NEUR3006 – Semester 1

Lecture 1: Neuron excitability

P1.1: Write down the Nernst Equation. Use arrows to identify each term and explain the key variables that influence resting membrane potential

- **All** living cells of the body have an electrical potential difference across their peripheral membrane (V_m)
- The ***resting membrane potential*** (resting V_m) is the V_m when the neuron is **inactive**.
    - Outflow of K^+ is balanced by an equal inflow of Na^+ (created by inward chemical and electrical driving forces acting on Na^+)

Key Regulators	**Equilibrium potential** - E_K and E_{Na} (when electrical potential = chemical force of ions)	**Features**
Role of potassium	-75mV	***Chemical driving force* for K^+ causes** K^+ to diffuse *down its* ***concentration gradient*** (from inside to outside).
Role of sodium	+55mV	V_m never reaches E_K → due to slow outward diffusion of K^+ being balanced by slow inward diffusion of Na^+ via leak channels
Relative permeability	**Resting V_m = -65mV** due to membrane being 20 x more permeable to K^+ than Na^+ (V_m closer to E_k than E_{Na})	

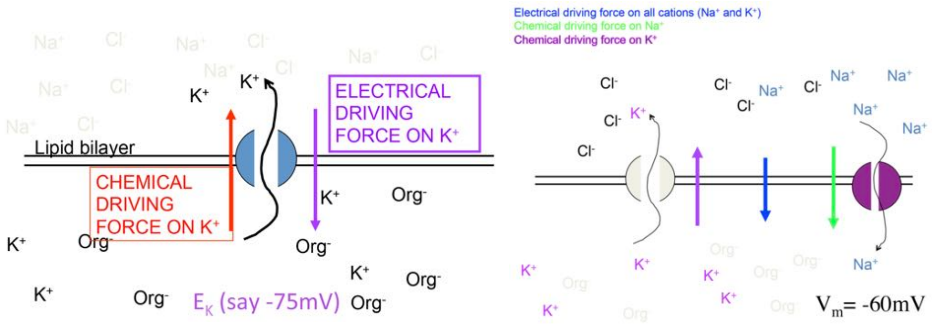
P1.2: Explain the relationship between the resting membrane potential and the Nernst Potentials for sodium, potassium and chloride ions

$$E_{K^+} = \frac{RT}{zF} \ln \frac{[K^+]_{ECF}}{[K^+]_{cytoplasm}}$$

- The Nernst potential for potassium (E_K) tells us **where potassium would take the V_m** if potential is potassium dependent [applies to all ions]

CHEMICAL AND ELECTRICAL DRIVING FORCE:

- The direction and magnitude of the chemical driving (vector arrow) depends on:
    - the **concentration difference** across the membrane
    - **temperature**.
- Ions **cannot cross the lipid bilayer** → require a protein channel.
- K^+ leakage channels on the peripheral membrane allow **continual slow leakage of K^+ out of the cell** → enough to polarize membrane and produce unbalanced negative charge for resting potential
- The V_m constitutes 2nd force: the *electrical driving force* which acts upon all ions



Goldman Equation

$$V_m = \frac{RT}{F} ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}$$

The Goldman Equation models the combined contributions of K^+, Na^+ and Cl^- ion movements to the resting V_m (Chloride ions also play a role in determining V_m)

Role of the sodium/potassium pump

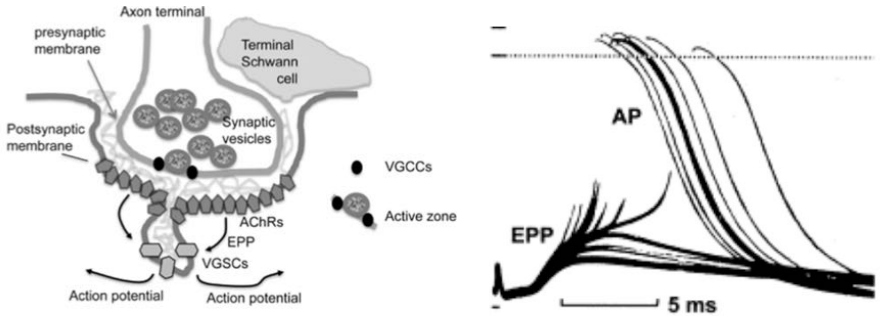
- The **Na^+/ K^+ -ATPase pump** actively transports 3 Na^+ out of the cell and 2 K^+ into cell but has minimal effect on V_m as it is very slow
- Inhibiting pump will cause V_m to become less negative → hence **Na^+/ K^+ -ATPase pump** maintains concentration gradients of K^+ and Na^+ over the long term

