation curves.
- characteristic is that the second contraction has a higher amplitude.
Tetanic contractions

- multiple impulses stimulating the motor unit (and in turn the muscle fibers) and not giving it time to relax between contractions
- the strength of contraction is increased as it is added to the previous contraction
- dependant on the frequency of stimulation
Tetanic contractions
Temporal recruitment

- frequency summation: the force exerted by the muscle is controlled by varying the frequency at which action potentials are sent to muscle fibers.
- if the muscle is stimulated with a higher frequency stronger contractions occur.
Muscle fatigue

- the amplitude of contraction decreases with fatigue because there is an incomplete relaxation of the muscle
- the relaxation period is increased