Lab 5: Physiology of the *in situ* Amphibian Heart

Lab Roles: Please make sure they are rotating roles, not just the PC, but the animal handler/tissue prep, experimenter, and the computer operator. Remind them to rotate each week.

Notes:

1) This is a live animal lab. Work quickly to make sure you complete al experiments before the tissue dies.

2) The group member who is monitoring the animal must apply ringer's solution to all *open cuts* throughout the experiment. Anything that dries out will die.

3) Carefully attach the fishhook to the heart at the tip (apex) of the ventricle. You are going into the ventricular wall, but don't go all the way through! If you puncture the heart your experiment is over.

4) Good electrical contact is essential to get a good ECG.
    1) Clean the hook electrodes. You may have to scrape to get shiny metal.
    2) Make a slit in the muscle belly of a large muscle, hook the electrode inside the muscle belly.
    3) Apply Ringer's to these cut areas too.
    4) Clean the hook electrodes with water and dry them with paper towels at the end of the experiment — they are expensive!

Cardiac muscle

This lab is about regulation of heart rate and force of contraction, both by extrinsic (temperature and stretch), as well as intrinsic (neurotransmitters, ions) factors.

The role of ions in AP's:

Remember that the membrane potential in neurons is negative (negative inside the cell, positive outside).

<u>Inside cells:</u> Overall negative, High K+, Low Na+
<u>Outside cells:</u> Overall positive, Low K+, High Na+

Action potentials occur when the Na+ channels are open, allowing the Na to flow into the cell and depolarize the membrane.

The reason why these particular neurotransmitters are chosen is because they are representative of the neurotransmitters of the sympathetic and parasympathetic nervous system, which regulate heart rate and contractile force.

Acetycholine (Ach) - decreases heart rate and force of contraction
In vertebrates Ach is inhibitory rather than excitatory.

causes hyperpolarization of cardiac muscle by opening K+ ion channels.
K+ flows out, making the membrane potential more negative (hyperpolarization).
Hyperpolarization inhibits action potentials by increasing the stimulus needed to move the membrane potential to the A-P threshold. (it has the opposite effect in skeletal muscle because there Ach receptors are coupled to Na channels, so encourages depolarization when open).

Epinephrine increases heart rate and contractility by stimulating the SA node and the AV node and enhancing Ca2+ ion release via second messenger pathways.

Pilocarpine stimulated release of Ach.

Atropine binds and blocks acetylcholine receptors, preventing Ach from decreasing heart rate.

Caffeine increases heart rate and force

cadmium chloride decreases rate and force, increases P-R interval

Ca+ is necessary for heart muscle contraction. Therefore Ca free solution decreases heart rate and force

K free increases heart rate and force because it makes the extracellular environment less positive (making it easier to depolarize the membrane)

K added decreases heart rate and force because it hyperpolarizes the membrane.

Temperature: low temperature reduces enzymatic activity, high temperature increases enzymatic activity

Starling's Law: heart rate contracts more forcefully when muscle is pre-stretched (this is a general property of muscle fibers). Muscle is stretched naturally when ventricles fill, resulting in a more forceful contraction and ejection of blood.