

**Figure 6.3:** Circumferential and uniaxial flow devices applying shear stress to the cell culture.

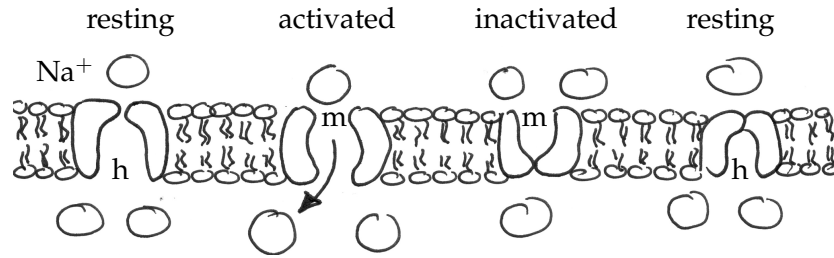
They are stimulated through a circumferential fluid flow generating by a rotating upper disk. This disk can either be conical or flat. Both devices generate an inhomogeneous fluid profile caused by a zero flow velocity at center that increases radially to reach the maximum flow velocity at the edge of the disk. Again, the interpretation of different shear stresses acting on the cell culture can be difficult. Sometimes, however, it might be advantageous to explore the impact of different shear profiles within one single sample.

### Uniaxial flow - Flow chambers

Flow chambers generate a uniaxial flow on top of a cultured substrate by using pressure gradients to drive the fluid. Fluid flow profiles can, in general, be either parallel or radial, but the parallel fluid flow chamber is by far the most common. Parallel flow chambers assume Poiseuille flow, i.e., they are designed such that the flow profile on the culture is laminar and the resulting shear stress is constant for a given flow rate. Parallel flow chambers are very attractive; they are relatively easy to handle and can be put under a microscope to observe the response of cells as the flow profile is changed or reversed. A potential disadvantage of flow chambers is that they apply fully developed laminar flow profiles. It is questionable to what extent laminar flow can fully mimic the *in vivo* observed stimuli.

## 6.3 Electrophysiology

The cell membrane can be understood as a boundary separating the internal working cell from its external environment. Maybe the most important feature of this membrane is that it is selectively permeable, regulating the permitted and restricted transport of materials into and out of the cell, see figure 6.4. In chapter 5, we have seen that the cell membrane is a 4-5nm thick double layer of phospholipid molecules. Among other aggregates that move around freely within this double layer, the cell membrane contains water-filled pores with diameters of about 0.8nm, as well as protein-lined pores called channels which allow the controlled passage of specific molecules. The intracellular and extracellular environment consist of a dilute aqueous solution of dissolved salts, primarily NaCl and KCl which dissociate into  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  ions.



**Figure 6.4:** The fast sodium channel has two gates, an activation gate (m-gate) shown at the top and an inactivation gate (h-gate) shown at the bottom. In the resting state (left), activation gates (m-gates) are closed and inactivation gates (h-gates) are open. Rapid depolarization opens voltage-gated m-gates enabling sodium to enter the cell (second from left). Upon repolarization, inactivation gates (h-gates) close to inactivate the channel (third from left).

The phospholipid bilayer acts as a barrier to the free flow of these ions maintaining a well-regulated concentration difference of ions across the cell membrane. Most cells have a potential difference across their cell membrane which is referred to as membrane potential. This implies that the membrane can selectively separate charge. We

