Lab 3: Electrophysiology in the periphery (EMG and muscle stimulation)

0. Assignments associated with this lab

- Comprehension questions (section 4)
- You do not have to turn in your completed code. However, you are asked to provide the figures generated by running your analyses (last question of the comprehension questions).
  - All figures that must be provided are underlined in the lab manual and flagged in the code templates
- **BIOE 566/EE 564 sections:** You must write a results section describing the findings for experiment 1 and experiment 3
  - Include all relevant figures and statistics for these experiments
  - You should use the IEEE conference paper template
  - Your results section should not exceed one page. Figures can extend onto a second page.

1. Equipment required

- Hardware
  - Musclebox pro
    - Connection cable to connect to computer
    - Connection cable to EMG electrode (1)
  - Computer with USB connection
  - Grip force strength training device with adjustable resistance
  - Surface EMG electrodes
  - TENS Unit 3000 stimulator
    - Stimulation leads (2)
    - Self-adhesive electrodes
  - Spikerbox pro with analog input adapters (provided)
  - Circuit components for force sensor input
    - 51 kOhm resistors (2)
    - 1.5 kOhm resistor (1)
    - 2 KOhm resistor (1)
    - 3 kOhm resistor (1)
    - Small breadboard and connecting wires
    - Force-sensitive resistor with soldered connection wires
    - 9V battery and battery harness
  - Connectors for routing TENS stimulation signal to spikerbox
    - Alligator clips (2)
    - Mono audio connector to wires (1)
  - Optional: Small-muscle EMG electrodes
- Software
  - Backyard Brains Spike Recorder
  - Matlab
  - Scripts outlines and functions for analysis

2. Background

This lab will give you hands-on experience with using electrophysiology (recording and stimulation) in the periphery and explore the physiology of the peripheral motor system. We will perform experiments to quantify how peripheral nerve stimulation recruits muscle fibers (experiment 1), and 3) quantify the impact of stimulation frequency on evoked muscle contraction (experiment 2), and probe how electromyography relates to muscle force production (experiments 1).

2.1 Peripheral sensorimotor anatomy primer

A review of muscle anatomy and its innervation are illustrated in figure 1. Muscles are composed of many individual **muscle fibers**. **Motor neurons** have cell bodies within the spinal cord, and project axons out to muscle fibers to cause contraction. Nerves contain the axons of these motor neurons, travelling along with axons of sensory receptor neurons (which travel from receptors in the periphery to the spinal cord). Each motor neuron innervates multiple muscle fibers. A motor neuron and the muscle fibers it innervates are called a **motor unit**.

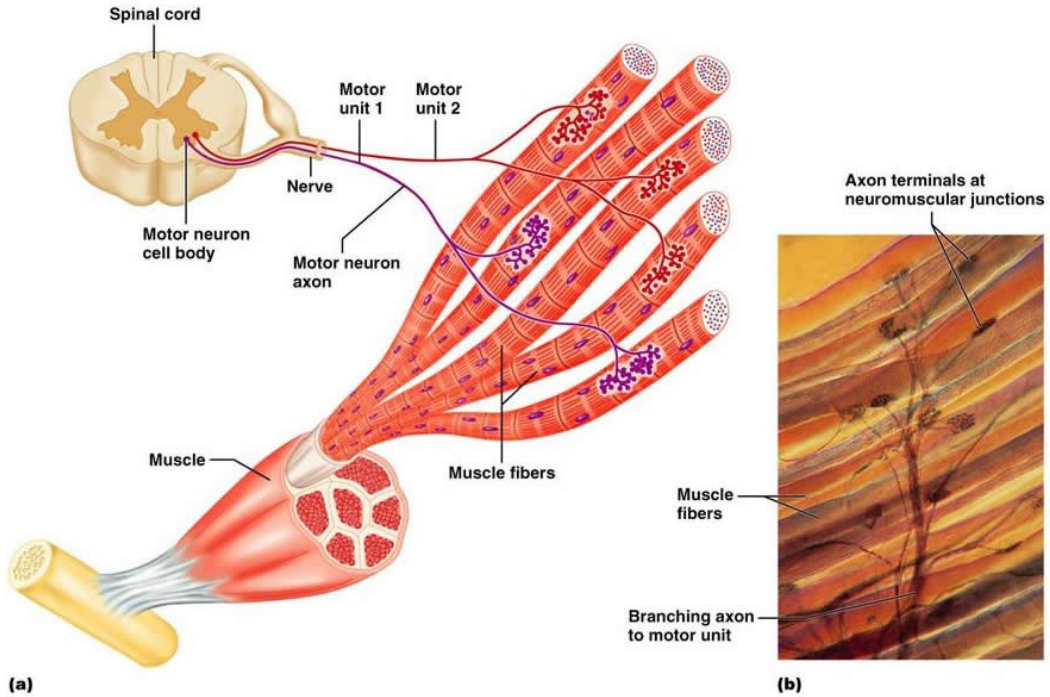


Fig. 1 Muscle anatomy. Motor neurons in the spinal cord innervate multiple muscle fibers. A motor neuron and the fibers it innervates comprise a motor unit. (a) Shows an illustration, while (b) shows an image of biological tissue. From http://www.rtmsd.org

2.1 Electromyography

In labs 1 and 2, we used electrophysiology to measure action potentials of neurons within a nerve. In this lab, we will use the same electrophysiology techniques to measure the activity of motor neurons at their projection to muscles. This is called **electromyography**, or EMG. EMGs capture the action potentials occurring at each muscle fiber for all motor units that are active within range of the electrodes.

Much like recordings in the brain and nerves, EMG can be performed with a variety of different electrode types and placements. We will be performing "Surface EMG" (**sEMG**), which measures EMG signals from electrodes

placed on the skin. The action potentials travelling along a motor neuron are too small to be reliably detected by sEMG electrodes. However, when the action potential reaches the motor endplates of the hundreds of muscle fibers in that motor unit, muscle action potentials occur nearly simultaneously in each fiber. The combination of these muscle action potentials—called a **motor unit action potential** (MUAP)—generates large electrical signals that can be easily detected.

