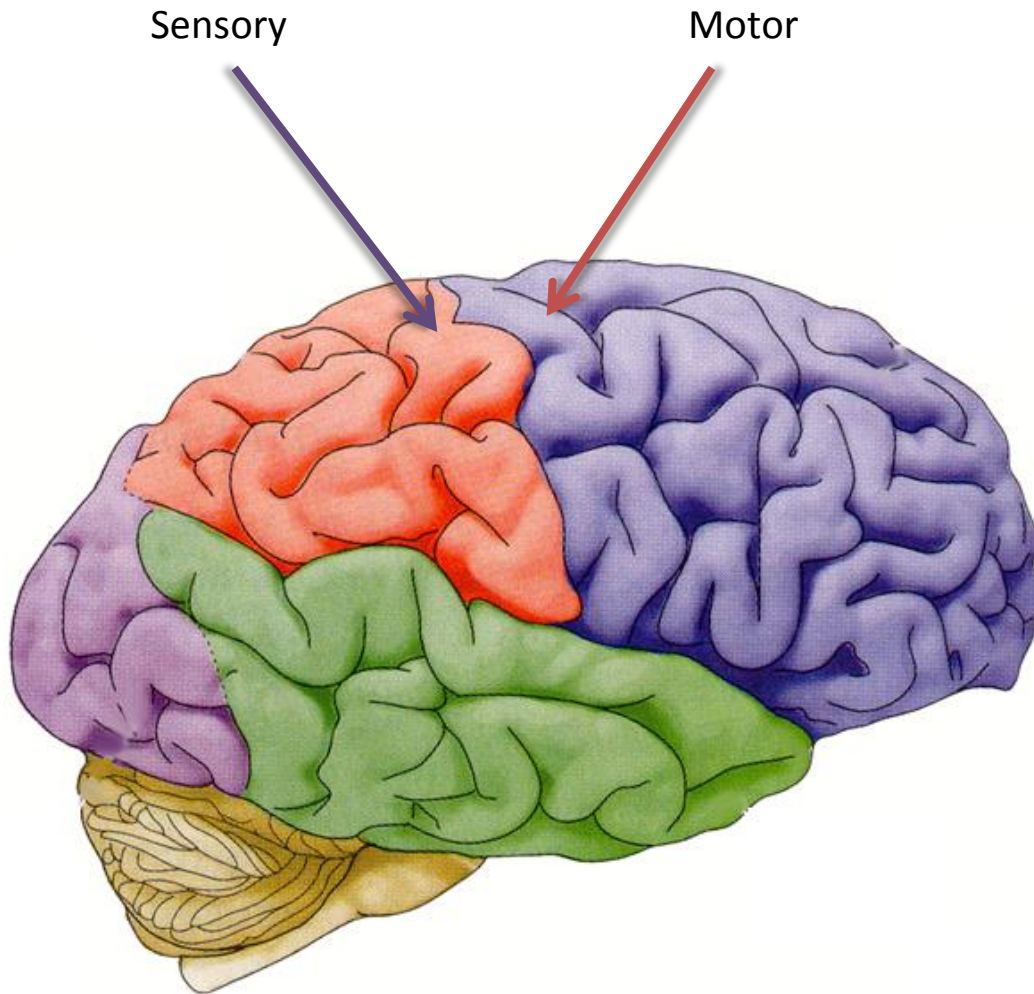


Neuroscience as a STEM Subject

A Workshop that Explores Sensory & Motor Function



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Rochelle D. Schwartz-Bloom, PhD (Duke University)

Sponsors: The Society for Neuroscience

Recording Electrical Activity (Action Potentials) that Make Muscles Work Using the SpikerBox

(adapted from www.backyardbrains.com)

Time	Difficulty	Prior Knowledge	Equipment
30 minutes	Beginner	It helps to know the basics about neurons, motor units, and action potentials.	EMG SpikerBox Cable Laptop or smartphone

Experiment: Muscle Action Potentials

In this experiment we will look at muscle activity using EMG (or electromyography) (myo means muscle). Muscle activity is the result of many muscle fibers contracting at once. When you add up the electrical activity from each fiber, you get a large distributed EMG signal that we can pick up using surface electrodes. But what about the signal to each fiber? How can we measure action potentials in a single motor unit?

What will you learn?

This experiment will show you how to use your EMG SpikerBox to record neural activity of motor units from the small *interosseous* (os means bone) muscles in your hand. Can you find other small muscles from which to record?

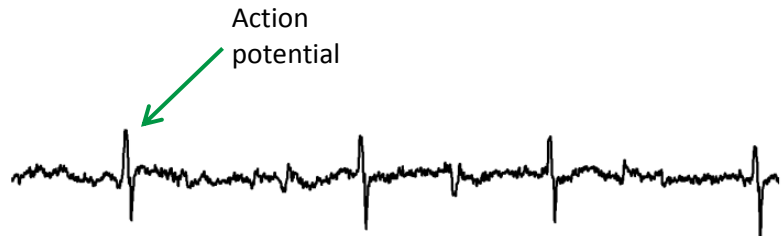
Background

There are two things that determine the strength of a muscle contraction: 1) the rate of action potentials that occur in the nerve cell and its accompanying muscle fibers, and 2) the "number" of motor units recruited. The more action potentials, the more contractions occur. Let's see how muscle fibers are recruited to form varying degrees of a muscle contraction.