P1.3: Explain the characteristic features of the axonal voltage-gated sodium channel that contribute to generating the action potential

Neuronal signalling is rapid:

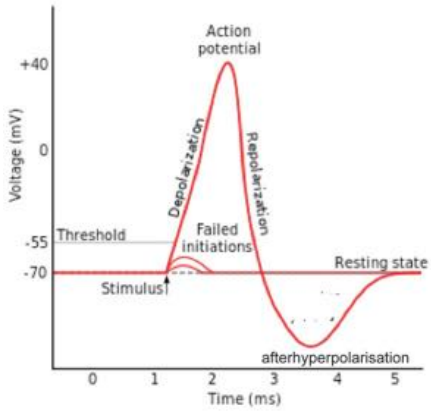
- Synaptic potentials and action potentials occur quickly over a **millisecond timescale** due to rapid changes in relative permeability of ions
- Sudden increases to Na^+ permeability will increase the rate of Na^+ influx, in the same proportion, causing depolarisation.
- Local increase in Na^+ influx produces an excitatory postsynaptic potential (EPSP) or **graded potential (not involved in Hodgkin Cycle)** to cause a transient rise in V_m above its resting level
    - Amplitude of the increase in V_m depends on **how many ligand-gated cation channels** open at the synapse.

P1.5: To what does the term 'synaptic potential' refer?



- Synaptic potential refers to the difference in voltage between the inside and outside of a postsynaptic neuron
- In motor neurons, depolarising synaptic inputs (EPSPs) trigger APs, which move down a *myelinated* motor neuron *axons* via *saltatory propagation* (100m/s)
- **The *neuromuscular junction* (NMJ) is a *chemical synapse*** that operates through:
    1. Depolarisation of the motor nerve terminal by AP causes the opening of *voltage-gated calcium channels* (VGCCs) releasing Ca^{2+} from ECF into axon terminal
    2. Ca^{2+} binds to sensor proteins on *synaptic vesicles* to trigger *exocytosis* of ACh from inside the synaptic vesicle into the synaptic cleft.
    3. ACh binds to and triggers the opening of acetylcholine ligand-gated cation receptors (AChRs) on the postsynaptic membrane to allow influx of Na^+ into postsynaptic membrane
        - Brief opening of AChR channels produces small (quantal) depolarisation called the ***miniature endplate potential***
        - *Each quantal response* sum together to produce the large amplitude postsynaptic depolarisation called the ***endplate potential (EPP)***
    4. **If EPP reaches threshold,** this **triggers an action potential** and thus, the **Hodgkin cycle** in the muscle fibre.

P1.4: Describe the properties of the axonal voltage-gated potassium channel that complement the role of the sodium channel in creating action potentials



What does the VGSC contribute to the action potential shown Here?

- *Action potentials are produced by the rapid opening of Voltage-gated Na^+ channels* (VGSC) when the graded potential causes the V_m to exceed the threshold value causing a brief, exponential rise in the *membrane permeability* resulting in rapid depolarization of the cell membrane.
- This is followed by a slow transient increase in the permeability of K^+ (causing repolarization and subsequent hyperpolarization)

What 2 gates are there on the VGSC and the properties of each gate type?

Gate	**Properties**	**Effect**	**Note:**
Activation gates	Open **rapidly** in response to depolarisation of the membrane	**Increased influx** of Na^+ causes neighbouring VGSCs to open (this is the *Hodgkin cycle*).	Both the activation and the inactivation gates **must be open** for Na^+ to pass through the channel
Inactivation gates	When the membrane is depolarised for a short time (< 1ms), these gates **close**	**Repolarisation** → stops influx of Na^+	

Explain: "hyperpolarisation" + "after hyperpolarisation" + "period of reduced excitability"

- *Voltage-gated K^+ channels* (VGKC) also respond to the depolarisation, but their activation gates are **intrinsically slower to open.**
- VGKCs open to increase the rate of K^+ efflux causing the V_m to return from +40mV (peak) to E_K
- **Repolarisation of AP** = Inactivation of VGSC and opening of VGKC
    - VGKCs are also **slow to close their activation gates** after membrane repolarization → explaining *afterhyperpolarisation* or the *period of reduced excitability*.

