SimMuscle

Physiological experiments on isolated frog muscle in a virtual laboratory

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Aims

Most of the recordings that you will make in this practical lesson will generate diagrams that you will also find in physiology textbooks. You will find similar diagrams in recordings of cardiac muscle contractions. These diagrams, such as isometric and isotonic maxima, are usually not easy to understand. However, once you have produced these diagrams through your own experiments, you should no longer have much difficulty understanding what they represent.

By proper physiological experimentation and appropriate documentation of your results, you should learn how macroscopic observations reflect underlying physiological and anatomical conditions; this is a principle that routinely applies in everyday clinical practice.

All the background knowledge that you require for performing this practical lesson is described in detail in textbooks while, here, you just need to make the right associations between theoretical facts and experimental observations.

Required background knowledge

Morphological/functional organization: muscle fibers, myofibrils, sarcomere, filaments.

Electromechanical coupling: action potentials, ryanodine and dihydropyridine receptors, the role of the calcium ion (Ca^{2+}), the actin-myosin cross-linking cycle, the role of ATP.

Innervation: motor end-plate, motor units, muscle fiber recruitment.

Mechanics: forms of muscle contraction, myoelectric superposition and tetanus, effect of muscle pre-stretching, isometric and isotonic maxima, muscle fatigue.

Preliminary excercises

Q: 1. Explain the following terms, using examples from daily life: isotonic, isometric and auxotonic contractions; support and stop twitching of muscle.

Q: 2. Describe the anatomical and functional structure of the contractile apparatus of the skeletal muscle.

Q: 3. Describe the underlying molecular mechanism of muscle contraction.

Q: 4. Why does the muscle power change with different degrees of pre-stretching?

Q: 5. What is the relationship between the isometric curve (muscle force at maximal tetanic contraction vs. relative muscle length) and sarcomere length?

Q: 6. Describe the processes of synaptic transmission at the muscle end-plate, from the nerve action potential to that of the muscle.

Q: 7. Describe electromechanical coupling, from muscle action potential to muscle contraction.

Q: 8. Draw the approximate time-dependent relationship between muscle action potential, the resultant (single) muscle contraction and the intracellular Ca^{2+} levels.

Q: 9. Name the neurotransmitter and its target receptor in the muscle end-plate, as well as the substances that can be used to block the neuromuscular transmission.

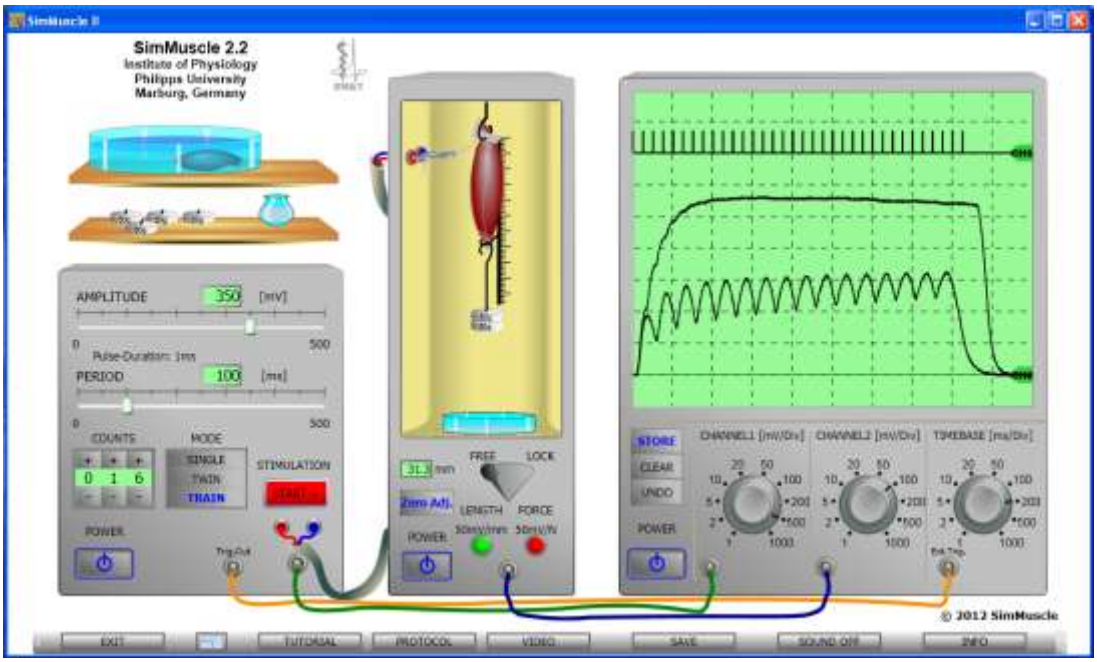
Q: 10. Name the two factors that physiologically control the muscle force. (In the experimental procedures, which of the stimulant parameters correspond to these factors?)

Q: 11. What effect does a fall in energy substrate (ATP, creatine phosphate) have on the twitch process?

Q: 12. Explain the cellular processes that underlie a physiological tetanus of skeletal muscle and explain why this does not occur in cardiac muscle.

1. The Virtual Laboratory

The virtual "SimMuscle" laboratory contains all the apparatus, in a simplified but quite realistic form, that you will need to perform the experiments. On one side, there is the 2-channel memory **oscilloscope** for displaying the stimulus and the resultant muscle contraction. On the other side, there is **stimulus apparatus**. In the middle, you will find the **suspension device** from which the nerve-muscle preparation will be suspended. This device includes an integrated **mechano-electrical converter** for generating electric potential from muscle power and muscle length changes.