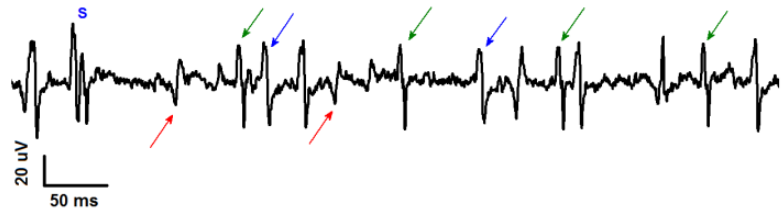
In a very weak muscle contraction, only 1 motor unit might be recruited to activate a muscle fiber. In general, relatively weak muscle contraction involves recruitment of motor units with the smallest number of muscle fibers first. These small motor units produce small twitch forces when they contract. If you increase the strength of the contraction you gradually recruit larger and larger motor units with greater numbers of muscle fibers. They produce larger and larger twitch forces. At the same time that you are gradually recruiting more and more motor units, you are also firing them faster and faster (more twitches per second equals more force!).

Take a look at the EMG recordings made by scientists studying the soleus (calf) muscle activity in a volunteer, shown below. Each "spike" is an individual action potential, the electrical impulse generated in the muscle fiber under "command" by the motor neuron.

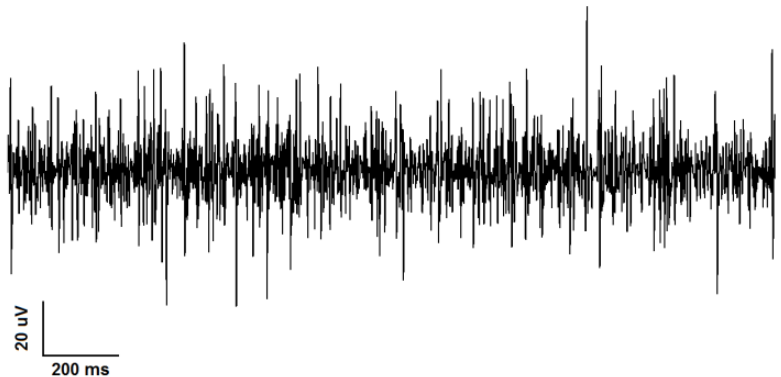
Very weak contraction:
Only 1 motor unit recruited



Increased voluntary contractions:
Increased firing rate of original
motor unit (green), and recruitment
of more motor units (red and blue).



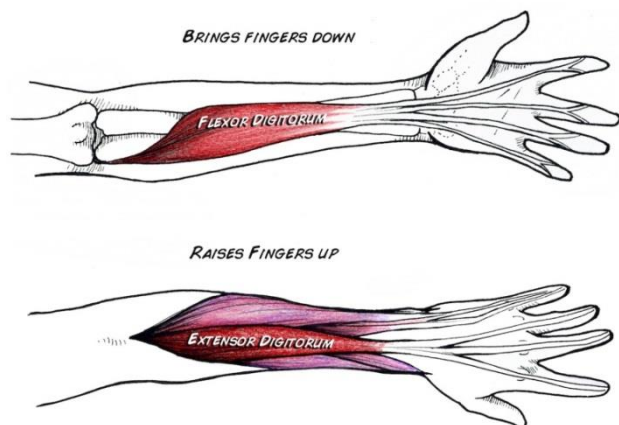
High-intensity muscle contraction:
Random firing of many motor units
at different rates.



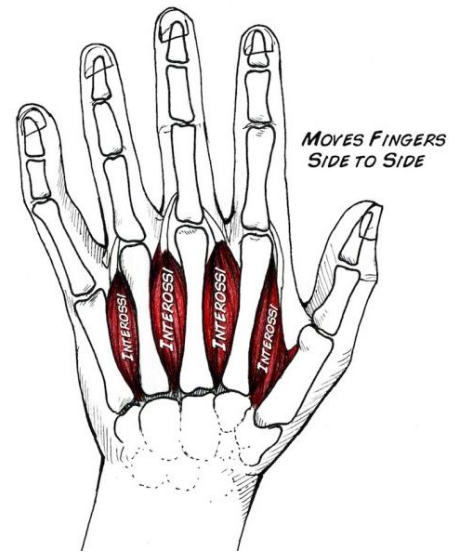
From: RA Mezzarine et al., (2013) DOI: 10.5772/54870

So all of these twitches are added together in the muscle. This is why motor units are so cool.... they convert the code of the nervous system into a perfectly adjusted muscle force that pulls on a tendon connected to a bone - and allow you to chew your food, kick a soccer ball, press a keyboard button, or hug a loved one.

We can use our EMG SpikerBox to record the individual muscle action potentials that occur as you contract muscles! One of the simplest EMG experiments takes advantage of the fact that most of the muscles that control your hand are in your forearm. Move your fingers up and down. These are controlled by the *flexor digitorum superficialis* and *extensor digitorum communis* muscles in your forearm.