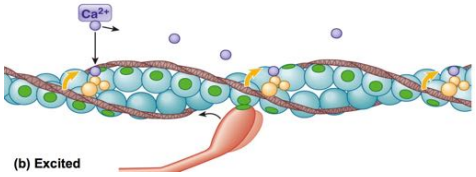
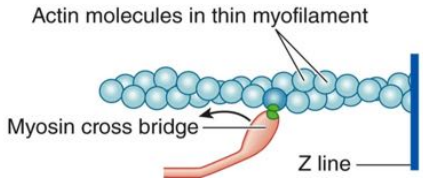
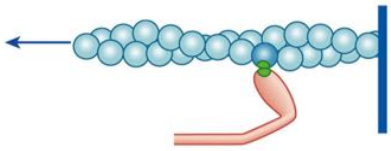
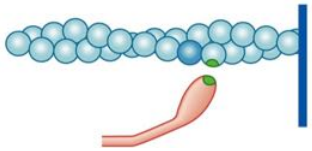
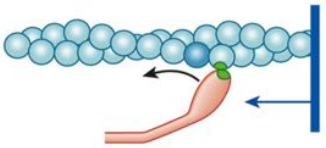
Explain "the Hodgkin Cycle"

The Hodgkin cycle represents a **positive feedback loop** in which an initial membrane depolarization leads to uncontrolled deflection of the membrane potential to near E_{Na}.

2. MOTOR UNITS AND MOTOR NEURON POOLS

1. Skeletal muscle is stimulated to contract by release of ACh into synaptic cleft of neuromuscular junction
2. ACh binds to nicotinic ACh receptors to create influx of Na^+ and efflux of K^+
3. Sufficient depolarization generates an AP along the sarcolemma
4. AP transmitted to Transverse-tubules which stimulate dihydropyridine receptors linked directly to ryanodine receptors on SR
5. Stimulation of ryanodine receptors release Ca^{2+} stored in SR into the sarcoplasm of the muscle fibre to allow contraction through the cross-bridging cycle
6. An *active transporter* pump protein works continuously to pump Ca^{2+} from the cytoplasm back into the SR → to end contraction

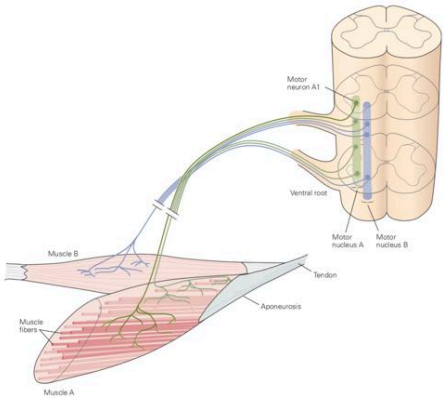
**Contraction continues as long as sufficient calcium, ATP and stimiulus is present (tetanus)*

1. Destabilisation	Ca^{2+} binds to troponin, tropomyosin fibres changes conformation to reveal actin binding site ** troponin stabilises tropomyosin in a cross bridge **blocking** position when Ca^{2+} not bound*	
2. Binding:	Myosin cross-bridges (thick filaments) can bind with actin molecules (thin filaments)	
3. Power Stroke	Cross bridge bends, pulling thin myofilament inward	
4. Detachment	Cross bridge detached at end of power stroke and returns to original conformation	
5. Binding	Cross bridge binds to more distal actin molecules and cycle repeats	

How much force is generated?

- **Amount of force** generated depends on **cytoplasmic $[Ca^{2+}]_i$,** which depends upon the **action potential frequency**.
    - Thus, higher frequencies cause an increase tetanic contraction force up to about 150Hz.
- Force produced by a skeletal muscle is dependent on:
    1. **# of fibres** contracting in a muscle
    2. **Amount of force** developed by each contracting fibre

P1.6 What is a motor unit and are they all the same?