A single MUAP generates signals much like the extracellular action potentials we recorded in labs 1 and 2 (a bi-phasic spike). Much like neuron action potentials, the waveforms of MUAPs will vary with electrode size and placement. With the right measurement setup, MUAPs can be "sorted" to distinguish the activity of individual motor units. Because sEMG electrodes are placed far from the source, they measure the summed activity of many MUAPs (a field-potential). Electrodes placed into the muscle ("percutaneous EMG") are typically needed to resolve the MUAPs of individual motor units.

sEMG is best performed using a *differential amplifier*, which serves to better suppress the background electrical activity in a muscle. Our Muscle Spikerbox uses a differential amplifier (the neuron Spikerboxes used in labs 1 and 2 do not). You will thus record sEMG by attaching two electrodes to the skin directly over a muscle and recording the potential difference between them as the muscle contracts. A third electrode is used as a ground, which may be attached anywhere nearby, usually not directly over a muscle (e.g. a boney portion of the body like the elbow).

2.3 EMG signal processing

In stronger voluntary contractions, the raw EMG recording gives an indication of the level of muscle activation, but there are two ways of extracting more information from the signal.

1. The sum of many asynchronous MUAPs appears as amplitude-modulated noise. The average value is zero (why?), but the variance gives a useful measure of overall activity. The root mean square (RMS) over a short time interval is often used as a quantitative measure of muscle activation. The raw signal can also be rectified (absolute value) and smoothed (low-pass filtered) for the same purpose.
2. To pick out useful frequency information from surface EMG data, investigators use a Fast Fourier Transform (FFT) to find power in the signal at different frequencies. Changes in parameters of the frequency spectrum can be used to characterize the muscle and contain different information about muscle activation. For example, the median frequency sometimes changes with muscle fatigue.

In this lab, we will be measuring EMG as we vary the force generated in a muscle and explore how well different features of EMG capture muscle activation.

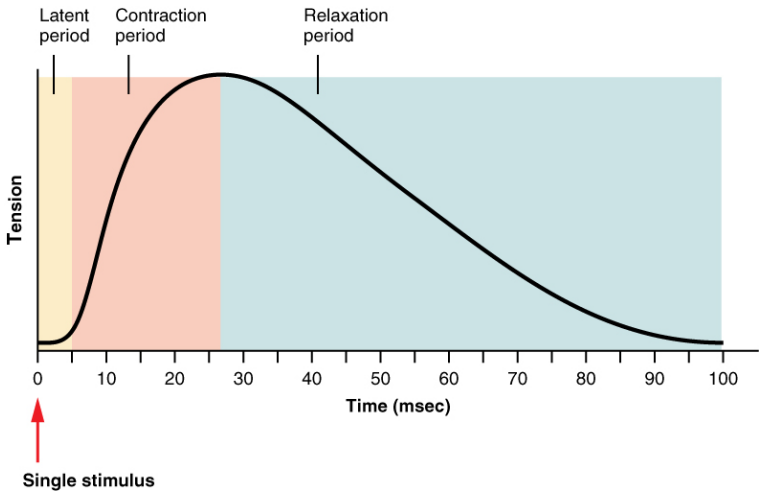


Fig. 2 Muscle twitch profile and parameterization. Figure from: OpenStax College, https://commons.wikimedia.org/wiki/File:1012_Muscle_Twitch_Myogram.jpg

2.5 Muscle twitches

When a motor neuron fires an action potential, all branches of its axon conducts the action potential, and all the muscle

fibers in its motor unit produce a **twitch**, almost simultaneously. A motor unit twitch can be characterized by several parameters: latency (the time from stimulus to rise of tension), twitch tension (the maximum force of the twitch), contraction time (the time from first force to maximum tension) and half relaxation time (time from maximum force to half of maximum force). Figure 2 illustrates an example of a muscle twitch force profile.

Twitch parameters (especially contraction time) are sometimes used to characterize fiber types. Generally, muscles have a mixture of fast-contracting and slow-contracting fibers. In mature vertebrates, all muscle fibers in a single motor unit all have the same fiber type (fast or slow), and the heterogeneity in fiber types active in a muscle reflects a mix of active motor unit types.

2.6 Peripheral nerve stimulation

Peripheral nerves contain both motor and sensory axons, with a range of diameters. When a large enough current is passed through a peripheral nerve, it produces action potentials on some of the axons by opening voltage-dependent ion channels. Larger axons present more cross-sectional area to the current, so they are often stimulated at lower stimulus intensity than are smaller axons. After a low-intensity impulse, the largest sensory and motor axons produce an action potential, exciting a sensation of movement and a small twitch.

You will be stimulating the flexor digitorum superficialis (FDS) in your experiments, a muscle on the anterior side of the forearm responsible for flexing the middle and proximal phalanges (bone segments) of fingers 2-5. Each section of the flexor digitorum superficialis corresponding to a single finger can be stimulated individually by a separate branch of the median nerve. The best results are obtained by applying an external stimulus to the nerve branch near its entry to the muscle, a location called the **motor point** (see fig. 6). We will be performing our stimulation through the skin, which is called **transcutaneous stimulation**.

2.7 Muscle force generation

A motor unit action potential (MUAP) is an all-or-none phenomenon, and every MUAP in skeletal muscle produces a single twitch. Yet, vertebrate skeletal muscles are capable of generating smooth, continuously variable forces. Primarily, vertebrates vary force by varying the number of motor units activated, a process called **recruitment**. The brain and spinal cord send command signals to pools of motor neurons that innervate a muscle. When your brain commands a small force, it sends excitatory signals through synapses to many motor neurons. Motor neurons vary greatly in size, and smaller neurons are more sensitive to a given amount of synaptic current than larger ones, so the smallest motor neurons are recruited first. The brain commands larger force by sending more excitatory synaptic activity, and additional motor neurons (with larger cell bodies) respond. The same pattern occurs in reverse as synaptic activity declines - larger motor neurons drop out first. This size-based ordered progression in motor unit recruitment is called the **Size Principle**.