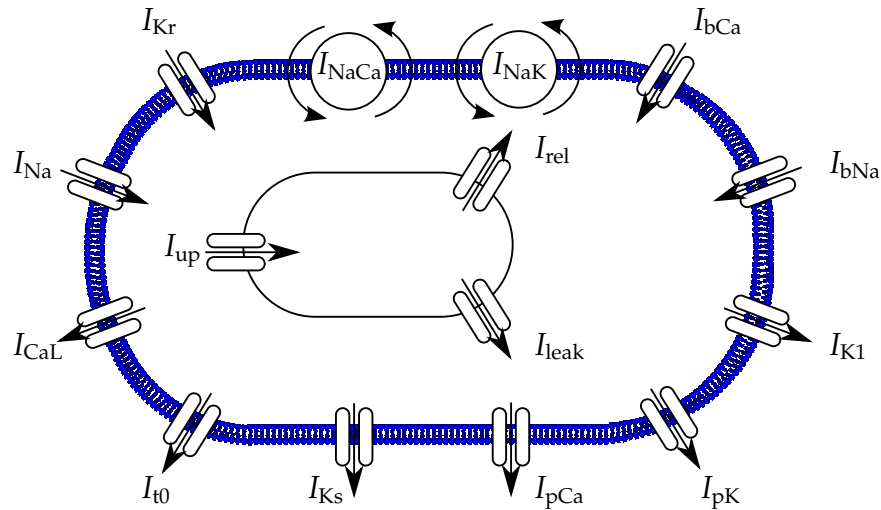
	$\text{Na}^+_{\text{int}}$ mM	$\text{Na}^+_{\text{ext}}$ mM	$\text{K}^+_{\text{int}}$ mM	$\text{K}^+_{\text{ext}}$ mM	$\text{Cl}^-_{\text{int}}$ mM	$\text{Cl}^-_{\text{ext}}$ mM	resting pot. mV
nerve cell	50	437	397	20	40	556	$\phi = -65$
skeletal muscle cell	13	110	138	2.5	3	90	$\phi = -99$
cardiac muscle cell	10	145	135	4	25	140	$\phi = -90$
red blood cell	19	155	136	5	78	112	$\phi = -8$

**Table 6.2:** Typical values for intracellular and extracellular concentrations of sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), and chloride ( $\text{Cl}^-$ ) ions.

can measure the potential difference by inserting an electrode into the cell and another one placed just outside the cell measuring the two voltages  $\phi^{\text{int}}$  and  $\phi^{\text{ext}}$  with a voltmeter. By convention, we measure the transmembrane potential by taking the difference of the interior and the exterior potential.

$$\phi = \phi^{\text{int}} - \phi^{\text{ext}} \quad \dots \text{ membrane potential} \quad (6.3.1)$$

In virtually all cells, we will find a negative voltage. Nerve cells, for example, have a membrane potential of about  $-70 \text{ mV}$ , with the negative sign indicating that the inside is negative, i.e., it has an excess of negative charges. This basically raises two questions: Why is there a potential difference across the cell membrane? And what are the mechanisms that are responsible for generating, maintaining, and regulating membrane potentials? Two main mechanisms that regulate the membrane potential through a controlled transport of charged ions across the membrane: (i) discontinuous passive transport through ion channels and (ii) continuous active transport through ion pumps. We will address these two regulatory mechanisms of the cell in detail in the sequel.

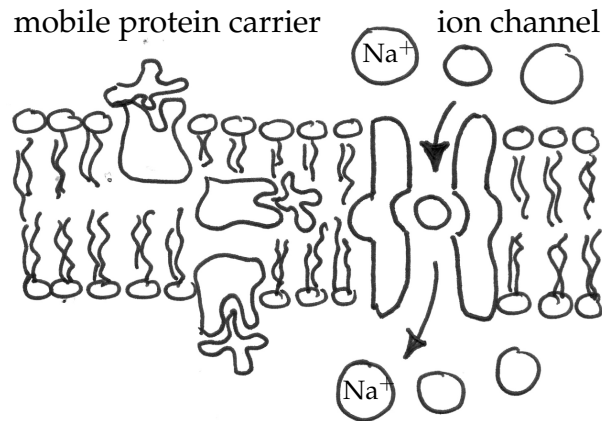


**Figure 6.5:** Human ventricular cardiomyocyte. In this model, the chemical state of the cardiomyocyte is characterized in terms of four ion concentrations: the free intracellular sodium, potassium, and calcium concentrations and the free calcium concentration in the sarcoplasmic reticulum. Ion concentrations are controlled through 15 ionic currents, the fast sodium current  $I_{Na}$ , the background sodium current  $I_{bNa}$ , the sodium potassium pump current  $I_{NaK}$ , and the sodium calcium exchanger current  $I_{NaCa}$ , the inward rectifier current  $I_{K1}$ , the rapid delayed rectifier current  $I_{Kr}$ , the slow delayed rectifier current  $I_{Ks}$ , the plateau potassium current  $I_{pK}$ , the transient outward current  $I_{t0}$ , the L-type calcium current  $I_{CaL}$ , the background calcium current  $I_{bCa}$ , the plateau calcium current  $I_{pCa}$ , the leakage current  $I_{leak}$ , the sarcoplasmic reticulum uptake current  $I_{up}$ , and the sarcoplasmic reticulum release current  $I_{rel}$ .