Two nerve-muscle preparations will be made available to you. Although this virtual laboratory allows you to avoid preparing the isolated tissue, you may watch a video of one such preparation being performed; you can access this video via the Info bar below the laboratory display. Also via this Info bar, you can access a copy of this handbook ("Tutorial") and the experimental report form ("Protocol"). The report form allows you to record your measurements and the experimental settings of the stimulus device, and to plot the response curves. Alternatively, you may enter your data in to the excel form that is integrated in to the virtual laboratory and, in this way, obtain automatically-derived response curves.

The suspension device and mechano-electrical converter

The preparation needs to placed on the suspension apparatus; you do this by clicking on the preparation, which is lying in nutrient medium in the petri dish, and, with the mouse, drag the preparation to the suspension apparatus. The preparation will be fixed in place via a piece of bone above and via a small moveable loop below. The nerve of the preparation lies over the two stimulus electrodes that are connected via a double-cabled wire to the stimulus machine.

The position of the moveable loop indicates any change in muscle length and this will be shown on the transmitter, such as the change in muscle length after hanging a weight from the muscle. To hang a weight from the muscle, click on the weight and drag it to the preparation, placing the hook of the weight through the loop. You can then hook on more weights, as desired. With increasing weight load, the muscle will stretch but by a reduced degree with each similar increase in weight (see exercise 4).

The integrated transmitter of the suspension device converts the mechanical parameters of force and length change in to electrical potentials that are transmitted to the oscilloscope, via the double-cabled wire (blue). The conversion factors are fixed at 50 mV per N (force) and 50 mV per mm (length change).

Using the toggle switch on the transmitter, ("Lock" or "Free"), you can set, by mouse klick, whether force or muscle length are to be measured (setting is indicated also by the green light): with "Lock", the position of the loop is fixed and there is no macroscopic change in muscle length with a stimulus, which would be cause a isometric contraction; with "Free", the muscle can move freely and, after a stimulus, an isotonic contraction occurs.

When the switch "ZERO ADJ" is activated (displayed in blue text), the actual muscle force or length will be registered as the respective "zero" value. If this switch is not active, then with every addition or removal of weight, a new baseline value will be registered, with a corresponding shift in the baseline displayed on oscilloscope. This setting can be used to derive the passive stretch relationship of the muscle.

Stimulus apparatus

The stimulus apparatus delivers voltage pulses of fixed duration (1 ms). The **strength of the pulse** ("Amplitude") can be set using the upper slide-control, while the lower slide control ("Period") allows the

frequency of delivery of more than one pulse to the muscle, if required, by setting the time period between pulses. The "Mode" switches allow selection of delivery of a **single pulse** ("Single") or a **double pulse** ("Double") or a **series of pulses** ("Train"). For a series of pulse, the **number of pulses** needs to be selected ("Counts"). When all settings are selected, the stimulus is delivery ("Stimulation") by pressing the red button ("Start").

The stimulus is delivered to the preparation via a double-cabled wire and the nerve is activated as the impulse reaches the electrodes. The stimulus also delivers a parallel signal to the oscilloscope via the green cable to channel 1 of the oscilloscope. An additional cable (yellow) connects the "Trig.Out" of the stimulus apparatus to the "Ext.Sig" of the oscilloscope and provides a signal to trigger the oscilloscope to provide a recording only during stimulation of the preparation.

Oscillograph

In order to obtain good recordings on the oscilloscope, the appropriate scale settings need to be made. This is done by setting the amplification of the stimulus and muscle contraction signals using the knobs, "Channel 1" (mV/division (div)) and "Channel 2" (mV/div), respectively. The time interval is the same for both channels and is set by the knob "Time Base" (ms/div), for example, to 20 ms/div, which would provide 10 sections over the width of the oscilloscope screen (=200 ms). The zero line for each channel is highlighted and can be shifted using the mouse.

It is adviced to have channel 2 on the first line at the bottom of the screen as the muscle force and length changes (shortening) will be projected upwards on the screen, with the exception of the passive stretch-force curve. The channel 1 carries the stimulus information and presents on the upper scale the time intervals of the impulses and the duration of a series of impulses.

Normally, as one set of recordings starts, the oscilloscope removes from the display the previous recording. However, by activating the "Store" switch, this is prevented and a series of recordings can be displayed on the screen. By activating the "Clear" switch, the screen presentation is removed and no longer displayed. The switch "Undo" allows the last recording to be removed from the display, for example, in order to correct the settings and perform a re-run.

2. The Experiments

In principle these experiments may be performed in any order. However, they are presented here in an order that allows specific aspects to be demonstrated step-by-step. In a first series of experiments, you examine the strength of single twitches as a function of the stimulus-strength. Then you demonstrate the time-dependent summation of muscle contractions with superposition of double stimuli and the development of tetanic contraction on trains of stimuli. The second series of experiments begins with recording the passive stretch-force curve of a skeletal muscle from which then the curves of isometric and isotonic maxima can be determined and plotted in a way as they typically are illustrated in conventional text-books. Finally, you can examine the effects of muscle fatigue

Stimulus-Dependency of the Muscle Twitch

As described earlier, the strength of a single skeletal muscle twitch is largely determined by the number of muscle fibers that are involved, which in turn depends on the number of neurones that have reached activation threshold; in other words the strength of contraction depends on the number of motor units that have been activated. With a fixed duration of stimulus (1 ms), the strength of a single muscle twitch is exclusively a function of stimulus intensity.