Recruitment by stimulating a peripheral nerve typically does not progress according to the size principle. Axons of motor neurons vary greatly in size, larger motor neurons having larger axons. Smaller axons, having less surface area, are less sensitive to stimulation. The largest axons are typically recruited first (at lowest stimulation amplitudes), and progressively smaller axons are recruited as we increase stimulation amplitude. Thus, the recruitment order for stimulation tends to be from larger to smaller, opposite of the size principle. (Sensitivity to stimulation through the skin also depends on proximity of axons to the electrodes and conductivity of the tissue near an axon, so recruitment order during transcutaneous stimulation does not typically follow any strict rule.)

If a muscle is stimulated a second time, before the first twitch has relaxed completely, then the second twitch force adds to the first, and the sum is larger if the two stimuli are closer in time. There is, however, a limit: a second stimulus will not cause a second action potential if it occurs during the absolute refractory period of the

first. Muscle force generation sums when trains of twitches are elicited by regularly-space stimulation. Stimulation trains spaced further apart than the duration of a twitch do not summate; trains with more closely spaced stimuli produce a force called **unfused tetanus** that varies periodically at the stimulus frequency, and trains at higher frequency stimuli produces smooth force, called **fused tetanus**.

2.4 Uses for EMG and muscle stimulation

sEMGs are used clinically to assess function of the motor system, for instance quantifying stretch reflexes by measuring timing and amplitude of EMGs after a tendon tap. EMGs are regularly used in research to study the relationship between activity in the central nervous system and muscle recruitment, and to study the biomechanics of the motor system.

EMGs are also useful control signals for some neural interface applications. The branching of one motor neuron to innervate many muscle fibers means muscles act like an amplifier (amplifying a single action potential in a motor neuron into a MUAP). EMGs provide high-amplitude measures of neural activity, even when acquired in a non-invasive way. EMGs can be used to control devices to restore movement, such as **myoelectric prostheses** (prosthetic limbs controlled in part with EMG activity) or **myoelectric interfaces** (e.g. computer cursors controlled with EMG). These can successfully restore function to people with some residual motor control. Some of the most successful demonstrations of myoelectric prostheses explicitly leverage the amplification properties of muscles by surgically implanting nerve fascicles into portions of muscle ("targeted nerve reinnervation" [1]). Because it monitors movement details, EMG is also a strong candidate biomarker signal for closed-loop neuromodulation devices for motor disorders (e.g. deep-brain stimulation for Parkinson's Disease).

Peripheral nerve stimulation is also used as a tool for clinical diagnosis of nervous system function and for therapeutic applications (e.g. for chronic pain). Muscle stimulation can be used to drive movements, restoring movement of someone's own body after injury. This is called **Functional Electrical Stimulation** (FES). FES has been quite successful in providing basic function (e.g. standing from sitting for quadriplegic individuals). Challenges limiting the technology primarily center around optimizing electrical stimulation to recruit muscle activity efficiently (why is this a problem?). Peripheral stimulation (especially in the spinal cord) can also facilitate plasticity to restore functions lost after injury (e.g. [2]). The physiological mechanisms and engineering to optimize these protocols are very active areas of research.

3. Experiments

IMPORTANT NOTE: Read all provided procedures and safety information in Appendix S before starting experiments. You must submit the safety quiz for this lab via Canvas prior to beginning any procedures.

3.0 Initial equipment setup (DONE AS A GROUP)

In experiments 1 and 2, we will be measuring movements evoked by stimulation using a force sensor. We will first work together as a group to build the circuit and instrumentation setup to read this new sensor information into our SpikerBoxes.

Setup force sensor circuit and input to SpikerBox

- We will use a force-sensitive resistor to quantify the evoked force. A circuit diagram of the sensor is shown in figure 3.

- Use your breadboard, battery, battery harness, resistors, and breadboard wires to put together the circuit illustrated in Figure 3a.
- While assembling your circuit, use the multi-meter to perform the following checks:
    - The voltage supplied to the force-sensitive resistor is ~3.8 V
    - The voltage output of the circuit (V_{out} in figure 3) is 0 when no force is applied to the sensor, and increases as you apply force to the sensor
    - The voltage output of the circuit does not exceed 3V when significant force is applied to the sensor
- **IMPORTANT: YOU MUST SHOW THE TA OR PROF. ORSBORN YOUR CIRCUIT MULTIMETER CHECKS BEFORE PROCEEDING TO CONNECT THE CIRCUIT TO YOUR SPIKERBOX. INCORRECTLY CONDITIONED INPUTS TO THE SPIKERBOX WILL DAMAGE THE CIRCUITRY.**