### 6.3.1 Passive transport - Ion channels

The chemical compositions of the fluids inside and outside the cell are different. Passive transport is driven by directed diffusion to equilibrate these differences. Passive transport is directed *along concentration gradients* from high to low. There are three major mechanisms of passive transport to passage molecules through the cell membrane: (i) osmosis, a process by which water is transported through the membrane, (ii) simple diffusion, diffusion of small molecules through pores and of lipid soluble molecules such as oxygen or carbon dioxide through the bilipid layer, and (iii) carrier-mediated diffusion, binding of molecules to a carrier molecule that take them through the membrane.

Ion channels are integral membrane proteins through which ions can diffuse through the membrane. The schematics of a typical sodium ion channel are illustrated in figure 6.6. The sketch shows that the pore through which the ion passes is usually ion specific and so small that only one single ion can pass through it at a given time. Although ion channels may have various different states corresponding to different configurations of their proteins, they are either fully open or fully closed. The passive transport through ion channels is therefore a discontinuous process. When the channel is closed, the passage of ions is blocked. When the channel is open, ion flow is driven by passive transport. The flow rate is determined by both the concentration gradient and the

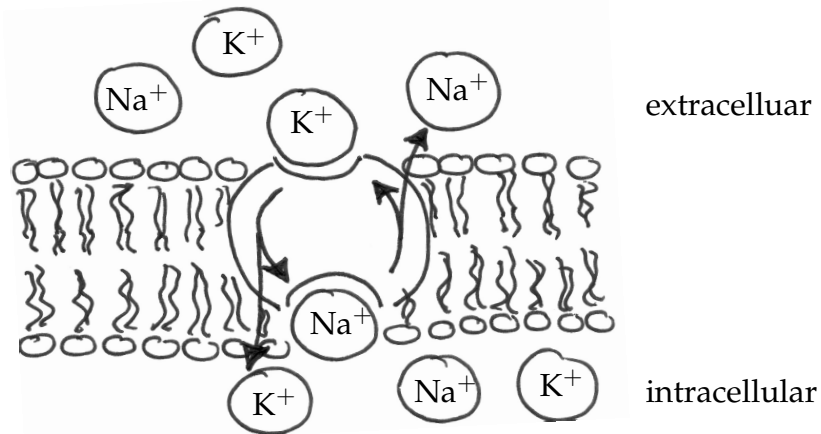


**Figure 6.6:** Passive transport through protein lined ion channel. Ion channels are specified for a particular class of ions and their pores are usually so small that only one ion can pass through it at a time.

membrane potential. Opening of ion channels can be gated by different mechanisms. The most common ion channels are voltage-gated channels, ligand-gated channels, or mechanically-gated, but light, pressure, and temperature might also gate the opening and closing.

### 6.3.2 Active transport - Ion pumps

The membrane is able to pump ions from the intracellular to the extracellular side and vice versa to regulate ionic concentrations at the expense of extra energy. Active transport is directed *against concentration gradients* from low to high. The cell membrane possesses a variety of different ion pumps to set up and maintain concentration differences. Typical examples are: (i) the sodium/potassium  $\text{Na}^+/\text{K}^+$  pump which uses energy stored in ATP molecules to pump  $\text{Na}^+$  out of the cell and  $\text{K}^+$  in, (ii) the  $\text{Ca}^{2+}$  ATPase which pumps  $\text{Ca}^{2+}$  out of the cell or into the endoplasmic reticulum, and (iii) the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger removing  $\text{Ca}^{2+}$  from the cell at the expense of  $\text{Na}^+$  entry. In contrast to ion channels, ion pumps generate a continuous flux of ions through the cell membrane. However, they influence the transmembrane potential only by establishing the relative ratio of extracellular and intracellular concentrations. This implies that even if the ion pumps were turned off for a while, the cell could still maintain the difference in transmembrane potential for hundreds or even thousands of cycles. Figure 6.7 illustrates the most important example of active transport, the sodium/potassium pump. It actively pumps sodium ions out of the cell against their steep chemical gradient and pumps potassium ions into the cell. The sodium/potassium pump is extremely important for cell survival. It is used to regulate the internal ionic composition of the cell and maintain its membrane potential. Almost one third of all the energy requirement of a typical animal cell is consumed by fueling this particular pump.



**Figure 6.7:** Active transport through cell membrane containing sodium/potassium pump. The  $\text{Na}^+/\text{K}^+$  pump is the most important ion pump that consumes up to one third of the total energy requirement of a typical animal cell to actively pump cells against concentration gradients.