From experience with this experimental set-up, the most powerful contractions that provide the most reproducible results can be obtained when the muscle is pre-stretched with 50 or 100 gram (weights 1-2). Your measurements should start with stimuli of low amplitude (about 50 mV), followed by increments of about 50 mV until the muscle contraction becomes visible in the display of the oscilloscope. You should then reduce the stimulus step-wise in order to accurately detect the minimum threshold (first visible contraction). The upper threshold is reached when there is no further increase in contraction with increased stimulus intensity. You should notice it is quite difficult to accurately detect the lower and upper thresholds of the stimulus-dependent contractions of your preparation. You should than make recordings at suitable intermediate stimulus values in order to obtain data points that span the lower and upper thresholds. You can than plot the values and obtain the stimulus intensity vs. contraction strength curve for your preparation. The plot should include values that are sub-threshold (stimuli that failed to evoke visible contractions) and values above the saturation or upper threshold (stimuli that failed to evoke

any visible increase in contraction strength). You should also include representative plots of single contractions in your experimental log.

Superposition of Double Stimuli

When successive stimuli are evoked within a short time interval, the 2 individual contractions will overlap (Superposition) if the second stimulus is evoked before the previous contraction has faded. This you can demonstrate by selecting a double stimulus (set mode switch to "Twin") and observe the contractions evoked after setting different values for the time interval (PERIOD) between the 2 stimuli. You should start your recordings with the time interval greater than the duration of a single muscle twitch, which is 200 to 300 ms, and then reduce the interval step-wise. An attempt should be made to determine the stimulus interval at which a maximal superposition is observed. Recordings of isolated single twitches and at about half-maximal superposition should be entered in your experimental log. In the STORE mode, you can select the appropriate time and amplitude settings.

Tetanic Contraction

The physiologically relevant form of skeletal muscle contraction is the tetanic contraction. In vivo, muscle fibers of the motor neurons are not activated by single action potentials but by prolonged bursts of action potentials that evoke superimposed single twitch contractions (see 0 Superposition) to form a more-or-less single smooth contraction of the skeletal muscle. In the following, you should aim to generate recordings that range from a single contraction, through transitional stages of superposition, to a smooth tetanus. The stimulus pulse should be fixed at a level that evokes the same supra-maximal contraction but varied in regard to impulse frequency. You can use either isotonic or isometric conditions. For you documentation, you should have recordings of an incomplete and a complete tetanic contraction (likely to occur at 10 Hz and 20 Hz), and of a sequence of still well separated single contractions (likely to occur at 4 Hz or 5 Hz), preferably with all three recordings on the same oscilloscope display (using the Store mode). You must note that the gain setting should allow for the tetanic stimuli causing about three-times greater muscle force or change in the length than that of a single muscle twitch. A suitable time scale setting is 200 ms/div, which gives a full oscilloscope display setting of 2 s. The number of pulse stimuli should be selected so that the entire sequence is displayed. For 2 s and 5 Hz, this would be 10 pulses. In order that the muscle relaxation is still clearly visible, the total duration of stimulus should be shortened by about 20% (which, in this example,

reducing by 2 pulses to a toal of 8). For each doubling of the frequency, you can double the number of pulses, such as by applying 16 and 32 pulses at 10 and 20 Hz, respectively.

Passive stretch-force curve of skeletal muscle

Because it is elastic, a muscle will change in length as it is stretched. But unlike an ideal spring, the changes in length are not proportional to the force of the stretch. You can demonstrate this by adding an increasing number of weights to the non-stimulated (passive) muscle preparation and record the change in length.

You should note that this can actually be done directly using the oscilloscope. The stimulus intensity should be to 0 mV and channel 2 should be set at maximum. Without activating a zero adjustment (ZERO ADJ), each additional weight will be observed to cause a shift in the baseline of channel 2 and this shift directly relates to the change in muscle length and can be used to generate the stretch-force curve.

Enter in Table 4, the weight force Fp [N] and the intrinsic passive length change ΔLp [mm]. Then measure for each degree of pre-stretching, the active forces ΔFa [N] and length changes ΔLa [mm] at each single muscle twitch and enter the values in the table.

You can begin to create diagram 4, simply by using the force and length changes of the active contractions (upper diagrams). The zero point that is indicated on the abscissa marks the initial length. It is not identical with the zero point of your coordinate system because you have to consider muscle shortening, ie negative values of ΔL.

In the 4 lower diagrams, the passive stretch-force curves are plotted and are, of course, identical. In order to create the classic isometric curves and isotonic maxima, you need to add to these passive values, the values of active force development (isometric maxima) or subtract the values of active muscle shortening (isotonic maxima). The resulting values of the isometric and isotonic maxima can be entered in the appropriate columns of the table. You should add labelling to the diagrams so as to achieve clarity (look for examples in your physiology textbooks), such as indicating the different curves.

Muscle fatigue

The aim of this experiment is to directly compare the contraction curves of a fresh and a fatigued muscle. The contrast is most marked in the freely-suspended muscle (isotonic contractions). Again, you should pre-

stretch the muscle (with one or two weights) as this optimally reflects the normal physiological condition of skeletal muscle. Then, by adding more weights to the muscle, you can quickly cause muscle fatigue.