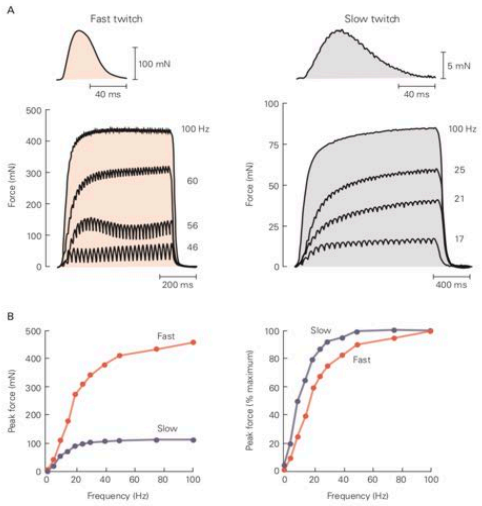


- Each muscle is controlled by a group of ***alpha motor neurons*** that constitute the ***motor neuron pool***
    - E.g. The motor neuron pool of a hind limb muscle = loose column of motor neuron cell bodies (***green*** *column)* in the ventral and lateral portion of the lumbosacral spinal cord.
    - ***1 motor neuron cell body in the ventral spinal cord*** *sends its axon to innervate a collection of muscle fibres (1 motor unit) in its target muscle*
- 1 **motor unit** = single motor neuron + the **group of muscle fibres** that it **innervates and exclusively controls**
    - One motor neuron can innervate a number of muscle fibres **BUT** each muscle fibre is supplied by only one motor neuron
    - Minimum unit of force production within the muscle

P1.7 How does the frequency code influence muscle force?

Motor unit types and force production

	Fast-twitch motor units (weightlifting**)**	**Slow twitch motor units (**Marathon**)**
Diameter	**Large**	**Small**
Myosin Type	Fast-type	Slow-type
Metabolism	Glycolysis (more anaerobic than fast twitch)	Oxidative phosphorylation (aerobic)
Contraction duration	**Short**	**Long** (can sustain tetanic contraction)
Force	Far **greater** force	**Less** force
Speed to develop max tension	**Fastest**	**Slowest**
# of muscle fibres	**Many**	**Few**
Subdivisions	• Fatigue resistant (aerobic) Fast fatiguing types (using anaerobic metabolism to produce a lot of force but only for brief contractions).	

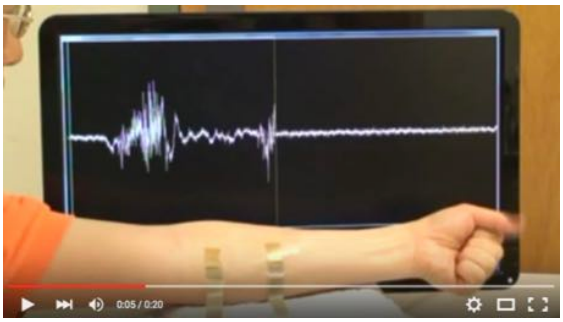


Single motor axons of either a ***fast-twitch or slow-twitch motor neuron*** *were individually stimulated and isometric contraction force was recorded with a force transducer.*

Note the* ***different scales* *on the graphs.*

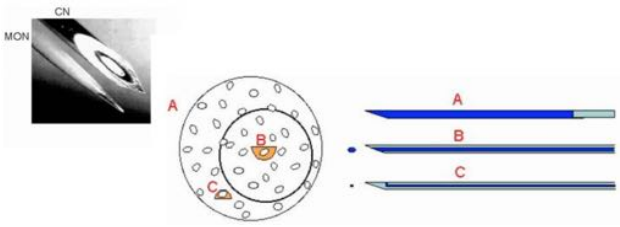
P1.8 What methods are needed to record CMAPs and single fibre EMG and what kinds of information can they reveal?

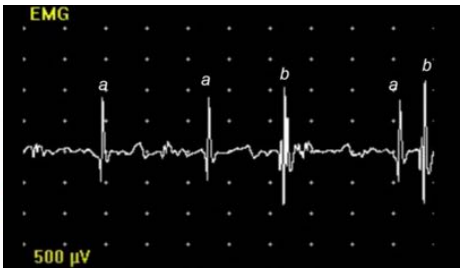
Compound muscle action potential (CMAP)