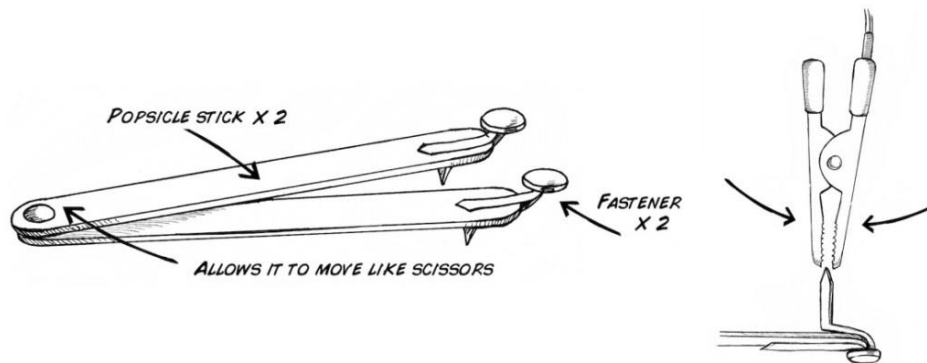


Now move your fingers from side to side. These are controlled by the *dorsal interossei* muscles which are actually some of the few muscles inside of your hand. Since the EMG SpikerBox is only detecting local electric activity of muscles, and we put electrodes on the top of your hand, would you expect a difference if you moved your fingers up and down vs left or right? Let's see!

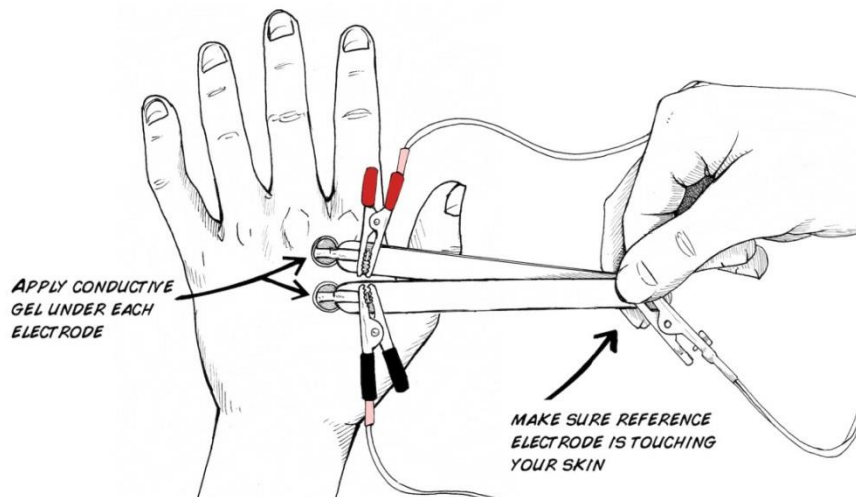


Experiment

1. Attach the Red and Black clips to one lead from each of the Brass fasteners in our "popsicle stick electrode" that came with your SpikerBox"

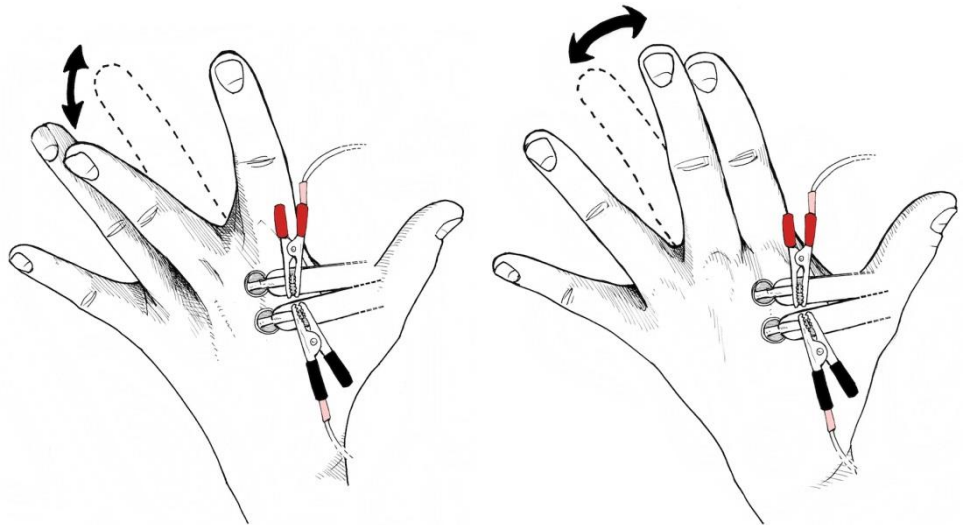


2. Locate an indent (valley) between knuckles on your hand.
3. Place the electrodes over the back of your hand between the knuckles as shown below. Make sure you put a dab of [conductive gel](#) in-between each electrode and your skin. This will increase your signal quality (without gel you will most likely just get noise).

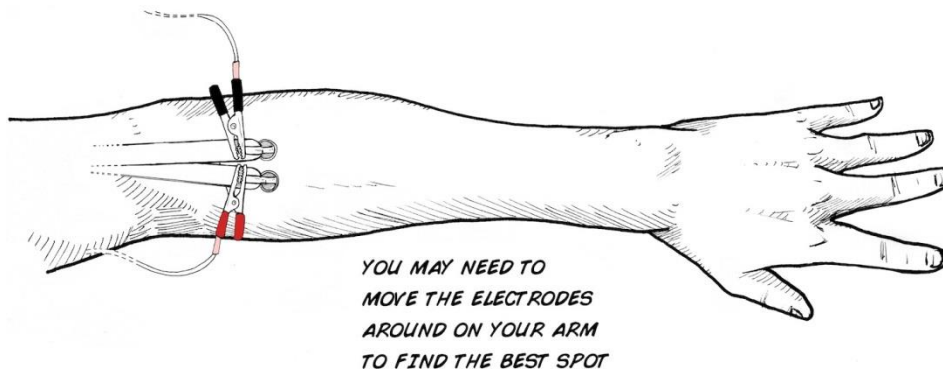


4. Hook up the EMG SpikerBox to the cables connected to the Brass Fasteners. You will also want to connect the white cable (naked, uncolored alligator clip) to a part of your body as this is our reference electrode. You can clip it to the brass tack at the end of your popsicle stick electrode and just hold the clip, for example, or you could clip it to a ring on one of your fingers.