Muscle fatigue can be demonstrated in different ways. You could compare single contractions before and after fatigue is induced by a prolonged tetanic stimulation. The oscilloscope should be set at 50 mV/div and 50 ms/div so that longer single muscle twitches can be displayed. Also, you can detect on the oscilloscope muscle fatigue developing during a tetanic contraction; but you would have to temporarily reduce the sensitivity (200 mV/div) and slow the time scale. On applying 200 pulses at a frequency of 50 Hz (total time 200 x 20 ms = 4000 ms), 500 ms/div (display time span of 5000 ms) is required for the contraction to be fully observed on the display.

During a tetanic stimulation, the declining force of contraction can be observed through progressive changes in the single twitches, in particular, the significantly reduced rate of contraction and relaxation. These changes, including decreasing contraction amplitude, can also be illustrated by simply making a continuous recording of a prolonged series of pulse stimuli. This should be over an extended period of time, starting separate single twitches, with a setting of 200 ms. As muscle fatigue develops after about 50 pulses, these individual twitches become flatter and wider and begin to overlap. With a time setting of 50 ms/div 2 muscle twitch curves can be selected and superimposed and so demonstrate this effect of muscle fatigue.

3. Practical notes on experimentation – in the virtual and real laboratory

Measurement of the nerve-muscle preparation

For recording skeletal muscle contractions, nerve-muscle preparations are preferred for practical reasons, particularly relating to the need to evoke muscle contractions electrically. This is normally achieved by stimulating the nerve that supplies the muscle. In principle, the muscle could be directly stimulated electrically by, for example, placing electrodes in the form of a loop around the muscle. However, this would require such a large stimulus intensity, to ensure activation of the innermost muscle fibers, that the muscle fibers closest to the electrode would be damaged. Also, because the position of the electrodes will move with each muscle movement, the reproducibility of the response is not guaranteed.

In preparing the nerve-muscle preparation you should make sure that a stump of the femur remains, as this allows the preparation to be fixed to the suspension device by attaching it via this piece of bone and avoiding damaging the muscle or nerve. Particularly important is that the nerve is comparatively thin and, therefore, allows all the neuronal fibers (even the thick fibers of alpha-motor neurones) of the nerve to be activated by a small and non-harmful current. The nerve can then be loosely placed above the suspended preparation and in contact with the stimulating electrodes, without interfering with the position of the muscle and affecting the contraction. Thus, indirect electrical stimulation allows the target tissue (the muscle) to be physically unhindered during activation. It should be noted that, in contrast to other physiological experiments involving nerve and muscle, here mechanical parameters are being recorded, not electrical parameters, and their response time-course is much longer.

Muscle pre-stretching

It is important to keep in mind that a special feature of skeletal muscle *in situ* is that, in its passive state, it is pre-stretched. This means that, when isolated from the body, skeletal muscle contracts and needs to pre-stretched, by attaching weights, in order to exhibit physiologically-relevant contractions of appropriate force. Tests can be performed on isolated muscle to determine the range of optimal pre-stretching and to generate the curves of isometric and isotonic maxima. Experience has shown that in regard to the isolated gastrocnemius muscle of the

clawed frog, pre-extension using a weight of 50 or 100 g provides the physiological length and allows the muscle to exhibit its normal range of contractions.

Muscle fatigue

Because of the absence of a blood supply and the normal level of nutrient supply, the isolated skeletal muscle fatigues much more rapidly than in situ, and once fatigued does not fully recover. For reproducible and physiologically meaningful results, experiments on isolate muscle must be performed systematically and unnecessary repetitive contractions and prolonged tetanic contractions should be avoided. Although there is short-term fatigue, from which the muscle may recover in a few minutes, there is usually some residual fatigue that remains. Returning the preparation, temporarily, to the Petri dish allows the preparation to recover to some degree. In the virtual laboratory, returning the preparation to the petri dish leads to an immediate and complete recovery and so guarantees the comparability of the experimental results.

Muscle deformation

Skeletal muscle is not an ideal elastic mechanical structure and also has plastic components. This can lead to the muscle failing to return to its original starting length after excessive and prolonged contractions. Also this deformation affects the reproducibility of the experimental results. In this virtual laboratory, these plastic effects were not taken into account in the computer simulation. This is because muscle deformation is not a physiological phenomenon but an artifact of the isolated stated of the real experimental model. For this reason, in the real isolated frog muscle preparation, it needs to be ensured that you do not strain the muscles for too long and with too many weights, such as in experiments aiming to derive the curves of isometric and isotonic maxima.

Supra-maximal stimuli of muscle

Besides in experiment 1, you should always apply supra-maximal stimuli, which means stimuli of strengths that are well above the maximum threshold, which you determined in the first experiment. This is important under real conditions to ensure that the stimulus always evokes a similar response because small changes, such as drying of the nerves, will occur that will slightly affect the stimulus conditions and more so at sub-maximal stimuli.

Single twitches versus tetanic contractions

In physiology textbooks, curves of isometric and isotonic maxima are usually shown for measurements of tetanic contractions in individual muscle cells. For a student experiment, the preparation of individual muscle cells is too difficult and time-consuming. In experiments on single muscle cells, fatigue is reached much more slowly than in isolated whole muscle preparations. This is because of the easier access of nutrients which encounter much shorter diffusion paths in the isolated cell than in isolated whole muscle preparations. Thus isolated cells are amenable to using tetanic contractions. In contrast, in isolated whole muscle preparations, it is preferred to perform single twitches rather than tetanic contractions in order to avoid excessive fatigue.