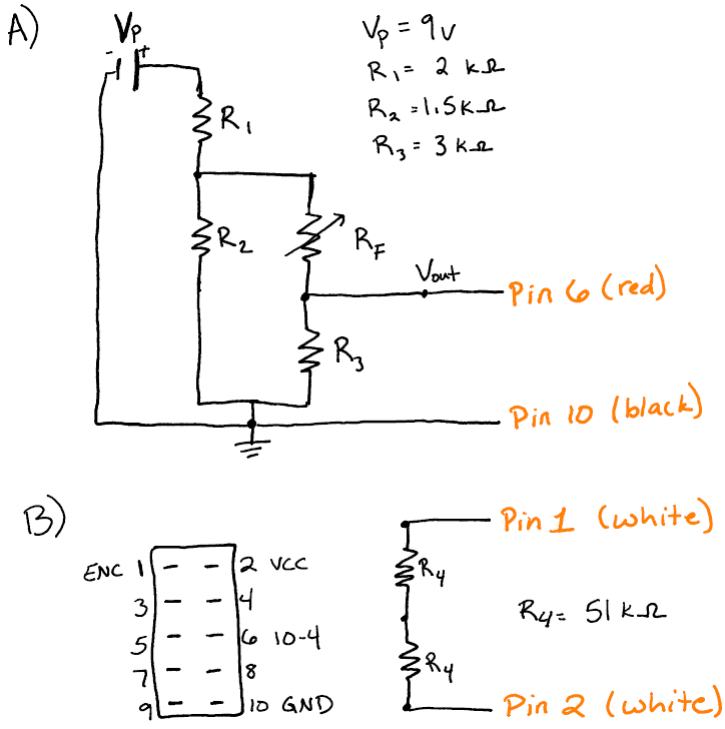


Fig. 3. A) Circuit diagram of the force sensor and its connectivity to the spikerbox pins. B) Top-down view of the spikerbox analog input interface pins (left) and pin connectivity

- Now, we will wire the output of the force sensor circuit into the analog input of the SpikerBox. The connectivity for doing this is illustrated in Figure 3.
    - Your SpikerBox analog input board is provided with 4 connection wires
        - Two white wires connected to pin 1 (ENC) and pin 2 (VCC)
        - One red wire connected to pin 6 (IO-4)
        - One black wire connected to pin 10 (GND)
    - Disconnect the power supply of your force sensor circuit
    - Wire your inputs by making the following connections
        - Place two 51kOhm resistors in series between pin 1 and pin 2 (the two white wires)
        - Connect the positive terminal of V_{out} to pin 6 (red wire)
        - Connect the ground terminal of V_{out} to pin 10 (black wire)
    - **IMPORTANT: SHOW THE TA OR PROF. ORSBORN YOUR INPUT WIRING BEFORE POWERING ON THE FORCE SENSOR**

- After confirming your circuit is wired correctly, check that you can read in force sensor measurements in SpikeRecorder
    - Power on the force sensor
    - Power on your SpikerBox
    - Turn on SpikeRecorder and connect to your device
    - You should see two lines appear—one green (reflecting the neuron input channel 1) and one yellow, reflecting the analog input

- Adjust your settings to remove low-pass filtering on the SpikeRecorder display
  - Click the gear on the left to open settings
  - On the frequency range dialog, set the lower frequency to 0
- Apply forces to your sensor and confirm that you see voltage fluctuations on the analog trace

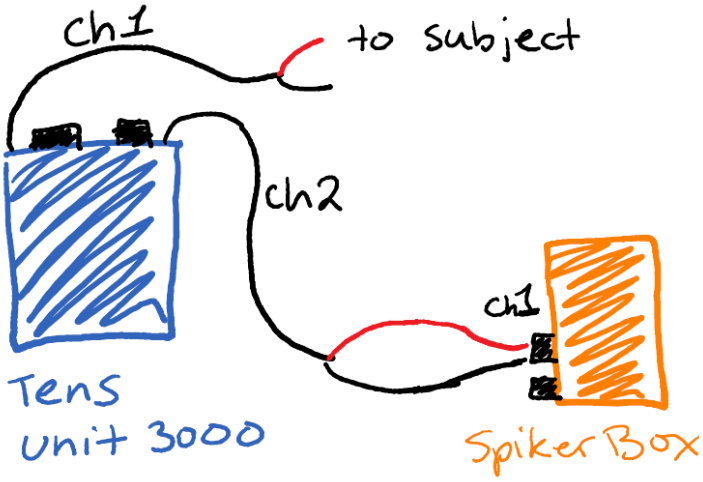


Fig. 4. Connectivity diagram for routing the stimulation pulses into the SpikerBox

Setup stimulator input to the SpikerBox

- We will also use spike input channel 1 on the SpikerBox to record the stimulator output. This allows us to know the precise timing of stimulation.
  - We will leverage the fact that the TENS Stimulator has two channels, whose amplitudes are different but that have the same timing.
  - One channel from the TENS will go to the subject, while the other will go into the SpikerBox
- The connectivity required is illustrated in figure 4.
- With the stimulator off, connect a simulator lead to channel 2 the TENS Unit 3000
- Plug the mono jack – to – wires adapter into channel 1 of the SpikerBox
- Use an alligator clip to connect the positive lead of the TENS (red) to the positive terminal of the SpikerBox input (red)
- Use an alligator clip to connect the negative lead of the TENS (black) to the ground terminal of the SpikerBox input (black)
- Turn on the TENS Unit channel 2 and confirm you see a large 'spike' on the channel 1 trace in SpikeRecorder

Change the frequency of stimulation on the unit and confirm that the spacing of these spikes changes in a corresponding fashion

3.1 Experiment 1 – Quantify muscle twitches evoked by electrical stimulation

We will now use our force sensor to explore how electrical stimulation recruits muscle activity. To do this, we will apply peripheral nerve stimulation to the median nerve and measure the evoked force profile using the force sensor we setup together in section 3.0.

Identify your stimulation site

IMPORTANT: Before applying stimulation for the first time, you must 1) be seated and 2) let the TA and/or Prof. Orsborn know.

- Connect a stimulator lead to channel 1 of the TENS Unit
- Set-up your stimulator settings to generate:

    - 120 µs pulses
    - 2 Hz pulse frequency
    - 'Normal' stimulation mode
- Place electrodes on your subject
    - Place a grounding electrode on the subject's hand, using one of the TENS Unit self-adhesive electrodes.
    - Place an electrode to serve as the positive stimulation site.
        - Use one of the smaller sEMG electrodes for the stimulation
        - Use an alligator clip to connect the positive lead of the TENS unit (red) to the stimulation electrode
        - We will be targeting the motor point for the flexor digitorum superficialis (FDS) to evoke twitches in the middle finger. The motor point for the FDS is illustrated in figure 5. Use this to select an initial location for your electrode.