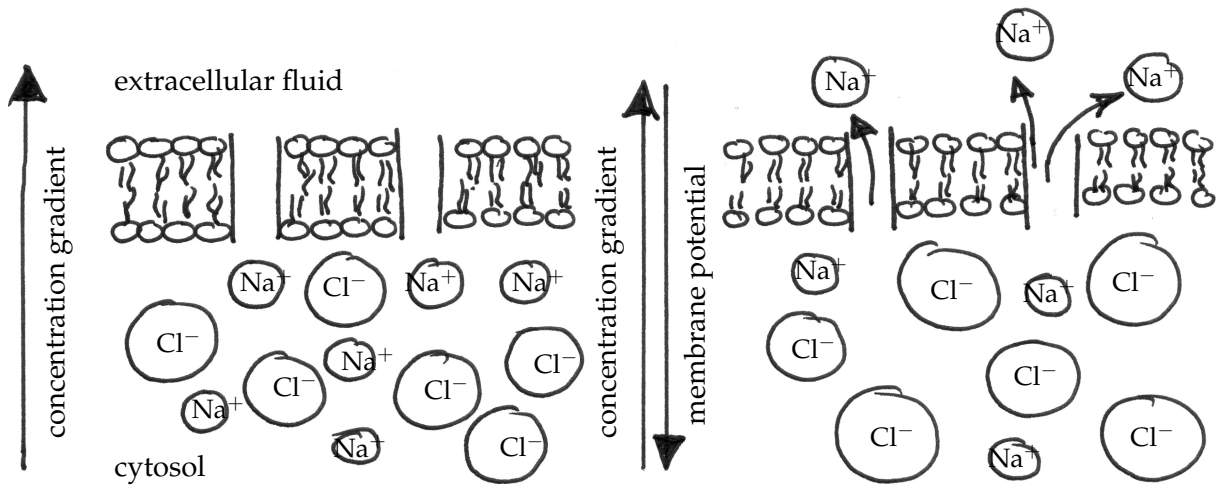
### 6.3.3 Membrane potential

We have seen that passive transport through ion channels and active transport through ion pumps regulate different compositions of the extracellular and intracellular fluid. On the two sides of the membrane, the concentrations of particular ions can be different. This concentration difference leads to a difference in charge. Let's illustrate how the membrane works by assuming two reservoirs representing the intracellular and extracellular spaces. The reservoirs are separated by a semipermeable membrane, which represents the cell membrane, see figure 6.8.

**Phase I - Electrically neutral state** Initially, both reservoirs contain the same ions but at different concentrations. However, both sides are electrically neutral so that on the intracellular and on the extracellular side, each positively charged ion, for example sodium  $\text{Na}^+$ , is balanced with a negatively charged ion, for example chloride  $\text{Cl}^-$ .

**Phase II - Selective permeability** Now, the membrane is made permeable to sodium but not to chloride. The concentration difference across the membrane initiates a passive transport of sodium to along the concentration gradients whereas the chloride concentration on both sides remains constant. This generates a charge separation across the cell which creates a transmembrane voltage, the membrane potential.

**Phase III - Resting state** Charge separation will continue and the membrane potential will grow. An equilibrium state is reached when this electric field exactly balances the diffusion of ions, or, in other words, the tendency of sodium to leave the cell along its concentration gradient is now matched by the tendency of the membrane voltage to pull sodium ions back into the cell. Theoretically, this equilibrium state will correspond to a configuration with more sodium ions on one side than on the other and neither side of the membrane will be electrically neutral. There is no more net sodium flux across the membrane and  $\text{Na}^+$  is in equilibrium. The membrane potential is said to be stable or resting.



**Figure 6.8:** Concentration gradient and membrane potential. In the electrically neutral state both sides of the membrane have the same number of positive and negative charges but the concentrations on both sides may be different. This causes a concentration gradient, left. In the resting state the concentration gradient is balanced by the membrane potential, right.

It is somewhat counterintuitive that in reality, the transmembrane flux of ions will significantly change the membrane potential whereas the concentration gradient will remain almost unchanged. Why is that? If sodium is leaving the cell, shouldn't this affect both, i.e., increase the membrane potential and decrease the concentration? It's important to realize that only a small number of ions, in our example sodium ions, need to move across the membrane in order to generate a significantly large membrane potential. It's therefore justified to assume that the ion concentration on both sides of the membrane remains almost unchanged, the solutions on both sides remain electrically neutral, and the small excess charge accumulates locally at the thin interface.