Isometric and isotonic contractions

In most of the experiments, it is irrelevant whether isometric or isotonic contractions are performed; the one exception is experiment 0 0 which aims to demonstrate the generation of isometric and isotonic maxima. However, for demonstrative reasons, isotonic measurements have the advantage that the muscle contractions are clearly evident in the muscle, itself.

The physiological diversity of the preparation

As applies to all biological preparations, there is variation between different preparations of the gastrocnemius muscle of the clawed frog, in regard to their response behaviour. This is due to biological variation arising from differences in anatomical and physiological features, such as muscle size. Additionally, there is experimental variation that affects measured response values, which can be crucially affected by, for example, the positioning of the specimen in the measuring apparatus, of the sensor and of the stimulus electrodes. A further source of variability in isolated preparations is the quality of the preparation. For example, if a lot of connective tissue is left around the nerve, greater stimulation currents will be required than with a well-exposed nerve. However, too much effort to expose the nerve may lead to nerve damage and reduce the efficient of some of the motor units and cause smaller than usual maximum contractions.

4. Physiological basis

In order to perform the practical class successfully and productively, you require a sound knowledge of the basic physiology of skeletal muscle, which you should have acquired from your physiology textbook and/or lectures. In the following, specific aspects of muscle physiology are presented that should be well-known to you but perhaps not fully understood in regard to their particular relevance to the process of muscle contraction.

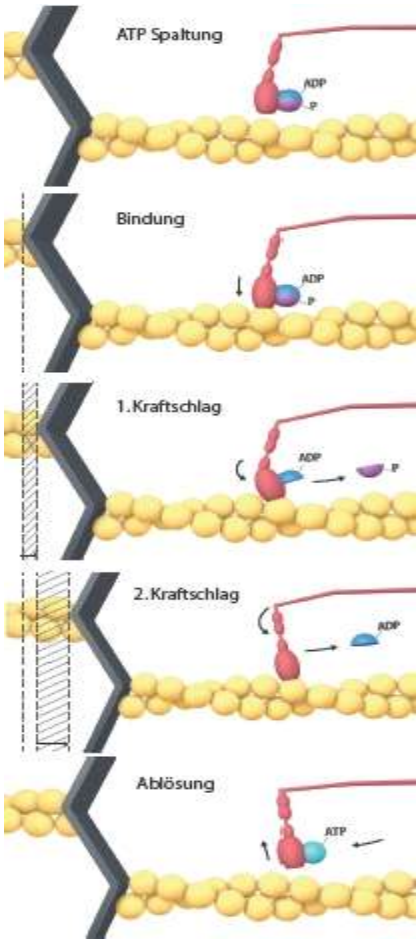


Figure 1: The Actin-Myosin Cross-Bridge-Cycle, illustrated by the movement of a single actin filament. You will find an animation at www.virtual-physiology.de.

The actin-myosin cross-bridging cycle

The contraction of a muscle cell depends not on the shortening of individual molecules but the sliding of molecules over one another. This is the general principle of contraction in all types of muscle and involves the filament molecules, actin and myosin, sliding over each other. This shortens the length of the sarcomere, which is the anatomical sub-unit of the muscle cell that lies between the Z-discs. The sliding of the actin and myosin filaments is initiated by a Ca^{2+}-dependent process. Calcium binds to a site on the actin filament, causing a conformational chain in the actin molecule that exposes a site for binding of the adjacent myosin filament. As myosin binds to actin, a molecule of ATP splits to ADP and inorganic phosphate and provides the energy that is used to turn the neck of the myosin. This causes a shifting of the myosin filament along the actin filament (ratchet-like) before another ATP molecule becomes bound to myosin, causing a further conformational change in myosin and releasing it from actin. In the continued presence of Ca^{2+} myosin binds again to actin and this cross-bridging cycling continues. This process is shown in figure 1 for a single myosin filament; the more myosin filaments involved the more observable macroscopically is the development of the muscle contraction.

Action potentials and Ca^{2+} - controlling muscle contractions

Skeletal muscle contraction is evoked by an action potential across the muscle cell membrane, which is in turn evoked in the motor end-plate in response to an action potential in an alpha-motor neuron arriving at the motor end-plate.

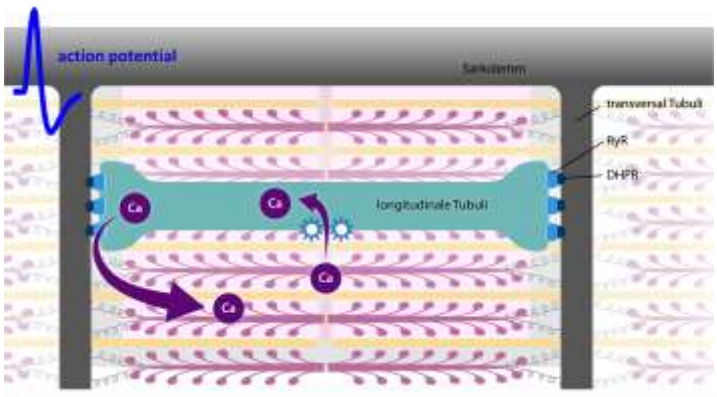
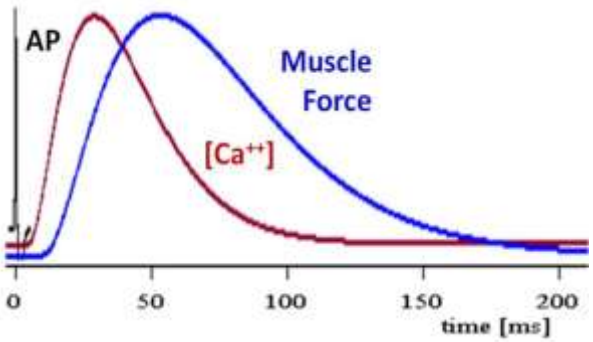