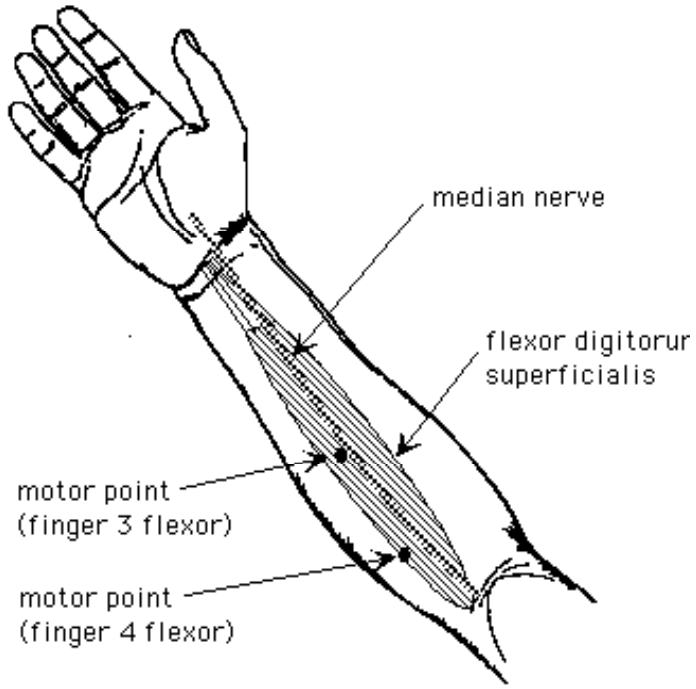


Fig. 5. Finding the motor point for flexors of fingers 3 and 4.

- Turn on your stimulation with a low amplitude. Allow the subject to control the stimulation amplitude. Gradually increase the stimulation amplitude until you see/feel an evoked muscle twitch.
- If you have found a good electrode placement for the FDS, you should see twitches of the subject's middle finger
- As needed, revise your electrode placement to refine the evoked movement
    - Always turn the stimulator off while adjusting electrode placement
    - You may be able to estimate the best direction to move your electrode by leaving the electrode placed on the skin and shifting its position relative to the underlying anatomy by shifting/stretching the skin slightly. You will often see significant shits in evoked twitches that can help identify the location of the motor point.
    - If you have difficulty finding the motor point for third finger flexion, try the motor point for fourth (ring) finger flexion, which is about 3 inches closer to the elbow and more medial (fig. 6).
    - Occasionally it is difficult to elicit a strong response with external stimulation, perhaps because the motor point is too deep or otherwise insulated from the skin, and you may want to try another volunteer.

Data collection

- Once your instrumentation (force-sensor and stimulation timing pulse inputs) is setup, and you have identified a stimulation set-up to evoke muscle twitches in one of the digits, you're ready to begin collecting your data to quantify stimulation-evoked muscle twitches.
    - Note: Be sure to have both channel 2 of the TENS unit on (with low amplitude) during your data collection to assure you collect the timing pulses for data synchronization
- We will measure, in separate recordings, the force evoked from stimulation with different amplitudes

- Before beginning, identify the range of stimulation values you will test
    - Find the smallest amplitude that evokes some visible movement
    - Allow the subject to slowly increase the amplitude of stimulation until you see large evoked movements or until the subject can no longer tolerate stimulation. This will define the maximum level of stimulation for your testing.
        - You may see that the number of fingers that move increases as you increase stimulation amplitude—why?
    - Divide this range into 4 different stimulation amplitude levels for testing
- Have the subject place their finger (the one moved by stimulation) on the force sensor
    - It is important to maintain contact between the finger and sensor, otherwise force generated by the twitch will not be registered.
    - We recommend the subject places their arm flat on the work bench and apply a small baseline force to the sensor to assure reliable measurement
- Record your data (repeat this procedure for each stimulation amplitude)
    - Start the stimulation, adjusting to the amplitude to be tested
    - Confirm proper subject hand placement on the sensor, and stimulation trigger pulse registration
    - Then start a recording, and capture data of several twitches. We recommend recording for ~1 minute (which gives you ~120 twitches)
- Use the table provided in appendix B to keep track of your experimental metadata

Data analysis

- We have provided an outline data analysis script called 'bioen466_lab3_winter2025_analysisExp1.mat'. Use this script to work through your data analysis
- The key steps of your analysis will be:
    - Load your data files (.wav files and event files)
    - Detect stimulation onset and trial-align your force measurements to stimulation onset
    - Quantify muscle twitch parameters of interest:
        - Onset latency
        - Maximum force
        - Contraction time
        - Relaxation time
    - Test whether these parameters change with stimulation amplitude
- The key plots you will generate through this script include
    - Raw trial-sorted force profiles
    - Trial-averaged force profiles
    - Twitch parameters vs. stimulation amplitude
- **Example figures of the data analysis steps required are included in appendix C.**

3.2 Experiment 2 – Quantify the effects of stimulation summation

We will now explore how electrical stimulation recruits muscle activity, shifting to the role of stimulation frequency. We will use the same set-up developed in experiment 1 to measure the time of stimulation and force evoked.

