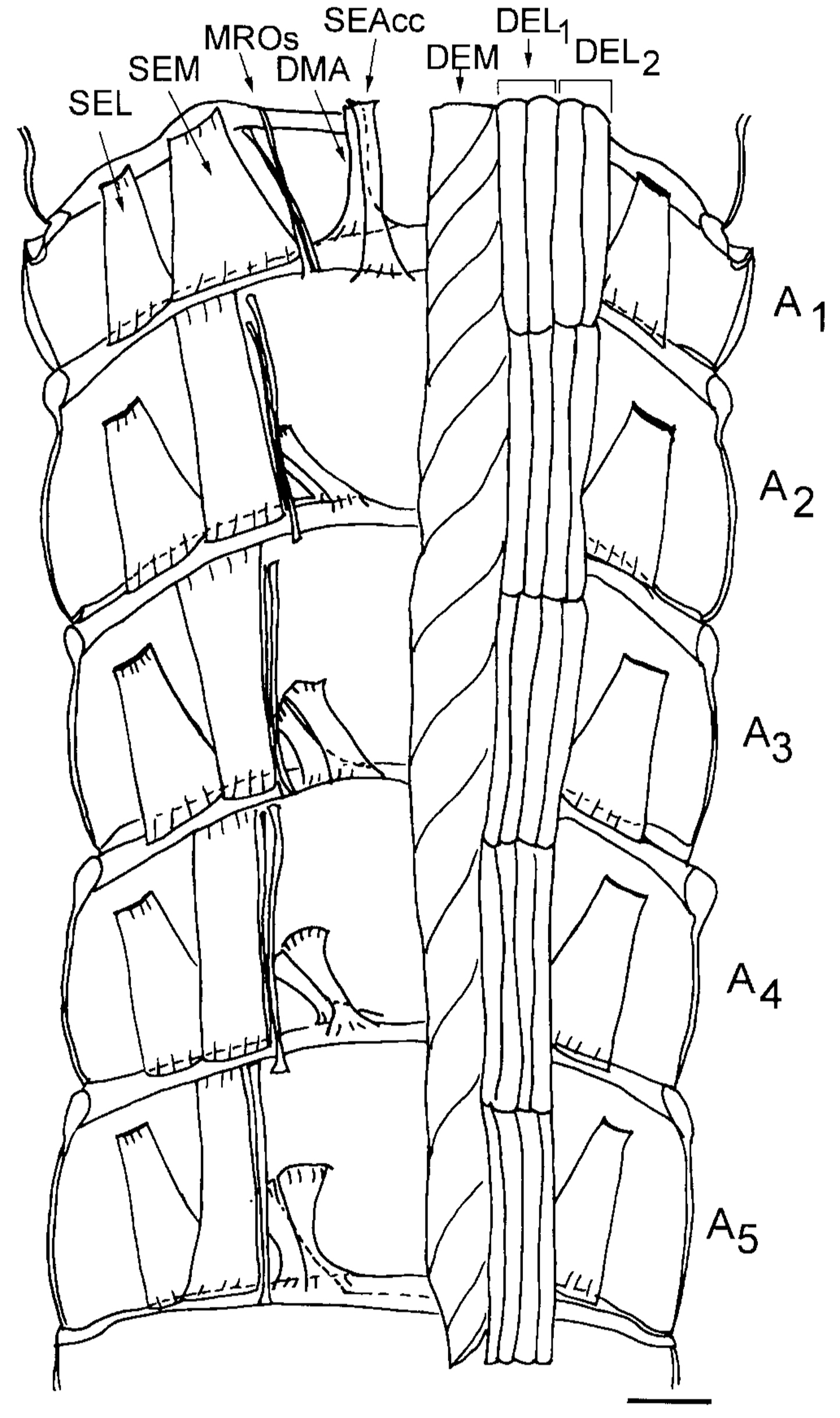
**Bio446/650 check off list for Feb 18, 2016**

**Part 1 of 2:**

Crayfish abdomen (L1,L2 maybe M muscles for resting membrane potentials.