Figure 2: Control of skeletal muscle contraction. On arrival of a muscle action potential in the transverse tubules (T-tubules), voltage-dependent Ca^{2+} channels (dihydropyridine receptors, localized at intervals in membrane of the T-tubules) are activated and, in turn, adjacent Ca^{2+}-dependent Ca^{2+}-channels (ryanodine receptors) in the terminal cisternae of the longitudinal system (sarcoplasmic reticulum) are opened. With the influx of Ca^{2+} and its accumulation in the cytoplasm, Ca^{2+} binds to troponin C of the actin binding sites for myosin heads and so starts the cross-bridge cycle. Simultaneously, Ca^{2+} is pumped by Ca^{2+}-ATPases back into sarcoplasmic reticulum.

The motor end-plate is a specialized synaptic structure that, unlike other synapses, allows one post-synaptic action potential to be evoked in response to each pre-synaptic action potential that arrives there. In the experiments to be performed here, it is possible to observe that a very brief stimulus, one that evokes for sure not more than a single pre-synaptic action potential, is sufficient to cause a single muscle twitch.



The time-span of a muscle contraction is significantly longer than the time-span of the action potential. For example, an action potential of 1-2 milliseconds can cause a muscle twitch lasting 100-200 milliseconds. This is because the actin-myosin cross-bridging cycling is not coupled directly to the muscle membrane action potential but indirectly via the action potential-induced increase in the intracellular Ca^{2+} concentrations.

Figure 3: The different time scales of a muscle action potential, intracellular Ca^{2+} and increase of muscle contraction by a single muscle twitch. The muscle contraction takes significantly longer than the action potential that triggers it, mainly due to the time lag in intracellular Ca^{2+} accumulation and the reverse pumping of Ca^{2+}. Muscle contraction is also slowed by the processes of the cross-bridges cycle and the mechanical properties of the muscle fibers (their elasticity and the damping effect of the surrounding fluid).

The action potential of the muscle fiber membrane is carried along the length of the muscle fiber by the membrane of the T-tubules that penetrate the fiber as invaginations of the muscle cell membrane (sarcolemma). The sarcolemma comes in to close proximity to the membrane of the intracellular structure, the sarcoplasmic reticulum, which stores high amounts of Ca^{2+} ions. By activating specific proteins (dihydropyridine receptors/ryanodine receptors) that are positioned at intervals to form molecular bridges between the sarcolemma and sarcoplasmic reticulum, an action potential (in the sarcolemma) causes extracellular Ca^{2+} to enter the muscle cell and intracellular, stored Ca^{2+} to be released (from the sarcoplasmic reticulum) into the cytoplasm of the muscle. High Ca^{2+} concentrations (10^{-7} moles/L) are required in the muscle cytoplasm to activate cross-bridge cycling. As cytoplasmic Ca^{2+} concentrations increase, the processes that pump Ca^{2+} back in to the sarcoplasmic reticulum are activated causing the cytoplasmic Ca^{2+} concentration to decrease. The processes controlling these fluxes of Ca^{2+} across the sarcoplasmic reticulum and that produce a "wave" of increased cytoplasmic Ca^{2+} require much more time than the duration of an action potential. In turn, the mechanical processes of actin-myosin sliding and muscle contraction and relaxation require much more time than the processes of Ca^{2+} release and re-absorption across the sarcoplasmic reticulum. A significant component of time required for a muscle contraction and relaxation in response to a single action potential is the delay caused by the combined effect of the elasticity of the muscle filaments and the mechanical resistance provided by the surrounding fluid. This can be seen as analogous to the response of a shock-absorber on a car; in response to a brief but powerful pressure applied to a car wing, the shock-absorber causes it to return to its original position without an over-swing but with a detectable delay.

It is important to realize here that the muscle forces or changes in muscle length that you can observe and measure are different to what is shown in a myogram. A myogram measures actual muscle action potentials, which are electric parameters, and not force or changes in length, which are mechanical parameters.

Superposition of single twitches and tetanic contractions.

The difference in duration of the short action potential and the much longer muscle twitch underlies the development of a tetanic contraction from single twitches. Action potentials can be generated at intervals that are much shorter than the duration of a single muscle twitch. If a second action

potential arrives before the previously evoked muscle twitch has subsided, a second muscle contraction will be added on top of the first contraction. When several action potentials arrive in a short period of time, a tetanic contraction will be formed by the single contractions superimposing and without a single contraction being detectable.

The muscle force resulting from repeated action potentials is not proportional to the frequency of action potentials for a number of reasons: (i) the elastic resistance of muscle fibers increases, (ii) an equilibrium will be reached between Ca^{2+} release and Ca^{2+} re-absorption, (iii) all the Ca^{2+} binding-sites will eventually become saturated, and (iv) the rate of cross-bridge cycling will reach a maximum, above which no further increase is possible. The force of a tetanic contraction is usually never more than 3-fold greater than that of a single muscle twitch. Much more important for increases in the force of the whole muscle is the recruitment of motor units. Under physiological conditions, a relatively low frequency of action potentials can lead to a tetanic muscle contraction through overlapping contraction of several motor units with alternating innervations. This is not observed in the isolated muscle preparation because the action potential, in this case, is evoked by an external stimulus.