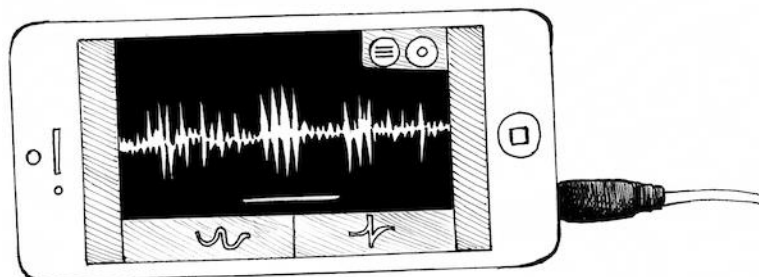
5. Turn on the EMG SpikerBox.
6. Move your middle or ring finger either side to side or up and down.



7. Now, try to isolate the muscles in your forearm responsible for up and down motion of your fingers! Can you find your *flexor digitorum superficialis*?



8. Plug in your SmartPhone (or iPad) and see if you can detect any motor action potentials (AP). You may find you have to "think about moving" so that you only generate a single twitch and a single AP! See if you can do it!



Muscle Contraction and Fatigue

(adapted from www.backyardbrains.com)

Time	Difficulty	Prior Knowledge	Equipment
30 minutes	Intermediate	It helps to know the basics about neurons and action potentials.	EMG SpikerBox Cable Laptop or smartphone Hand strength gripper

What will you learn?

You will use the EMG SpikerBox to record your forearm muscles while doing isometric muscle contractions until fatigue/failure occurs. You will measure the EMG amplitude during the contractions to learn about changes in muscle cells and neural signals during fatigue.

Background

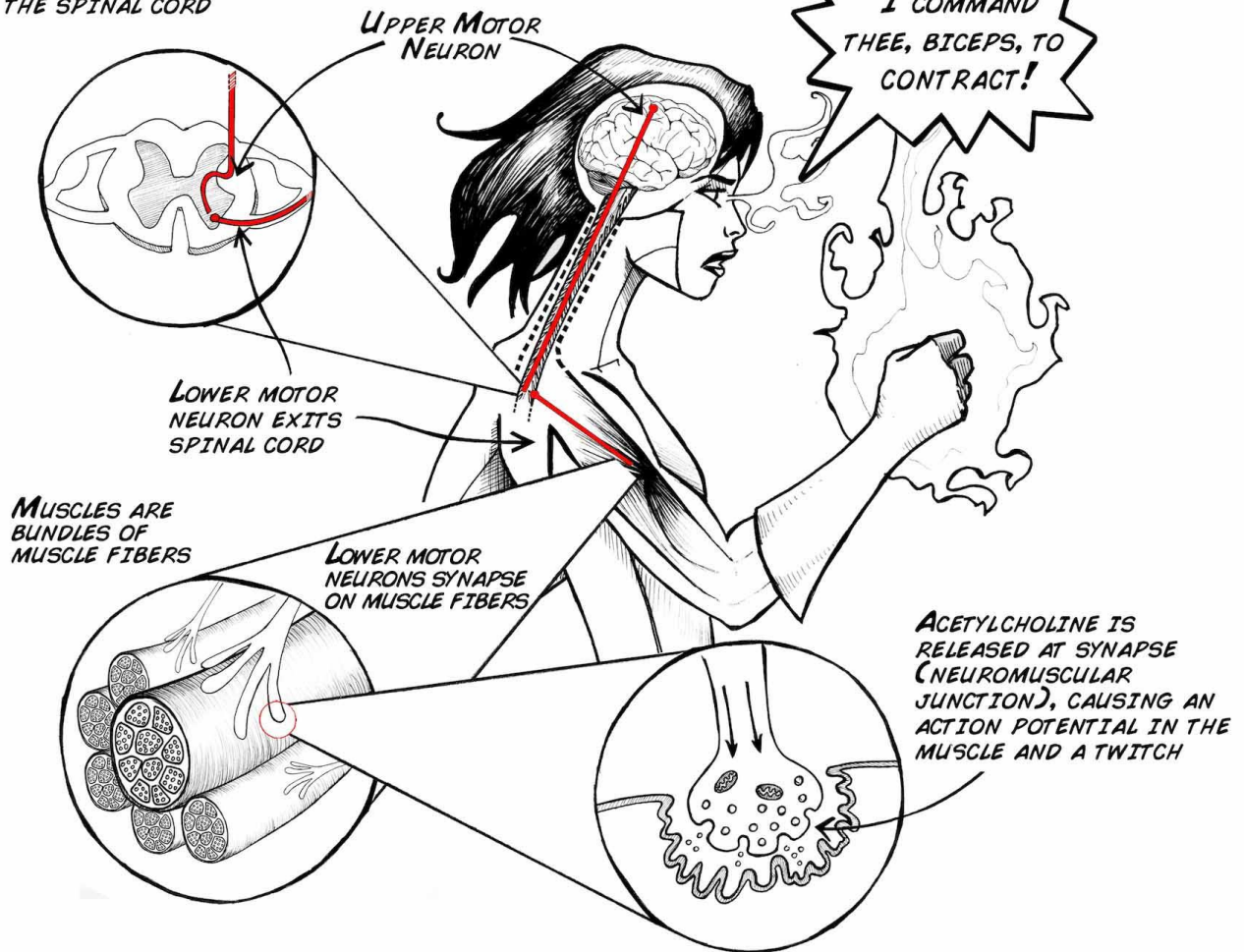
Our muscle system is the largest system in our body (40%-50% of our weight). This system includes your heart, which is a pump made of specialized cardiac muscle, and the smooth muscles in your guts, allowing food to move.

