HELPING STUDENTS TO UNDERSTAND THAT OUTWARD CURRENTS DEPOLARIZE CELLS

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The physiology of excitable membranes is a fundamental topic in neuroscience and physiology courses at graduate and undergraduate levels. From the building blocks of ionic gradients and membrane channels whose permeability is selective and variable, we build the concepts of resting potential, action potential, and propagation in neurons and muscle fibers. Many students have an intuitive understanding of the movements of ions and the associated changes in membrane potential. For example, potassium ions leaving a cell through potassium-selective channels become unbalanced positive charges on the outside of the cell (and leave unbalanced negative charges on the inside), thus producing a potential across the membrane with the inside negative with respect to the outside. Later, when we discuss the local circuit currents that underlie propagation or the basis for extracellular stimulation, we make the general statement that ''outward currents depolarize cells.'' Students respond with utter disbelief. Two simple additions to a discussion of membranes are suggested that permit the formulation of a consistent set of rules that apply to everything from the resting and action potentials of nerve and muscle through synaptic potentials and stimulation techniques.

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THE PROBLEM FOR STUDENTS

The physiology of excitable membranes is taught at our institution at the graduate (medical and graduate students) and undergraduate (health-related professions students) levels in neuroscience and general physiology courses. This includes lectures on cell membranes, ion gradients across cell membranes, and the means and mechanisms by which ions cross membranes.

Nearly every student at the medical/graduate level has had some prior training in membrane physiology. For most, this training emphasized the basis for the resting and action potentials in excitable cells. These concepts can be intuitively understood in terms of unbalanced charges. Ions cross a membrane through selective channels and produce a charge imbalance that is measured as the membrane potential. As an example, potassium ions move down their concentration gradient from inside to outside, leaving behind unbalanced negative charges (hence, the negative resting potential). Similarly, sodium-selective channels open to permit sodium ions to enter a cell, producing a different imbalance where positive charges accumulate to exceed negative charges (hence, the upstroke of the action potential).

In the past, students would begin to experience conceptual problems when the lectures turned to the basis for propagation and stimulation techniques. A demonstration that the initiation of an action potential in an axon occurs in the vicinity of an extracellular

cathode (and not an extracellular anode) continues to be the best provocateur of panic. In fact, this result is perfectly consistent with the depolarizing effects of local circuit currents that cause propagation. The students, however, are looking for the place (anode or cathode) where the electrodes are putting positive charges into the cell. To ''help,'' students were given a general statement to commit to memory: ''Outward currents depolarize.'' This notion slams head-on into the intuitive view of currents crossing membranes and their associated voltage changes. It can take weeks to undo the confusion. For some, resolution never comes.

THE PROBLEM FOR FACULTY

In the face of this annual agony (for both the students and the faculty), some faculty prefer to avoid the conflict altogether. They argue that we are teaching extracellular stimulation for historical purposes only and that it can easily be removed from the lectures. The local circuit currents are not nearly as troublesome because the positive charges have already entered the cell. They only have to move down the core and make the inside of the adjacent region of axon more positive. In our former problem-based learning track, the conflict never came up because students satisfied themselves and their tutors by reaching only this level.

Some faculty manage the problem by distinguishing current flows as ''active'' or ''passive'' in regions of membrane. In such presentations, an active inward current (e.g., due to the opening of voltage-dependent sodium channels) is depolarizing, but a passive inward current (e.g., due to an extracellular anode) is hyperpolarizing. To determine the effect on the membrane of an inward or outward current, one just has to keep track of whether the current flow is through active or passive membrane. This usually is not hard for students to do. Problems, which I feel are serious, arise in this style of coverage. First, there are currents that are difficult to classify. One is the ionic current in the simple concentration cell (discussed in more detail below). Because this is clearly an artificial situation and, more importantly, because it is covered before any discussion of active versus passive current flows, classification can be avoided. More difficult would be classification of the outward current through chloride channels in the t-tubule membrane of skeletal muscle cells. We teach that this is not a voltage-activated current, but it is a significant contributor to the repolarization of skeletal muscle membrane. In the active versus passive style, it behaves like an active current (outward current driving the membrane toward more negative potentials), but it is not. The second problem I have with classifying currents as active or passive is that it encourages students to neglect some passive currents but not others. In the region of an axon where voltage-dependent sodium channels are open or opening, an active inward current is all that gets considered. The passive return currents in this region are ignored, but the passive current paths in the region of resting membrane ahead of the action potential are stressed as the basis for the depolarization that will activate sodium channels for propagation. Not only does this take one away from complete electrical circuits but, by neglecting some passive current flows, one loses the ability to get a quantitative sense of the voltage change that will result from a given active current. One instance in which this can occur is in a discussion of synaptic potentials and the ''inhibitory'' effects of channels that are opened to shunt active inward depolarizing currents (e.g., from excitatory synapses or action potentials propagating in dendrites). I agree that a separation of active and passive currents gets one farther than with simple charge accumulation, but there are just too many conditions and qualifications for me to be comfortable with it.