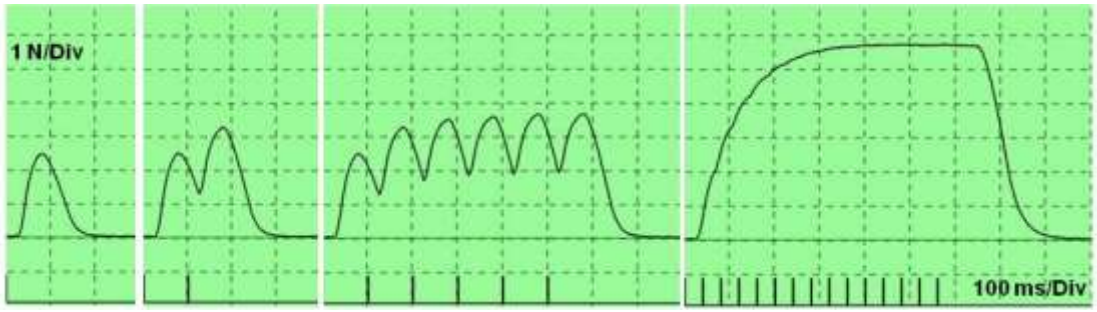


Figure 4: single muscle twitch; incomplete superposition of 2 and 6 muscle twitches; and complete (smooth) tetanus.

It should be noted here why tetanus cannot occur in heart muscle. This can be easily explained by reference to the duration of events underlying heart muscle contractions. The heart muscle action potential lasts 300-400 milliseconds (ms) and is of a longer duration than the heart muscle contraction. Therefore, after the cardiac muscle action potential is over, the cardiac muscle contraction is already over. So, if a second action potential was to arrive immediately, there would be no previous contraction present on which a second contraction could superimpose. Thus it is not the refractory period, as one often written in physiology text books, that prevents superposition of heart contractions as occurs in the skeletal muscle. Additionally, the action potential of the skeletal muscle has a refractory period

due to the different time relations between duration of action potentials and single twitches. (For the occurrence of sustained but uncoordinated heart contractions that occur during cardiac arrest see "SimHeart").

Effect of Pre-stretching Muscle

The contraction strength of a muscle is very dependent on the degree of pre-stretching but how contraction strength is affected by pre-stretching varies between different types of muscle. The effect of pre-stretching can be readily observed in the isolated skeletal muscle. When a skeletal muscle is detached from a tendon, it is clearly seen to become shorter. If the isolated skeletal muscle is then stretched by attaching a weight, as will be seen in this practical class, it can return to its in vivo length and it acquires a contraction strength that is close to that which it had in vivo.

