**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 just need to hook a few muscle fibers, don’t go all the way through the ventricular wall! If you puncture the heart your experiment is over.
4. Good electrical contact is essential to get a good ECG.
   1. Check the hook electrodes. If they are not clean, ask your TA.
   2. The ECG is hooked in the muscles of the arms and leg. Make a slit in the skin and in the muscle belly of a large muscle, hook the muscle fibers in the 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 (positive outside the cell, negative inside).

Inside cells: Overall negative, High K+, Low Na+

Outside cells: 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 messengers of the sympathetic and parasympathetic nervous system, which modulate 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** stimulates 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 (and additionally when there is more venous return, such as during vigorous exercise), resulting in a more forceful contraction and ejection of blood.