*Schematic drawing from a ventral view of the dorsal part of the crayfish abdomen showing the extensor musculature of each segment. The dorsal membrane abdomen muscle (DMA) and the superficial extensor accessory muscle head (SEAcc) occur in segments 1 through 5 of the abdomen with a different orientation for each segment. With the exception of segment 1, these muscles have their attachment sites at their anterior end to the calcified tergite and at the posterior end in the articular membrane. In segment 1, the homologous muscles have their anterior attachment sites to the articular membrane located between the thorax and abdomen. The illustration was based upon photographic montages of methylene blue stained preparations. On the left side of the figure all the deep extensor muscles have been removed to show the dorsal superficial extensor muscles. Scale = 2.35 mm. (Taken from Sohn et al. 2000).*

If you are bored here is a movie

<http://www.jove.com/video/2322/membrane-potentials-synaptic-responses-neuronal-circuitry>

Normal saline (5.4 mM K) RP\_\_\_\_\_\_, RP\_\_\_\_ RP\_\_\_\_\_\_ AVG \_\_\_\_\_

20 mM K RP\_\_\_\_\_\_, RP\_\_\_\_ RP\_\_\_\_\_\_ AVG \_\_\_\_\_

40 mM K RP\_\_\_\_\_\_, RP\_\_\_\_ RP\_\_\_\_\_\_ AVG \_\_\_\_\_

60 mM K RP\_\_\_\_\_\_, RP\_\_\_\_ RP\_\_\_\_\_\_ AVG \_\_\_\_\_

Think about how to graph RP vs. K+ and log , semi log etc… and why

Hint:

The Nernst equation is generally considered for ions across a membrane generating an electromotive force as commonly shown as:

X = ion of interest

V = equilibrium voltage for the X ion across the membrane

R = gas constant [8.314 J/(mol•K)]

T = absolute temperature [Kelvin]

Z = valence of the ion

F = Faraday's constant [9.649 × 10^4 C/mol]

For the K+ ion at 20oC and transformation of ln to log10 along with filling in the constants, one arrives at:

Let us assume that only K+ is permeant by diffusion. [Kin] is the K+ concentration on the inside of the cell and [Kout] is the K+ concentration on the outside of the cell.

As an exercise estimate [Kin]. \_\_\_\_\_\_\_\_\_\_\_\_\_\_

Assume for this calculation, membrane potential is only dependent on the K+ equilibrium potential.

Given the [Kout]= for the saline used is 5.4 mM. Also, assume membrane potential is -70mV.

In the experiment we will measure a cell’s resting membrane potential and determine how it is influenced by altering [Kout]. The slope of the hypothetical line relating membrane potential and [Kout] is 58. After collecting data on the resting membrane potential at various [Kout] (range from 5.4 mM to 100 mM) we will plot the observed values to determine if there is a match with the hypothetical line. We will use the average resting membrane potential obtained at 5.4 mM [Kout] for initiating the hypothetical and observed lines for comparison.

Considering that a membrane can be permeable to more than one ion at rest, as well as at various depolarized states, one uses the G-H-K equation to take into account the permeability (P in the equation) for various ions. The G-H-K equation will reduce to the Nernst equation if a membrane is permeable to only one ion.

Here is a generalized G-H-K equation for Na+, K+, and Cl- ions:

Since Cl- has a negative charge, the concentration term is inverted in this equation for the inside and outside. This allows the Z (ion charge) to be left off.