Data collection

- We will measure, in separate recordings, the force evoked from stimulation with different frequencies
- Before beginning, identify the range of stimulation values you will test
    - Allow the subject to select a stimulation amplitude that evokes reliable, clear twitches in a single finger at 2 Hz frequency and is comfortable
    - Allow the subject to slowly increase the frequency of stimulation until you see a larger, smooth contraction evoked (tetanus). This will define the maximum frequency for your testing.
    - Divide this range (2Hz to your maximum) into 6 different stimulation frequencies for testing
- Have the subject place their finger (the one moved by stimulation) on the force sensor
    - It is important to maintain contact between the finger and sensor, otherwise force generated by the twitch will not be registered.
    - We recommend the subject places their arm flat on the work bench and apply a small baseline force to the sensor to assure reliable measurement. Try to keep this force as constant as possible.
- Record your data (repeat this procedure for each stimulation frequency)
    - Start the stimulation, adjusting to the amplitude to be tested
    - Confirm proper subject hand placement on the sensor, and stimulation trigger pulse registration
    - Then start a recording, and capture data. We recommend recording for ~1 minute (which gives you ~120 twitches)
- Use the table provided in appendix B to keep track of your experimental metadata

Data analysis

- We have provided an outline data analysis script called 'bioen466_lab3_winter2025_analysisExp3.mat'. Use this script to work through your data analysis
- The key steps of your analysis will be:
    - Load your data files (.wav files and event files)
    - Detect stimulation onset and trial-align your force measurements to stimulation onset
    - Quantify muscle twitch amplitude
    - Quantify the absolute force measured
    - Test whether twitch amplitude, measured force change with stimulation frequency
- The key plots you will generate through this script include
    - Raw trial-sorted force profiles
    - Twitch amplitude and force vs. stimulation frequency

3.3 Experiment 3 – EMG as a measure of muscle force

We will now measure EMG and quantify its relationship to muscle activation. To begin, set-up our recording equipment and experimental subject:

- Plug the **MuscleBox** into the computer, plug in the battery, and turn it on (via the 'Volume' knob)
- Plug in your electrode-connection cable to channel 1.
- Open the Spike Recorder software and confirm you are streaming correctly and can save a file

Place electrodes to record Flexor Carpi Ulnaris (FCU) and confirm signal quality

- Select one of your group members to be the research subject
- We will record activity of the flexor carpi ulnaris, whose anatomy is shown in figure 6

- Place electrodes targeted to record this muscle. A reminder that our EMG measurements are differential measurements between two recording electrodes on the target muscle, and a ground on a boney portion of the body (we recommend the elbow)
    - Recording electrode 1 (red lead)
    - Recording electrode 2 (red lead)
    - Ground electrode (black lead)
- Ask the subject to make movements to assess your signal quality and electrode placement. Check whether you have muscle-specific recordings by having the subject:
    - make movements that should activate the muscle, and confirm that an EMG signal registers
    - make movements that should not activate the muscle, and confirm that you do not detect a significant EMG signal
        - If you do see substantial EMG when you don't expect the muscle to be active, what does that mean?

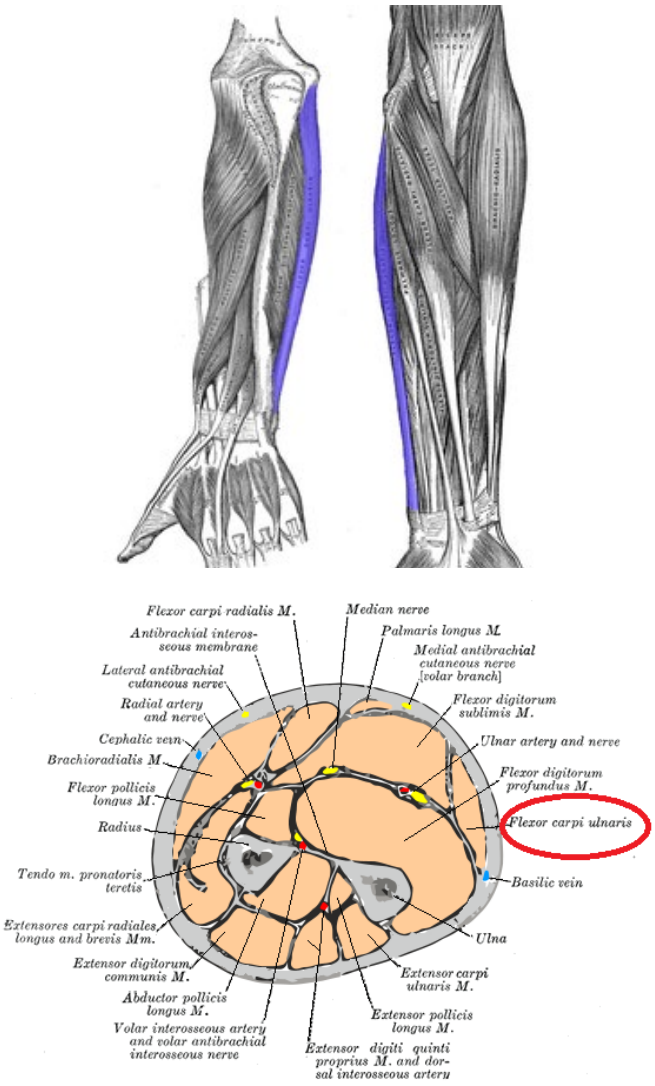


Fig. 6 Flexor carpi ulnaris (FCU) is a superficial finger flexor. Its primary actions are flexing the fingers and adducting the hand. Top: dorsal (left) and ventral (right) views of the forearm with the FCU highlighted in blue. Bottom: cross-section of the forearm to illustrate location of muscle body. Images from [3].