But to make voluntary actions such as lifting a soldering iron or kicking a soccer ball, you use your skeletal muscles! Your skeletal muscles allow you to do all the wonderful movements with which you pass your days. Your muscles contract and enable movement by sliding microscopic actin and myosin protein filaments across each other, with a full support cast of other players including proteins (troponin and tropomyosin), ions (Na^+ , K^+ , Ca^{2+}), energy carriers (ATP), and blood circulation to deliver O_2 and remove CO_2 .

When you move a muscle, you actually involve the brain in the process (unless it is a reflex movement). An area of the brain that controls muscle movement is called the "motor cortex". The cortex in the brain involves thinking, so when you think about moving a muscle, the neurons in the motor cortex start off the process. Their axons descend through the brain and into the spinal cord—they are aptly called "upper motor neurons". They make contact (or form a synapse) with another set of neurons in the spinal cord called "lower motor neurons". The axons of lower motor neurons project outside of the spinal cord at a level that is close to the level of a nearby muscle. See the cool picture below to get an idea of this arrangement.

The lower motor neurons then synapse with a muscle to make a "motor unit." A motor unit is a single motor neuron and the multiple muscle fibers it innervates. A muscle fiber is a very special type of cell that can change its shape due to myosin/actin chains sliding across each other to make the muscle contract or relax.

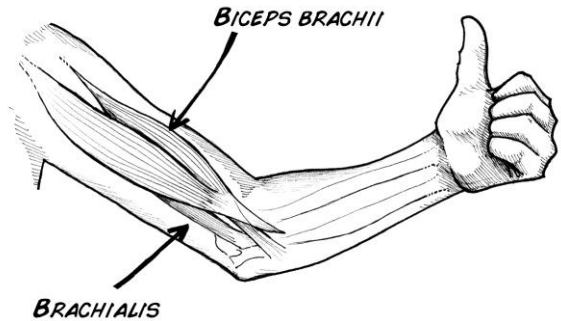
UPPER MOTOR NEURONS RUN DOWN THE CORTICOSPINAL TRACT WHERE THEY SYNAPSE WITH LOWER MOTOR NEURONS IN THE SPINAL CORD



A single motor neuron can synapse with multiple muscle fibers. In general, a large, powerful muscle like your bicep has motor neurons that innervate 1000s of muscle fibers, whereas small muscles that require a lot of precision, such as your eyeball muscles, have motor neurons that only innervate ~10 muscle fibers.

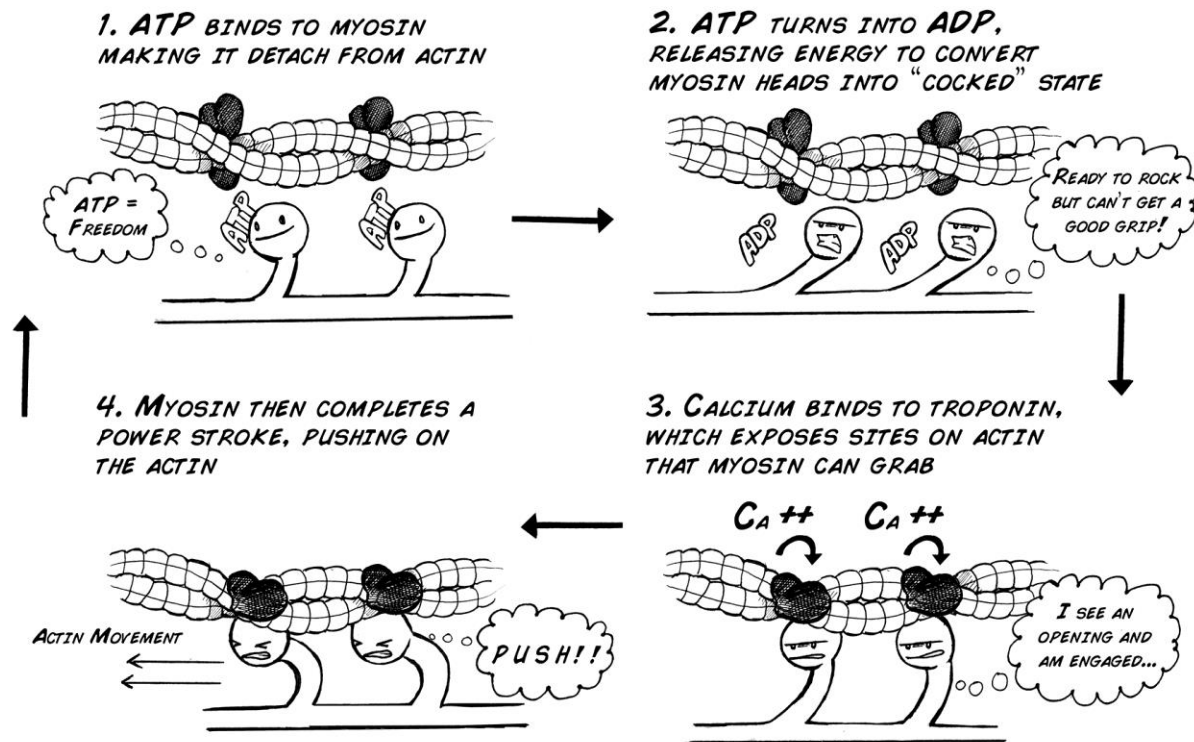
When a motor neuron fires an action potential, this causes a release of acetylcholine at the synapse between the neuron and the muscle (this synapse is also called the Neuromuscular Junction). This acetylcholine then causes changes in the electrical properties (the “electrical potential”) of the muscle. Once the electrical potential reaches a certain level or threshold, an action potential gets generated in the muscle fiber! This is the first step of the cellular cascade that ultimately causes muscle contraction.