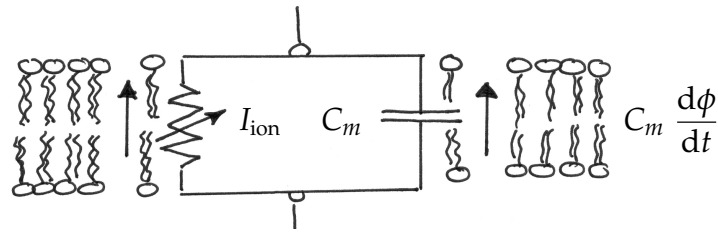
An illustrative model of the cell membrane is an electrical circuit in which the membrane itself acts as a capacitor in parallel with a resistor, see figure 6.9. According to this model, for a constant capacitance, the sum of ionic currents  $I_{\text{ion}}$  and capacitive currents  $C_m d\phi / dt$  must be zero.

$$C_m \frac{d\phi}{dt} + I_{\text{ion}} = 0 \quad (6.3.2)$$

Here,  $C_m = c_m A$  where  $c_m$  is the capacitance per area measured in farad per meter squared, i.e.,  $[c_m] = [F / m^2]$ . The capacitance of the cell membrane is typically of the order of 0.01 - 0.1 F / m<sup>2</sup>. Remember that one farad F is defined as the amount of capacitance for which a potential difference of one volt V results in a static charge of one coulomb C, i.e.,  $[F] = [C] / [V]$  and one coulomb C corresponds to  $6.24 \cdot 10^{18}$  ions of elementary charge. Moreover,  $A$  is the membrane surface area,  $\phi$  is the membrane potential, and  $I_{\text{ion}}$  is the ionic current. The most challenging task is to determine a good model for the ionic currents  $I_{\text{ion}}$  and we will address this issue later in this chapter. Equation (6.3.2) can be used to estimate how much current would need to flow to discharge the cellular membrane.

**Potential and concentration during cardiomyocyte action potential upstroke**

Let's calculate how many charged sodium ions have to move through the cell membrane of a cardiomyocyte during an action potential upstroke. This will give us an idea how much the intracellular sodium concentration changes. Assume the membrane potential during a typical upstroke increases by  $\Delta\phi=100$  mV in  $\Delta t=2$ ms. With this information, we can determine the sodium current  $I_{\text{Na}^+} = c_m A \cdot \Delta\phi / \Delta t$  for a given capacitance per area  $c_m$  multiplied by a particular cell surface area  $A$ . Assume the capacitance per area is  $c_m=0.02$  F/m<sup>2</sup>=0.02 C/[V m<sup>2</sup>]. Let's approximate cardiomyocytes to have a shape of a cylinder with radius  $r = 5\mu\text{m}$  and length  $L = 100\mu\text{m}$ . Their surface area then is  $A = 2\pi \cdot r^2 + 2\pi \cdot r \cdot L = 2\pi \cdot [5 \cdot 10^{-6}]^2 \text{m}^2 + 2\pi \cdot [5 \cdot 10^{-6}] \cdot [100 \cdot 10^{-6}] \text{m}^2 = 3.299 \cdot 10^{-9} \text{m}^2$ . Solving  $I_{\text{Na}^+} = c_m A \cdot \Delta\phi / \Delta t$  yields a sodium current of  $I_{\text{Na}^+} = [0.02 \text{ C/V m}^2] \cdot [3.299 \cdot 10^{-9} \text{m}^2] \cdot [0.1 \text{V}] / [0.002 \text{s}] = 3.299 \cdot 10^{-9} \text{ C/s}$ . Here C represents the unit Coulomb. Next, we need to calculate the number of ions  $n$  that are required to generate this charge. For sodium, every ion has one elementary charge, and one Coulomb then corresponds to  $1\text{C} = 6.24 \cdot 10^{18}$  ions, thus  $n = I_{\text{Na}^+} \cdot \Delta t \cdot 6.24 \cdot 10^{18} \text{ ions / C}$ . So in our case,  $n = [3.299 \cdot 10^{-9} \text{ C / s}] \cdot [0.002 \text{ s}] \cdot [6.24 \cdot 10^{18} \text{ ions / C}] = 4.12 \cdot 10^7$ . This means that it requires the movement of 41.2 million sodium ions across the membrane to change the membrane potential of cardiomyocyte by 100 mV in 2 ms! Okay, but now, what does this mean for the intracellular sodium concentration? Assume the intracellular sodium concentration in cardiomyocytes at rest is about  $C_{\text{Na}^+}=15$  mM. Remember that 1 M = 1 mol/L and that 1 mol corresponds to  $6.022 \cdot 10^{23}$  ions. So  $4.12 \cdot 10^7$  ions would correspond to a change in concentration of  $\Delta C_{\text{Na}^+} = n / [V \cdot 6.022 \cdot 10^{23} \text{ions / mol}]$ . With the assumed radius of  $r = 5\mu\text{m}$  and length  $L = 100\mu\text{m}$ , the cardiomyocyte volume is  $V = \pi \cdot r^2 \cdot L = \pi \cdot [5 \cdot 10^{-6}]^2 \cdot [100 \cdot 10^{-6}] \text{m}^3 = 7.8540 \cdot 10^{-15} \text{m}^3 = 7.8540 \cdot 10^{-12} \text{ L}$ . The change in concentration then results as  $\Delta C_{\text{Na}^+} = [4.12 \cdot 10^7 \text{ ions}] / [7.85 \cdot 10^{-12} \text{L}] / [6.022 \cdot 10^{23} \text{ions / mol}] = 7.77 \cdot 10^{-5} \text{ mol / L} = 0.0777 \text{mM}$ . So, what is the relative change of sodium ions in the cell?  $\Delta C_{\text{Na}^+} / C_{\text{Na}^+} = 0.0777 \text{mM} / 15 \text{mM} = 0.0052 = 0.52\%$ . Since the normal inside concentration of sodium is approximately 15 mM, an amount of 0.077mM entering the cell during the action potential upstroke only corresponds to 0.52 % extra sodium ions. That's seems like nothing!! In summary, approximately 40 million sodium ions must cross the membrane to move the membrane potential by 100 mV in 2 ms, and that this constitutes only some 0.5% of the sodium already present in the cell. The effect of sodium ion motion on the intracellular sodium concentration is therefore indeed negligible!