Other strategies have tried to divert students' attention away from current flows altogether in lectures on resting potential, action potential, and propagation. One method is to emphasize conductance changes and how these will drive the membrane potential toward or away from the equilibrium potential associated with the particular channel. Students are asked to work almost exclusively with the parallel conductance equation (shown below), which contains no current terms.

Another approach is to work with a spherical cell for much of the presentation during which students are frequently reminded that inward and outward currents exactly match each other at all times, yet the membrane generates perfectly good action potentials. The spherical cell can later be compared with an axon, where a net inward current exists at the place on the axon that has the peak of the action potential.

Students are told that the ''hyperpolarizing'' effect of a net inward current can be seen as the reduced amplitude of the action potential peak in an axon compared with the peak amplitude in a spherical cell. Few students are impressed by this, and it has never been enough to convert the ''nonbelievers.''

Lest I give the impression that every attempt to cover this material is somehow doomed to fail, I know many instructors who have had great success with their own styles of coverage, and I am not suggesting that they abandon their successful ways. There are many, however, who have had or are having difficulty getting this material covered. My paper is aimed at them and their students. Failed attempts are powerful reinforcers of the positions that extensive coverage is unnecessary or that there is not enough lecture time to do the job adequately. I think this material is so fundamental that thorough coverage is essential. What follows are the main points in my strategy for developing a general set of principles that describe the behavior of membranes in a reasonable time frame (I use 4 h of lecture to cover the structure of membranes, the resting potential, the action potential, and propagation).

ONE STRATEGY THAT WORKS

I start by talking about our goal of developing a single set of rules to describe all of the circumstances that will come up in our lectures and how these rules will apply to later lectures on synaptic potentials, gap junctions, and neuronal and muscle electrophysiology. I suggest that our set of rules will go beyond what they have already learned for the resting potential and action potential. I stress that this set of rules is not simply going to be a longer list; rather, it will be a more general set of rules. I try to prepare them for the inevitable shock by comparing this material to any other topics they will encounter in medical school. Surely they understand that they will be learning about the human organism at a higher level than they have previously. In this context, I do not suggest that their views of ions simply crossing a membrane and unbalancing charges are incorrect, just that they are incomplete.

Despite the rare student who makes the remark, ''I thought I was done with physics when I left college,'' I find the use of equivalent circuits very effective. I use them earlier than many people, and I do some things with them that others do not. The result is a simple description of the membrane, current flows, and the associated voltage changes. There are no special conditions. It takes some work, however, by both the faculty and the students to achieve understanding. Once achieved, the students have a solid foundation in electrophysiology. There is a risk involved. Our goal is achieved mostly by mentally exercising with a few simple ideas. Medical students often prefer to memorize facts rather than work with concepts until they are comfortable with them. For this set of lectures, we have a review session in which students work through homework problems after every two lectures. They must think about the ideas. If they go only halfway, they will be worse off than if we had just given them a list of special conditions to memorize. Some of the books listed in the references have excellent problems for students to do, with answers. We modify these, and some of the faculty have made up their own problems over the years. Working through problems is how we force the students to work with the concepts.

USE EQUIVALENT CIRCUITS EARLY

My set of lectures starts with a discussion of the structure of membranes: the lipid bilayer and its role as a barrier to aqueous solutions, and channels in the membrane that are selectively permeable to particular ions. From here, we develop the idea of a diffusion potential. The typical theoretical or experimental setup is a chamber divided by a selectively permeable membrane (the concentration cell). Solutions containing the permeable ion are present on either side of the membrane, with a high concentration on one side and a low concentration on the other (Fig. 1). By the process of diffusion, the ion (e.g., potassium) will move from the high concentration compartment to the low concentration compartment until an electrical force develops to the point at which it opposes further diffusion. If the concentrations are specified (along with the temperature), the equilibrium potential of the ion (E_{ion}) can be calculated with the Nernst equation (for general textbooks, see Refs. 1, 4, and 5; for advanced textbooks, see Refs. 2 and 3)