- EMG records the small extracellular electrical currents generated as the muscle action potential propagates from the NMJ to the end of each muscle fibre
- ***Compound muscle action potential*** **(CMAP)** is a simple form of EMG that involved:
  1. Electrodes are attached to the skin surface directly over the muscle
  2. CMAP triggered through voluntary or artificial electrical stimulation of all the motor axons in the nerve that innervates the muscle
  3. Measuring potential difference between 2 recording electrodes
- **CMAP represents the summed electrical currents produced BY all the active muscle fibres within the muscle**
  - Area of CMAP waveform = relative # of muscle fibres or motor units activated at any given time (i.e. greater area = greater # of active muscle fibres)

Single Fibre EMG





Activity of individual motor units detected by:

- **Bipolar needle electrode** inserted into the muscle close to individual muscle fibre ⟶ record single fibre APs
- Different extracellular action potential waveforms = 2 distinct motor units in the same muscle (e.g. a and b)
- ***How would you use such a trace to estimate the average action potential firing frequency of motor neuron/motor unit 'a'?***

Measure time between consecutive similar waveforms to determine frequency at which motor unit is activated by spinal cord during voluntary activation of the muscle.

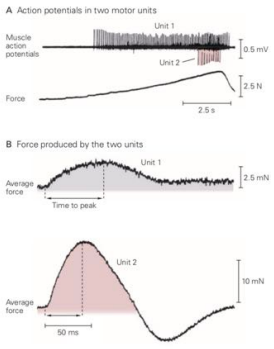
P1.9 What is the typical motor unit recruitment sequence?

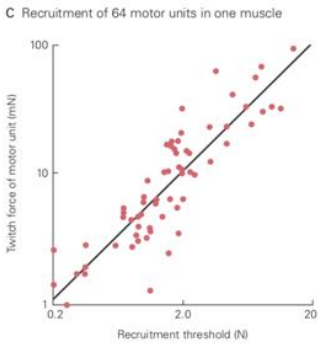
Typical recruitment sequence involves:

- Slow twitch (low force) units recruited first
- More powerful force-producing motor units recruited later

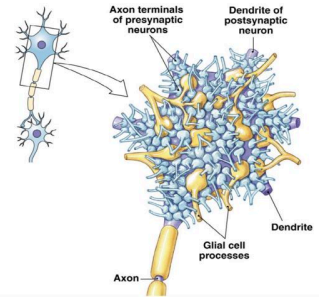
Graphs show that:

- Motor unit 1 = **low force** motor unit
- Motor unit 2 = produces **more force**





3. SYNAPTIC INTEGRATION AND MOTOR UNIT RECRUITMENT



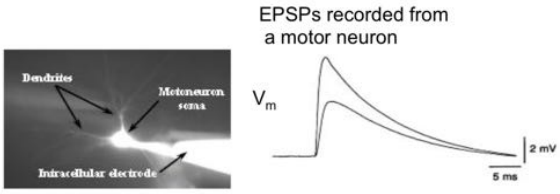
Intracellular recordings of motor neurons:

1. To study synaptic inputs → a microelectrode is pierced through cell membrane
2. A negative resting V_m recorded → because as whole surface of the motor neuron (soma and dendrites) is covered by synaptic inputs → *see figure*
3. Most **synapses are *glutamatergic*** → meaning that the presynaptic nerve terminal (*bouton)* releases vesicle-loads of glutamate.
4. These quanta of glutamate bind and activate ***glutamate* ligand-gated cation channels *receptors*** (AMPA or NMDA) on the postsynaptic membrane to cause depolarization via an influx of Na^+
5. This depolarization/graded potential is recorded as an *excitatory postsynaptic potential* (EPSP) using the intracellular electrode.

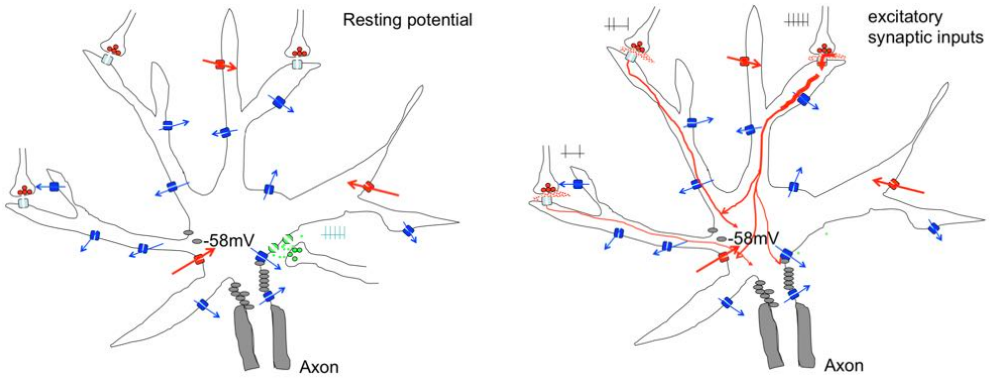
P1.10 What are EPSPs and what is meant by summation?