Aims of this exercise

In this experiment we will measure the membrane potential of a crayfish muscle cell and apply the principles discussed above to address:

1. How to measure a cell membrane potential with appropriate instrumentation and technique.

2. Ion permeability of the muscle cell membrane and how it contributes to the membrane potential.

Plot the measures obtained for the resting membrane potentials at each [K+]out used using Excel. See if the observed and hypothetical lines are matched in their slope. To plot the values use a semi-log plot with the x-axis of varied [K+]out as a log and the y-axis of the membrane potentials (as shown below). (Download free graph paper if needed <http://incompetech.com/graphpaper/logarithmic/>)

graph.tif

As early as 1902, Bernstein was dealing with the issues of a resting potential in the axon of a squid. It is intriguing to consider how these early ideas and observations of Berstein (1902) and Nernst (1888) later influenced research in membrane physiology. (See review by Malmivuo and Plonsey, 1995; also available on the www <http://www.bem.fi/book/> ). There are still, to this day, breakthroughs being made about ion channel function and properties of biological membranes that are very significant in understanding the cellular physiology which relates to the function of tissues, organs and systems.

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**Part 2 of 2:**

The abdominal extensor muscle preparation used to demonstrate the resting membrane potential is also ideal for demonstrating induction of synaptic responses at the NMJs from the various muscles. Some muscles in crustaceans are selectively innervated by either a phasic or a tonic motor neuron, although some single fibers can be innervated by both phasic and tonic excitatory motor neurons, such as for extensor muscle in the crayfish walking legs (Atwood, 2008) and most other limb muscles (Wiersma, 1961a). By selectively stimulating phasic and tonic motor neurons, physiological differences in the EPSPs may be measured. Phasic motor neurons produce rapid twitching of muscle fibers and evoke EPSPs on the order of 10–40 mV. The phasic response can depress rapidly with 5–10-Hz trains of stimulation. The tonic motor neurons give rise to smaller EPSPs that can be facilitated in the presence of a higher frequency (10–50 Hz) of stimulation. Structurally, the presynaptic phasic and tonic terminals at the NMJs are different (Atwood and Cooper, 1996; Bradacs *et al.,* 1997; Cooper *et al.,* 1998).

An additional NMJ preparation presented is used for monitoring intrinsic motor activity and sensory stimulus induced motor activity from the CNS. This is the superficial flexor muscle on the ventral side of the crayfish abdomen. This preparation will also be used to monitor the sensory-CNS-motor-muscle circuit and the effects of neuromodulators (Strawn *et al.,* 2000).

In each of the abdominal segment (except the last) there are three functional groups of muscles: (1) those controlling pleopod (swimmerets) movement, (2) three extensor muscles and (3) three flexor muscles. The flexors and extensors are antagonistic groups of muscles which bring about either abdominal flexion or extension by causing rotation about the intersegmental hinges. The phasic musculature occupies most of the volume of the abdomen, while the tonic muscles comprise thin sheets of fibers that span the dorsal (extensors) and ventral (flexors) aspect of each abdominal segment.

In crayfish, the tonic abdominal flexor muscles of crayfish are innervated in each half segment by five motoneurons and by a peripheral inhibitory neuron. The excitatory motoneurons use glutamate as a neurotransmitter. Glutamate depolarizes the muscle fibers by causing an increase in permeability primarily to sodium ions. The inhibitory neurons release gamma-amino butyric acid (GABA), which usually hyperpolarizes the muscle fibers by causing an increase in permeability to chloride ions. In some crustacean muscles (mainly in limbs), the peripheral inhibitory neurons make synaptic contacts with motor neuron terminals as well as with the muscle fibers, and reduce the amount of transmitter released by the motor neuron (presynaptic inhibition) (Dudel and Kuffler, 1961). This phenomenon is not present in the tonic flexor muscles of crayfish.

Each abdominal ganglion (except the last) has three roots on each side. The first root contains axons of neurons innervating the pleopod musculature and sensory axons; the second root contains axons innervating phasic and tonic extensor musculature and sensory axons; and the third root, which leaves the nerve cord several millimeters caudal to the ganglion, contains axons innervating phasic and tonic flexor musculature. There are two branches of the third root. The deep branch (IIIa) innervates only phasic flexor muscles. The superficial branch of the third root (IIIb) in each half-segment contains six axons, which innervate the tonic flexor muscles.