$$E_{ion} = \frac{RT}{zF} \ln \frac{[ion]_{outside}}{[ion]_{inside}}$$

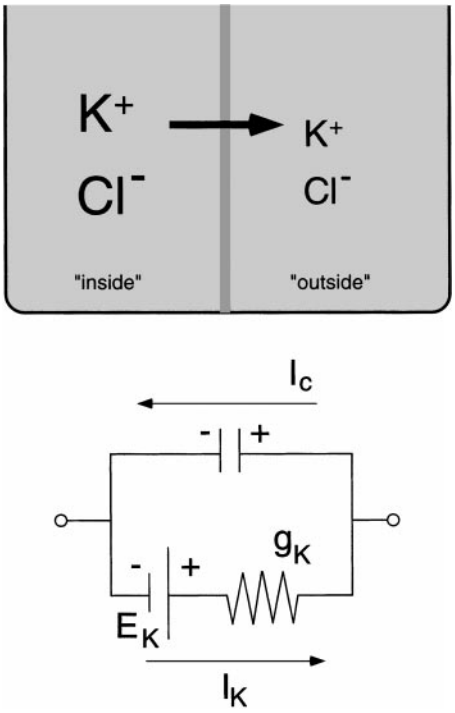

FIG. 1.

Use of an equivalent circuit to represent a simple diffusion chamber or concentration cell. *Top*: a typical theoretical or experimental apparatus consisting of 2 compartments separated by a membrane selectively permeable to potassium ions (K^+). A high concentration of potassium chloride (KCl) is present in the "inside" compartment, and a low concentration is present in the "outside" compartment. Here is where students begin thinking that K^+ simply moves from inside to outside (arrow). In fact, this movement is 1 part of a complete circuit of current flow. Cl^-, chloride ions. *Bottom*: an equivalent circuit used to reinforce the point that currents only flow in a complete circuit. Concentration gradient is represented as a battery (E_K), channels for K^+ are collected into a single resistor (g_K), and the membrane itself is drawn as a capacitor. Movement of K^+ across the membrane (I_K) is part of the current in the circuit. The other part of the current (until the potential across the capacitor reaches E_K) is a capacitive current (I_C). With this diagram students see that the outward K^+ current is matched by an inward current. By using a capacitor, they can keep track of the charges. At equilibrium, the inward and outward current components are both through the K^+ channel.

where $[ion]_{outside}$ and $[ion]_{inside}$ are the ion concentrations "outside" and "inside" of the compartments, R is the gas constant, T is absolute temperature in °K, z is the ion valence, and F is Faraday's constant.

Because the charges were initially balanced on each side of the membrane (each side in our example is a solution of potassium chloride), students readily see that the movement of potassium across the membrane unbalances both sides. Positive charges move from "inside" to "outside," so the inside becomes negatively charged and the outside becomes positively charged.

When we discuss sodium channels, the students see that exactly the opposite happens. Sodium moves down its concentration gradient from outside to inside through sodium-selective channels. This leaves unbalanced negative charges on the outside and adds unbalanced positive charges to the inside.

All this is largely a review for many students. My first attempt at altering students' perception of current flows and potential changes is at the chamber used to illustrate the diffusion potential. The intervention is simply to get them to develop an electrical representation of the components and events in the chamber. The concentration gradient is drawn as a battery. The students have already used the Nernst equation to calculate the battery's magnitude and sign. Next, we represent the set of channels as a resistor and show a vector for current flow from the positive pole of the battery (potassium is our diffusible ion) through the resistor. Some students in every group are satisfied at this point that the circuit diagram accurately depicts the chamber. The rest recognize that we do not yet have a complete circuit, and we finish the diagram by drawing a capacitor to represent the membrane. In the theoretical experiment in which ions are suddenly permitted to cross the membrane through channels, we can identify a current through the capacitor in our circuit that is exactly opposite the ionic current through the channels.

The use of an equivalent circuit at this point does two things that are critical for us to reconcile students' intuitive picture of a membrane with our later preaching that outward current depolarizes. First, it gets them to think in terms of circuits and not simply ions collecting in the solution on one side or another of a membrane. Put another way, the circuit impresses on students the fact that any inward current must be balanced (somewhere) by an equal outward current.