Excitatory Postsynaptic Potentials (EPSPs)

- EPSPs are graded potentials that cause the V_m to rise a few milliVolts (mV) above resting V_m for many *milliseconds*.
- Most EPSPs **do not** depolarise the motor neuron to threshold.
- **SUMMATION** = Many EPSPs received simultaneously by active excitatory glutamatergic synapses can sum together to reach threshold and trigger an AP.
- *Generally, the higher the V_m goes above threshold, the greater the frequency of action potential firing (up to a limit).*



EPSPs following stimulation of Ia afferents fibres

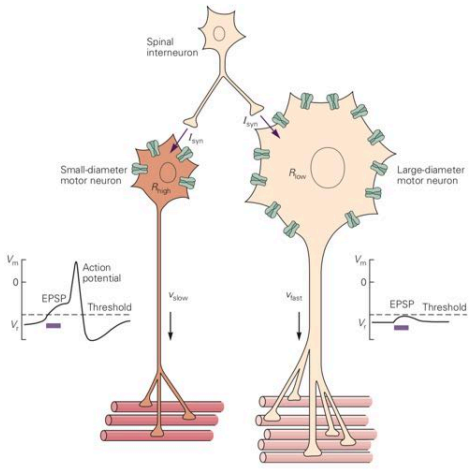


More excitatory inputs (bouton synapses) to motor neuron = **higher amplitude** (frequency coding)

P1.11 Explain the size principle and its underlying neurophysiological mechanism

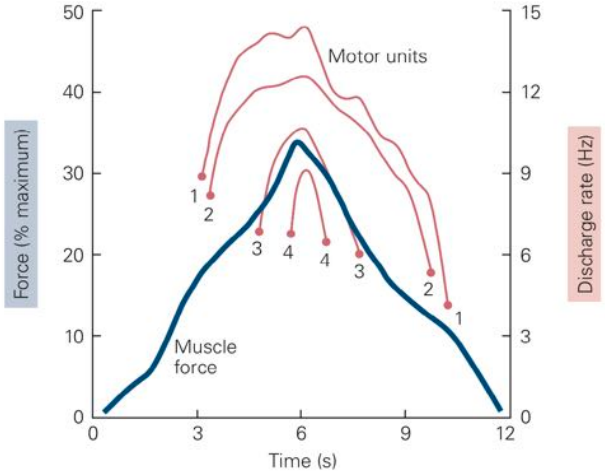
SIZE PRINCIPLE for motor neuron recruitment

- **Leakiness of neuron measured as its input resistance**
- Smaller motor neurons have higher input resistances as smaller area of peripheral membrane for depolarizing current to leak out → hence less current needed to bring it to threshold
- **More depolarising current** is needed to bring the large motor neurons up to threshold
- During voluntary activation, the increasing amount of depolarising postsynaptic current activates small motor neurons 1st (i.e. those with highest input resistance) followed by the next largest



P1.12 Suppose you try to lift a very heavy bag. What is happening to the electrical activity of your motor neurons and motor units?

Population and frequency codes in motor tasks



Recruitment and firing rates of motor units in a knee extensor muscle during a voluntary increase

Scenario	Response
1. Small **force is needed**	**Weakest but most enduring motor units are activated 1st** *(e.g. motor unit 1 reaches 20% of max force → but contributes to increase in total force)*
2. More **force is required**	Increased frequency of firing → greater tetanic force from **same slow-twitch motor units** → stronger motor units begin to be recruited
3. Even more **force is required**	more powerful motor units are recruited → each unit increases its firing rate to help as necessary to achieve the required voluntary force for the task

SUMMARY:

- The motor neuron pool of a muscle can steadily increase the force produced by its muscle by **progressively activating more and more motor units (according to the size principle)** and by **increasing the firing frequency** *(and contraction force)* of the motor units that are already active.