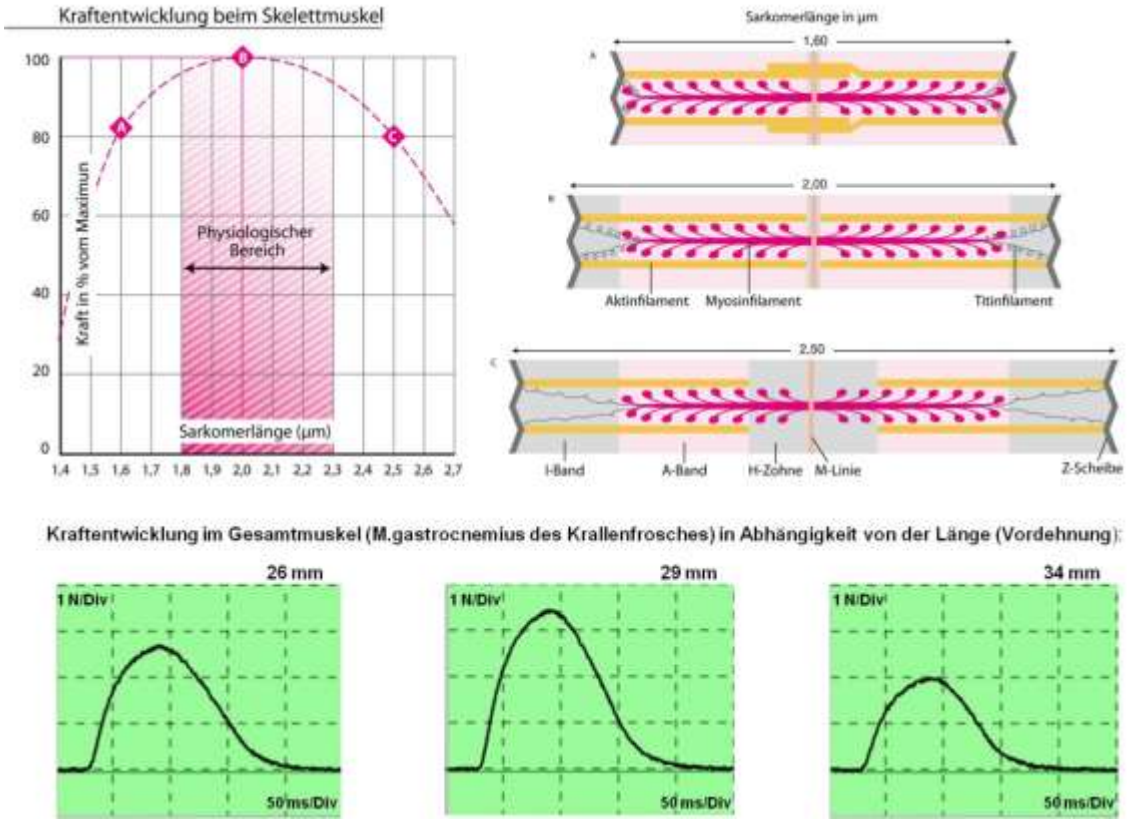


Figure 6: Force development in a skeletal muscle fiber in relation to sarcomere length (above left). The maximum force is set to 100%. Under physiological conditions, muscle contractions occur within a narrow range around the maximum force. Above right is shown the optimal degree of actin-myosin interaction with moderate pre-stretching (middle), compared to the overlap of actin filaments that occurs in the totally unstretched muscle (top) and with severe over-extension with a proportion of myosin heads without access to sites for actin binding (below). The lower diagrams show the corresponding recordings of an isolated whole muscle (from the laboratory SimMuscle) with the different degrees of pre-strain.

This has a lot to do with the stretching of the skeletal muscle allowing a maximal overlapping of the myosin and actin filaments, with optimal stretching allowing each myosin head to have access to a binding site on actin. Also, stretching the muscle increases the Ca^{2+} sensitivity of the myosin-actin interaction. Without pre-stretching, the actin filaments overlap and prevent myosin from binding or may even cause contractions in the wrong direction. Over-stretching the muscle reduces the access of myosin heads to actin.

It is important to note here that this phenomenon, as it is seen in vivo, differs between skeletal muscle, heart muscle and smooth muscle (see also SimHeart and SimVessel). Whereas the normal basal, unstimulated pre-stretched state of skeletal muscle provides for maximal muscle strength, heart muscle and smooth muscle have a considerable reserve of potential muscle strength when in their basal state. The strength of the heart muscle is increased as it is stretched during filling of a heart chamber (the Frank-Starling mechanism) and intestinal and vascular smooth muscle only become active as they are stretched in response to increased luminal pressure.

Muscle fatigue

The experience of muscle fatigue after prolonged strenuous exercise is a common experience. Several factors are involved in the experience of muscle fatigue. In real life, psychological factors mostly play a role in strenuous exercise and allow the feeling of "fatigue" to set in before true muscle fatigue occurs. Of course, with the isolated skeletal muscle such factors can be ignored. However, muscle fatigue is reached much sooner in the isolated skeletal muscle than in the skeletal muscle in situ. This is because of the absence of a blood supply and consequent lack of sufficient energy supplies and of adequate removal of metabolic waste products.

It is possible to recognize whether an isolated muscle is fresh or fatigued.

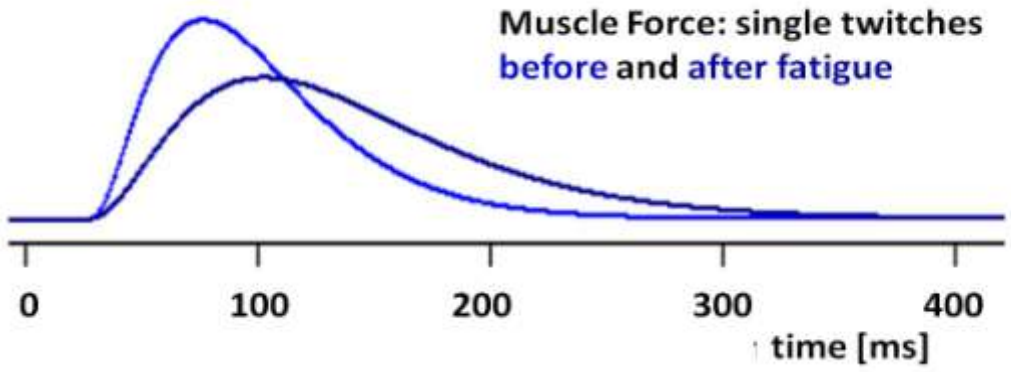


Figure 5: Single muscle twitch of an isolated skeletal muscle, before and after fatigue.

Usually, muscle fatigue in an isolated skeletal muscle is reached after 100 stimulated tetanic contractions. The form of the muscle contraction is clearly different when comparing recordings before and after 100 tetanic contractions (Figure 3). Not only is there a reduction in muscle power but also a much more prolonged contraction, which is usually observed before any clear decline in muscle power is observable. Although much of what causes muscle fatigue is unclear, the reduction in the intra-cellular levels of ATP is clearly an important contributory factor. ATP is needed not only for cross-bridge cycling (for releasing the myosin head from its binding site on actin and for inducing the "bending" of the myosin head) but also for pumping Ca^{2+} back in to the sarcoplasmic reticulum. A slowing down of both these processes would delay muscle contraction and movement would be slow and imprecise. Those myosin heads that are still bound to actin will not be able to contribute to muscle contraction and muscle power would be lost. In the complete absence of ATP, all the myosin heads will remain fixed to their binding sites on actin. This is seen in the facial muscles of a corpse, causing the fixed stare. A deficit of ATP in skeletal muscle may also cause muscle cramp.

There are several other candidate factors that may contribute to muscle fatigue. These include the increased intracellular levels of ADP and inorganic phosphate, which directly relates to the decreased ATP levels, and the decrease in the pH of the muscle tissue. In particular, raised levels of inorganic phosphate affect not only the cross-bridge cycling process but may also slow the rate of Ca^{2+}reabsorption. However, the underlying mechanisms of these effects are unknown.