When you contract a muscle, this is the result of many muscle fibers firing action potentials and changing shape. We can look at action potentials recorded during muscle contractions with our EMG SpikerBox.



How does an action potential cause the muscle to contract?

When a muscle cell fires an action potential due to a motor neuron command, this causes a release of calcium (Ca^{2+}) inside the muscle fiber from the sarcoplasmic reticulum. The Ca^{2+} then flows into the area where the muscle proteins actin and myosin reside (the sarcomere), initiating a complex cellular reaction with ATP that allows the myosin to pull on the actin. The movement of myosin pulling on actin in the sarcomeres is called a "[sliding filament model](#)" and consists of 4 steps.



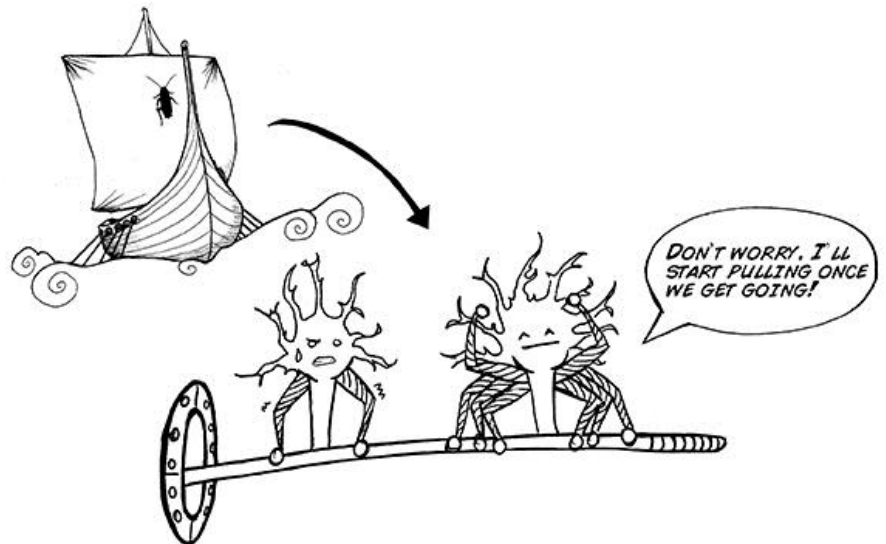
As long as calcium and ATP are available, the actin and myosin will continue pulling on each other and the twitching will continue. Note that the calcium is rapidly transported back into the sarcoplasmic reticulum where the process must be initiated again by the muscle firing an action

potential to cause another twitch. The summing together of many of these incredibly tiny "pulling events" produces a twitch (a very tiny, very fast force). When many twitches occur in a row, the twitches sum together and produce a larger force.

ATP is continually provided in the muscle by breaking down glucose (see the "[Oxygen Experiment](#)" at www.backyardbrains.com for an explanation of this metabolism). If glucose isn't available, fatty acids can be used to make pyruvate and keep the Krebs cycle and the oxidative phosphorylation pathway operating. As long as oxygen (O₂) is present and can be readily transported to the muscle cell, the oxidative phosphorylation pathway can produce ATP at incredible rates. This is called **aerobic contraction**, meaning "using oxygen."

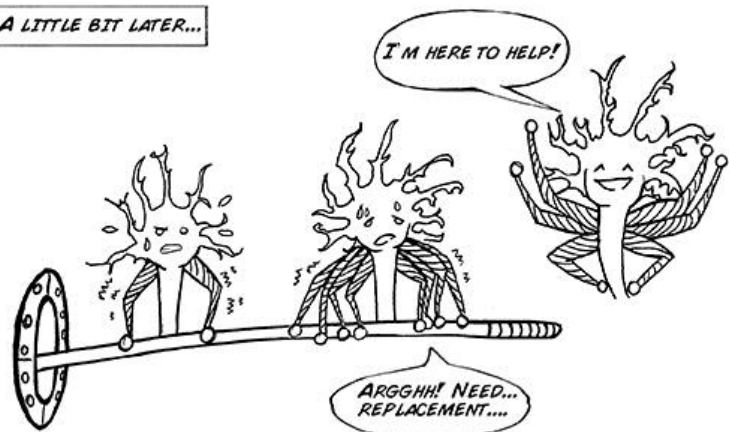
Recruitment of motor units during muscle contraction

To achieve great things, like lifting a heavy weight, motor units join together in a systematic way to supply the force required to achieve strength. This teamwork among motor units is called "Orderly Recruitment" by scientists, and as stated before, motor units with the smallest number of muscle fibers begin contracting first during a movement, followed by the motor units with the largest number of fibers afterward, to allow for a smooth, strong muscle contraction.