Collect data to quantify relationship between EMG and load force

- Use the grip force strength trainer device to vary the force generation of the subject's grip
- Record separate files for each level of force
- For a given level of force, ask the subject to generate force (by squeezing the gripper) several times, generating multiple 'trials' (~5-10)
    - Use the event-logging in SpikeRecorder to denote the time of force onset within a trial
    - Assure the subject relaxes fully between trials
- Use the table provided in appendix A to keep track of your experimental metadata

Data analysis

- We have provided an outline data analysis script called 'bioen466_lab3_winter2025_ analysisExp3.mat'. Use this script to work through your data analysis
- The key steps of your analysis will be:
    - Load your data files (.wav files and event files)
    - Trial-align your data

  - Calculate EMG features of interest: Raw EMG, RMS (amplitude), spectral features
  - Relate selected features to force generation
- The key plots you will generate through this script include
  - Raw trial-sorted EMG traces
  - EMG feature vs. grip force for:
    - Raw EMG
    - RMS
    - 2 frequency bands of interest

4. Comprehension Questions

1. Surface electromyography (sEMG), as we performed, measures the activity of many motor unit action potentials. Is it possible to measure action potentials in individual motor units with electrophysiology? If so, what would we need to change about our experimental set-up to accomplish this?

2. Describe the relationship you observed between EMG and load force in experiment 3 (1-2 sentences).

3. Based on your results, would you be able to use sEMG to "decode" the weight of the load in the hand? What, if any, limitations would this decoder have? (Hint: Is there a difference between the muscle activity when you grip something while keeping your hand still vs. while moving?)

4. Consider the variability across evoked muscle twitches in experiment 1. What are the largest sources of variability in your measurement? How could you improve your experiment to reduce this variability? **Note**: Be sure to think carefully about what things actually changed in your setup across the different measurements you made!

5. Based on the Size Principle, what would you expect the relationship between contraction time and twitch force to be? (1 sentence) In your stimulation experiments, what was the relationship between stimulation amplitude and contraction time? (1 sentence) What hypothesis does this support about how electrical stimulation recruits motor units?

6. A common problem observed in Functional Electrical Stimulation (FES) for evoking muscle activity is rapid fatigue of muscles. Based on your results, explain why.

7. a) Based on what we've learned about muscle twitch recruitment dynamics, in experiment 2 we **expect to see** that average twitch amplitude (INCREASES, DECREASES) as stimulus frequency increases, and average overall force (INCREASES, DECREASES) with stimulus frequency.
(note: you may have seen different relationships in your data.)

   b) Briefly (2-3 sentences) explain why.

8. Append the underlined figures from each experiment generated by running the data analysis scripts to your comprehension questions.

5. References

[1] Kuiken TA, Li G, and Englehart K. (2009) Targeted muscle reinnervation for real-time myoelectric control of multifunction artificial arms. *JAMA* 301(6): 619-628 doi: 10.1001/jama.2009.116

[2] Inanici, F, Samejima, S, Gad, P, Edgerton, VR, Hofstetter, C, and Moritz, CT. (2018) Transcutaneous electrical spinal stimulation promotes long-term recovery of upper extremity functions in chronic tetraplegia: a case study. *IEEE Transactions on Neural Systems and Rehabilitation Engineering (TNSRE)*. 26(6) 1272-1278. https://doi.org/1109/TNSRE.2018.2834339.

[3] https://en.wikipedia.org/wiki/Flexor_carpi_ulnaris_muscle

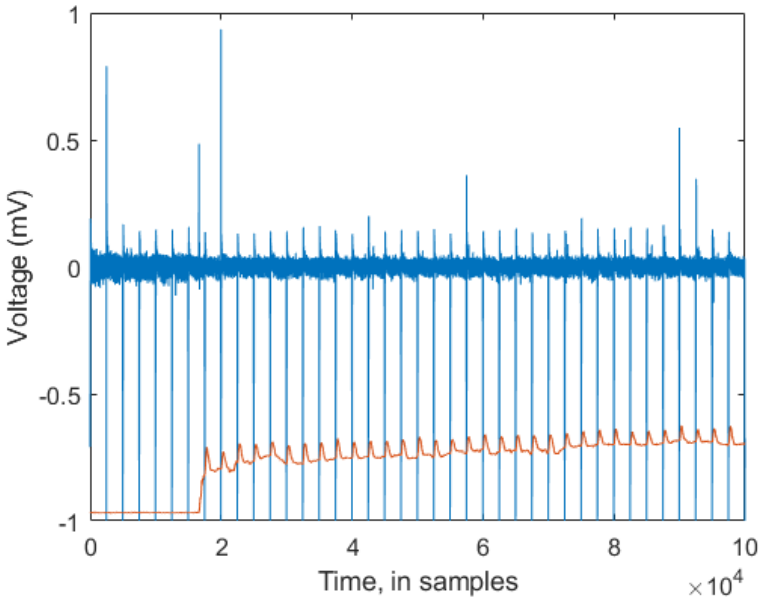
Appendix A: EMG Data logging table

Recording #	Grip force	Notes and observations

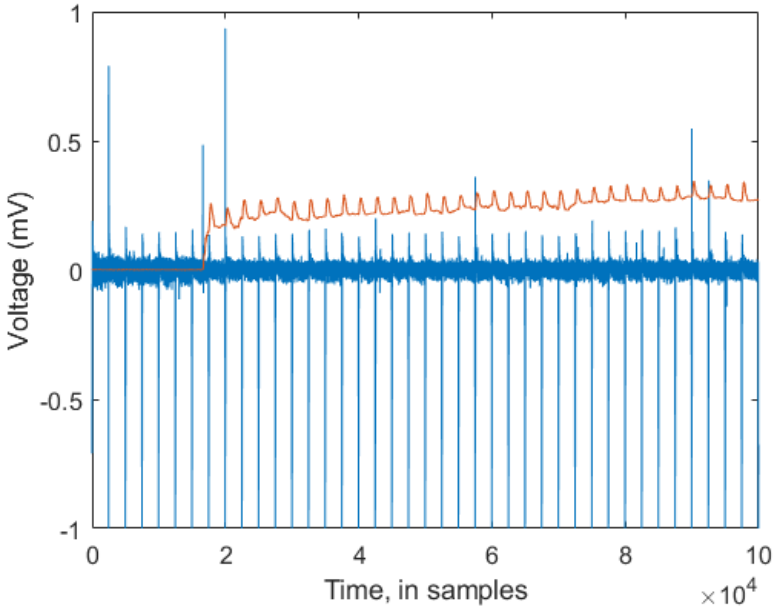
Appendix B: Stimulation Data logging table