**Figure 6.9:** Electrical circuit model of the cell membrane.

Normally, cells are net negative inside the cell which results in a non-zero resting membrane potential. The membrane potential of most cells is kept relatively stable. Nerve cells, skeletal, and cardiac muscle cells, however, are specialized to use changes in membrane potential for fast communication, primarily with other cells of their type. Within a millisecond, their membrane potential changes from positive to negative and back. This feature is referred to as action potential.

### 6.3.4 Action potential

The action potential is a self-regenerating pulse-like wave of electro-chemical activity that allows some cell types to rapidly carry signals over long distances. A typical action potential is initiated by a sudden change in the transmembrane potential. As the membrane potential is depolarized, both sodium and potassium channels begin to open generating an inward sodium current balanced by an outward potassium current. For only small perturbations, the potassium current wins and the membrane potential returns to its resting state. For sufficiently large perturbations of approximately 20 mV, however, the sodium current wins producing a positive feedback. The cell produces an action potential, we say the cell fires. One very important feature of the action potential is that its amplitude is independent of the degree of stimulation. Larger stimuli do not generate larger action potentials. This characteristic property of action potentials is referred to as *all or none response*, either the fires or it does not.

The initiation and propagation of electrical signals by the controlled opening and closing of ion channels is one of the most important cellular functions. Its first quantitative model was proposed half a century ago and awarded the Nobel Prize in 1963 [19]. Although originally developed for neurons, this theory was soon modified and generalized to explain a wide variety of excitable cells. To gain a better understanding of these models, let's take a look at equation (6.3.2) which we can rephrase as follows.

$$\dot{\phi} = -\frac{1}{C_m} I_{\text{ion}} \quad \text{with} \quad I_{\text{ion}} = I_{\text{Na}} + I_{\text{K}} + I_{\text{Cl}} + I_{\text{Ca}^{2+}}. \quad (6.3.3)$$

Here  $\dot{\phi} = d\phi / dt$  is the change in the transmembrane potential,  $C_m$  is the transmembrane capacitance, and  $I_{\text{ion}}$  is the total ionic current. This current results from the flux of sodium  $I_{\text{Na}}$ , potassium  $I_{\text{K}}$ , chloride  $I_{\text{Cl}}$ , and calcium  $I_{\text{Ca}^{2+}}$  ions across the cell membrane. If we measured the transmembrane potential of different cell types found in the heart and plotted it over time, it would look somewhat like the illustration in figure 6.10. Apparently, different cell types seem to have different action potentials.