In addition, a motor unit can be recruited to replace an already active motor unit that is experiencing fatigue.

A LITTLE BIT LATER...



So...how does this all relate to your muscles getting tired?

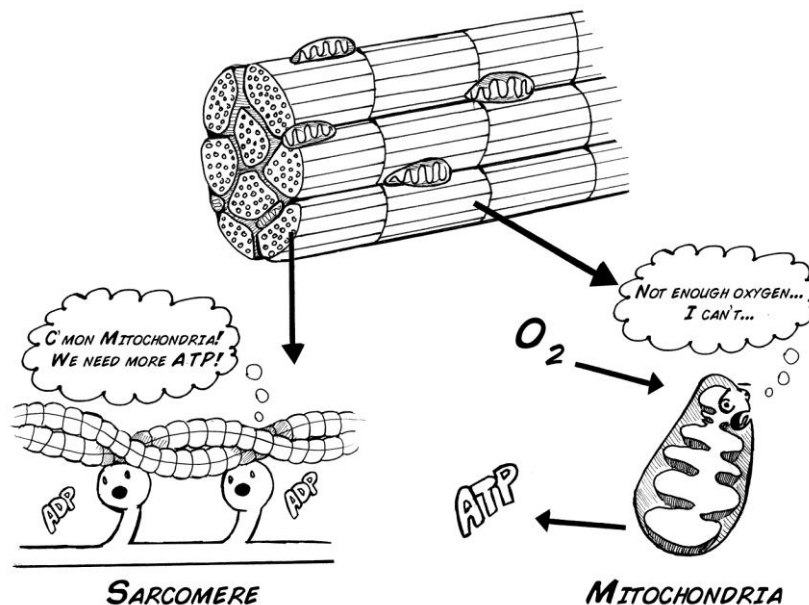
What happens when the muscles get fatigued?

Muscle Fatigue occurs when the muscle experiences a reduction in its ability to produce force and accomplish the desired movement. The factors that explain fatigue are complex and after more than 100 years of investigation, they are still a topic of active research.

For example, short term fatigue (failure to lift a heavy weight, do more push-ups, etc.) is different than long term fatigue such as a marathon run, a 100 mile bicycle ride, or a full-day hike through the Rocky Mountains of Colorado.

We do understand though some of the basic reasons that muscles become fatigued during high intensity exercise, most notably that the demand for oxygen can be greater than the supply. The blood flow to the muscle can be reduced because

- 1) muscles intensely contracting can reduce blood flow and thus oxygen availability, or
- 2) the muscle is simply working so intensely that there literally is not enough oxygen to meet demand (a sprint at top speed).



For advanced students:

If such O₂ isn't available as an electron acceptor, the Krebs cycle and electron transport chain cannot operate, and the muscle must gain ATP from other sources. For example, for rapid, intense activity, phosphocreatine (synthesized from amino acids) can serve as

a phosphate donor to allow ATP formation. This is called **anaerobic contraction**, meaning "not using oxygen."

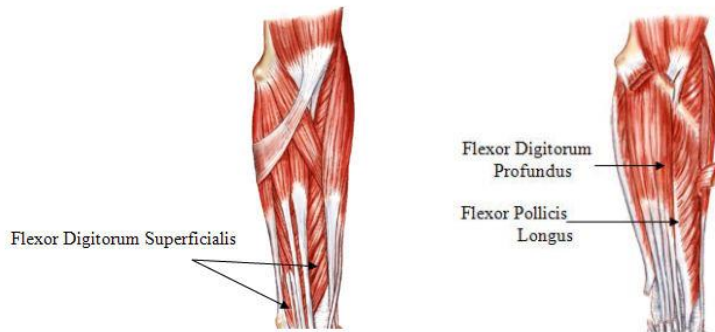
However, anaerobic contraction can lead to build-up of metabolites and waste products, and a significant increase in the acidity (decreased pH) inside the muscle cell, which can interfere with the many biochemical reactions necessary for the actin and myosin to produce force and slide against each other. This chemical change is thought to be the cause of the "stinging" or burning sensation you feel in your muscles as you become fatigued (such as in arm wrestling or in the last few reps of a difficult weight lifting set).

We can observe the effects of these fatigue processes, albeit indirectly, by examining the amplitude of the EMG signal during a muscle contraction. As fatigue progresses,

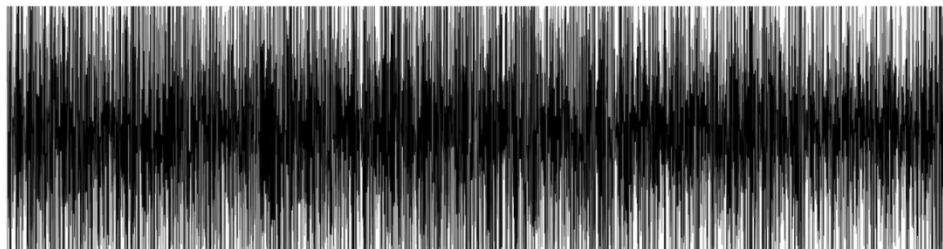
- 1) the firing rate of motor neurons drops, which in turn drops the number of action potentials the muscles themselves then fire, leading to a reduction in strength, and
- 2) muscles can often also continue generating action potentials due to neural drive, but the muscle is unable to contract due to molecular fatigue events in the muscle fibers, which in turn leads to a reduction in strength.