Recording #	Stimulation amplitude	Stimulation frequency	Notes and observations

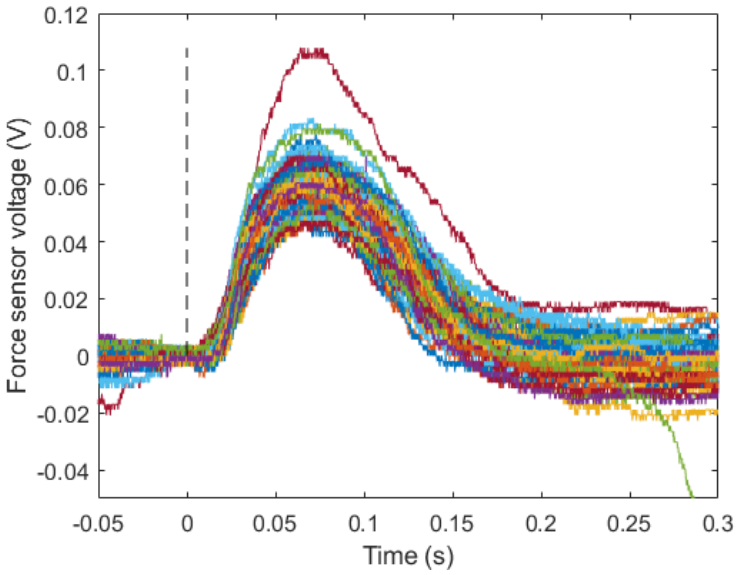
Appendix C: Example data analysis figures for experiment 1



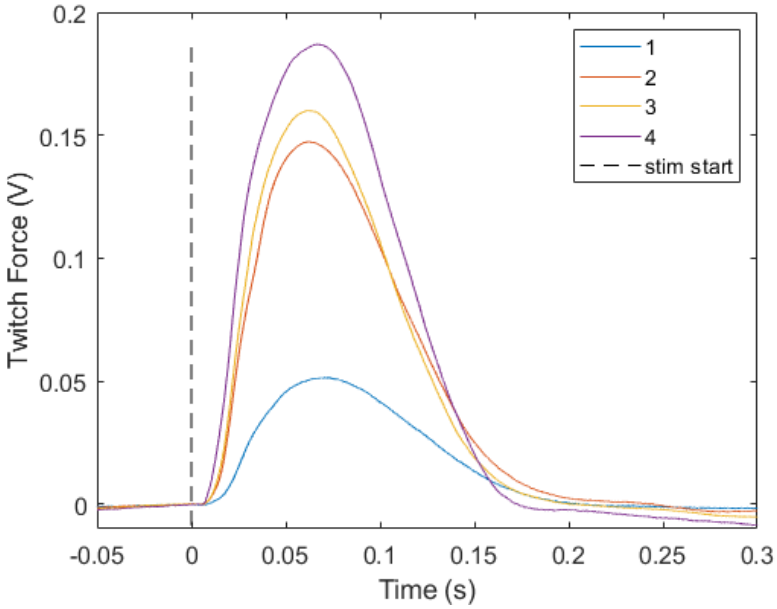
Raw data output prior to offset correction. Blue is the stimulation pulses, red is the force sensor output. Note: in this example, the subject did not put their finger on the force sensor until part-way through the recording.



Raw data output **after** offset correction. Note that the force trace (red) now begins at 0 and the forces are all positive (>0).



Trial-aligned force output that has been offset-corrected for one example stimulation setting. Each line represents a single stimulation pulse. Time zero is when the stimulation pulse starts. Note that our force for each trial starts close to zero. This should be the case if you have correctly accounted for trial-to-trial offsets. (see analysis)



Trial-averaged twitch profiles for 4 different stimulation amplitudes (1 = lowest, 4 = largest).

Appendix S: Lab Safety Considerations for Lab 3

This appendix describes the safety considerations for the procedures to be performed in this laboratory session. Read this appendix in full ***prior to beginning your experiments****. If you have any questions regarding the lab procedures or safety considerations, ask the TA or Professor Orsborn prior to starting your experiments.*

Once you understand these considerations in full, complete the safety quiz on Canvas to indicate that you have read it and understand the procedures. The quiz must be submitted prior to beginning your experiments

Transcutaneous Electrical Nerve Stimulation

Our experiments use transcutaneous electrical nerve stimulation (with the TENS Unit 3000) to evoke muscle contractions. TENS is generally safe for most people, though care should be taken to avoid possible injury.

- Do not place electrodes over broken or damaged skin
- Do not place electrodes over the front or sides of the neck or on the face
- Do not place electrodes over areas of reduced sensation
- All skin must be clean and dry
- No water or liquids allowed near the stimulator unit
- Always increase stimulation amplitudes gradually to assess response
- Do not touch the electrodes or stimulation leads while the stimulation unit is on. This includes direct touch with your hands or contact with any metal object.

Most people tolerate transcutaneous electrical nerve stimulation well. Be aware that there are some possible side-effects:

- Possible allergic reaction to the adhesive pads
- Buzzling or tingling sensation during stimulation that some may find unpleasant
- In rare cases, the sensation induced by stimulation can cause a vasovagal response (vasovagal syncope), which is a rapid drop in heart rate and blood pressure that leads to feeling light-headed or fainting

People with the following conditions should <u>NOT</u> receive transcutaneous electrical nerve stimulation:

- Pregnancy
- Pacemakers or other implanted electronic or metal medical device
- Epilepsy
- Heart disease or other heart condition

If you have one of these conditions or do not want to receive stimulation for any reason, it is not required to be the subject in your team's experiment.