The neurons innervating the tonic flexor are spontaneously active, unlike the phasic efferent neurons, and in a good preparation, they will continue to fire for many hours after the abdomen has been removed from the animal. For a review of the historical nature of the discoveries made in these abdominal preparations see Atwood (2008). The cell bodies of four of the motor neurons and of the peripheral inhibitory neuron innervating the tonic flexor muscle in any half segment are located in the ganglion of that segment. The cell body of the remaining motor neuron is located in the next caudal ganglion. These neurons may be reliably distinguished from each other on the basis of extracelluarly recorded spike amplitudes. If the tonic flexor muscle from one half segment is removed along with the two ganglia containing the neurons innervating this muscle, five neurons usually show some degree of spontaneous activity. These neurons are numbered on the basis of relative extracellular spike amplitude, in ascending order. f1 to f4 are motoneurons and f5, the largest spontaneously active neuron, is the peripheral flexor inhibitor. f6, the largest motor neuron, is an excitatory motor neuron which is seldom spontaneously active.

The spontaneous nature of tonic motor neuron activity can be modulated by exogenous application of compounds or by providing a sensory stimulus to the cuticle within the same segment that is being monitored for motor nerve activity.

Record the spontaneous activity of the EPSPs. Note the different sizes of the EPSPs and if IPSPs are present.

NOTES \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Very carefully take a small paint bush and by hand stimulate along the cuticle edge within the same segment that one is monitoring the spontaneous activity. Note a change in frequency of the responses and if different size EPSPs appear that were not there prior to stimulating the cuticle.

outline of brush prep.tif

*Preparation with stimulating brush and nerve roots. (modified from Strawn et al., 2000)*

NOTES \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

The stimulation can be repeated after carefully exchanging the saline bath with one containing a neuromodulator such as serotonin (1 microM) or saline bubbled with CO2. Note the effect on the activity profile for a given stimulus. Also note if exchanging the saline back to fresh saline returns the activity to its initial condition.

NOTES: effects of 5-HT \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Maybe we will also try GABA on the prep.

NOTES: effects of GABA \_\_\_\_\_\_\_\_\_\_\_\_\_\_

The phenomena of the spontaneous activity of the 3rd motor root has been a topic since the 1960’s when Eckert (1961) examined if the tonic firing static muscle receptor organ (MRO) within the same or neighboring segment could account for the spontaneous motor drive. In these earlier studies it became apparent that the activity was driven within the ventral nerve cord (VNC) possibly from higher centers (Eckert, 1961; Kennedy and Takeda, 1965a,b; Strawn *et al.,* 2000). Since the presence of CO2 stopped the spontaneous activity, one can assume somewhere in the drive to the motor neurons there might be gap junctions or even glutamatergic excitatory drive. The NMJs are blocked or present a decreased sensitivity to glutamate in the presence of CO2, so it is likely that they maybe blocked as well within the CNS (Bierbower, 2010; Bierbower and Cooper, 2010; see also Badre *et al*., 2005).

The action of various neuromodulators is also readily studied at the various types of NMJs (Cooper and Cooper, 2009; Griffis *et al.,* 2000; Southard *et al*., 2000; Strawn *et al.,* 2000) presented in addition to the influences on various aspects of the CNS circuitry. It has been suggested that the 5-HT and octopaminergic neurons may function as ‘gain-setters’ in altering the output of neuronal circuits (Ma *et al.,* 1992; Schneider *et al.,* 1996; Hörner *et al.,* 1997; Edwards *et al*., 2002). Much work remains to be done before we can fully understand the effects of neuromodulators on individual target cells. Given that different neuromodulators may work in concert with one another, analysis of their mixed action is an area for future research (Djokaj *et al.,* 2001). In addition, few studies, particularly in the vertebrates, address the effects of neuromodulators on entire pathways which can regulate a specific behavior. In this sensory-CNS-motor unit preparation one can examine the influence of both sensory input and neuromodulators on the activity of the motor neurons (Kennedy *et al.,* 1969).

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