Experiment: Hand Gripper Isometric Test

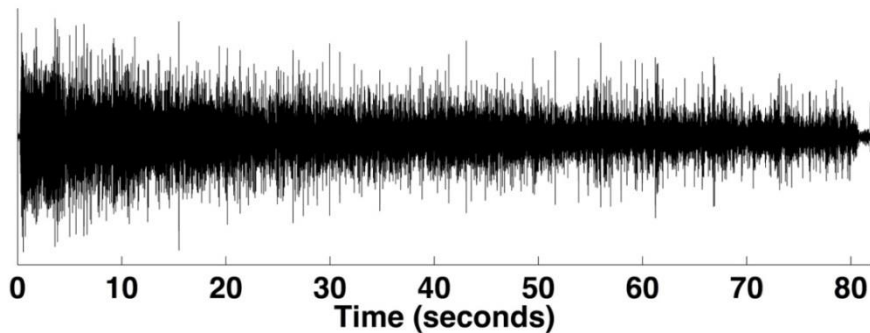
In this experiment, we are going to record the group activity of 1000's of muscle fibers within the forearm muscles involved in holding a hand grip, which can tire easily. Take a hand-gripper and squeeze it hard--look at which muscles are contracting on your forearm. There are 3 flexor muscles involved:



1. Remove the sticky backing from your EMG electrodes, and place these surface electrodes on two sides of the belly of one of your flexor muscles.
2. Hook up the EMG SpikerBox leads (the red and black alligator clips) to the two surface electrodes on your flexor muscle.
3. Place the reference electrode (White) anywhere on the body. You could clip it to a metal ring on your finger or you can also simply hold the reference electrode in your hand.
4. Plug the electrodes into your EMG SpikerBox, and hook up your SpikerBox to either your mobile device (e.g., iPhone or iPad) or PC. To use your SmartPhone you will need to have the free [Android](#) or [iPhone](#) apps installed. If you are using a computer, download the [Backyard Brains PC app](#) or [Audacity](#) program for PCs or MACs.
5. Turn on the EMG SpikerBox and listen for changes in activity. Do you notice a difference when you flex your muscles?
6. Use a [hand-dynamometer](#) or [hand gripper](#) (you should buy one in the 50-100 lb (25-45 kg) range), and squeeze the grip for as hard as you can for as long as you can.
7. Look at the recording of your EMG during this task. Reduce the gain (volume) of your EMG SpikerBox so that the signal is not clipping on your recording software (a clipping signal looks like this):



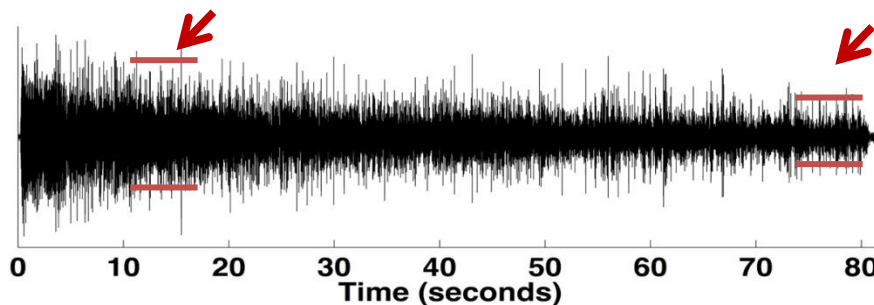
8. Observe the amplitude (height) and firing rate (number of impulses) in the EMG. What do you see over time? The gross signal may look similar to this:



Data analysis of action potentials during biceps muscle fatigue

You can use a program like [Audacity](#) to do quantitation, or you can do it “by hand” as shown below. On your Smartphone device, you can record the firing pattern with the app, and save it to your device for replay later. When you replay, you can pause the recording anywhere to make a measurement with a ruler.

Amplitude: measure an average height of action potentials for a certain time period, e.g., 5 seconds



Frequency: measure the number of action potentials (spikes) per second (this can be done in a program such as [Audacity](#) to expand the time scale)

Measurement	Rest	Grip-time 1	Grip-time 2	Grip-time 3
Amplitude (mm)				
Frequency (Hz)				

Next Generation Science standards that apply to these activities:

HS-LS1 From Molecules to Organisms: Structures and Processes

HS-LS1-2. Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.

HS-LS1-7. Use a model to illustrate that cellular respiration is a chemical process whereby the bonds of food molecules and oxygen molecules are broken and the bonds in new compounds are formed resulting in a net transfer of energy.

Science & Engineering Practices

- Developing & Using Models
- Planning & Carrying Out Investigations
- Constructing Explanations & Designing Solutions

Disciplinary Core Ideas

- LS1.A: Structure and Function

Crosscutting Concepts

- System & System Models
- Structure & Function
- Stability & Change

HS-PS4 Waves and their Applications in Technologies for Information Transfer

HS-PS4-1. Use mathematical representations to support a claim regarding relationships among the frequency, wavelength, and speed of waves traveling in various media.

HS-PS4-2. Evaluate questions about the advantages of using a digital transmission and storage of information.

HS-PS4-5. Communicate technical information about how some technological devices use the principles of wave behavior and wave interactions with matter to transmit and capture information and energy.

Science & Engineering Practices

- Asking Questions & Defining Problems
- Using Mathematics & Computational Thinking
- Constructing Explanations & Designing Solutions

Disciplinary Core Ideas

- PS4.A: Wave Properties
- PS4.C: Information Technologies & Instrumentation

Crosscutting Concepts

- System & System Models
- Cause & Effect
- Stability & Change