Second, by using a capacitor, students can visualize the charges actually going somewhere.

VOLTAGE DROPS ON "ISOLATED" CHANNELS

Sometime later in the course of the lectures, the membrane is represented as a collection of different types of selectively permeable channels. The variety of channels that gets discussed depends on the lecture and the audience. In the simplest case, the membrane is described as containing voltage-dependent channels for sodium and potassium ions. Students are comfortable with the membrane of a cell containing sodium and potassium channels having a membrane potential that lies somewhere in between the equilibrium potentials for each ion. Put another way, they see that a membrane separating solutions of sodium and potassium will sit at the potassium equilibrium potential if there are no sodium channels open (regardless of the number of open potassium channels), or it will sit at the sodium equilibrium potential if there are no potassium channels open. With some of each channel type open, clearly the potential lies somewhere in the middle. The parallel conductance equation expresses this as a straightforward average for the steady state

$$V_m = \frac{g_{Na}E_{Na} + g_K E_K}{g_{Na} + g_K} = \frac{\frac{g_{Na}}{g_K}E_{Na} + E_K}{\frac{g_{Na}}{g_K} + 1}$$

where V_m is membrane potential, g_{Na} and g_K are sodium and potassium conductances, respectively, and E_{Na} and E_K are sodium and potassium equilibrium potentials.

A parallel conductance model (Fig. 2) is used to represent these two channel types in an equivalent circuit. The circuit consists of three parallel branches, one for each ion type and a third for the membrane capacity. On each ionic branch, a resistor and battery depict the concentration gradient and channels for the particular ion.

By this time, students have heard that the membrane capacity does not pass current unless the potential across it is changing. I can put a potential across the entire circuit (e.g., resting potential), and students are comfortable that this is the potential across the capacitor. We can put some representative charges on the plates to keep ourselves oriented. Resting current flows are shown in the circuit through the sodium and potassium branches, and students recognize that the currents on each ionic branch are equivalent. The second critical intervention comes here. I make the point that the resting potential must be the potential across each branch of the circuit. We have seen that this is so across the capacitor. Each ionic branch consists of a battery, whose potential has already been

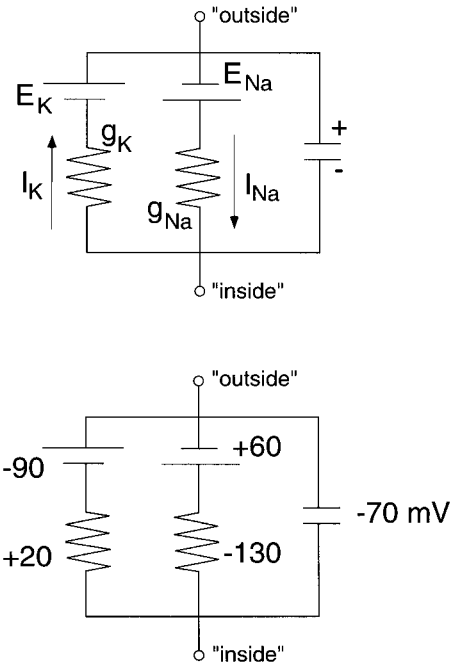

FIG. 2.

Use of an equivalent circuit to "isolate" inward and outward currents. *Top*: a simple equivalent circuit containing selectively permeable channels for sodium (Na^+) and K^+. If voltage across the circuit is stable (e.g., resting potential), current flows are entirely through the ionic branches (arrows). E_{Na}, Na^+ equilibrium potential; g_K, Na^+ conductance; I_{Na}, movement of Na^+ across membrane. *Bottom*: if numbers are used for resting potential (shown as potential across capacitor) and for E_{Na} and E_K, values for voltage drops on Na^+ and K^+ channels can be calculated. The inward Na^+ current produces a large voltage drop with the inside negative, and the outward K^+ current produces a small voltage drop with the inside positive. These values can also be used as a solution to the parallel conductance equation. Given that g = current (I)/voltage (V), if we take a value for I of 1, then $I_{Na} = -1$, $I_K = +1$, $g_{Na} = 1/130$, and $g_K = 1/20$. $V_m = [(60/130) + (-90/20)]/[(1/130) + (1/20)] = -70$ mV. The same basic procedure is followed for other conditions such as the peak of the action potential.

calculated, and a resistor, across which there is a potential drop that is calculated with some very simple arithmetic. The magnitudes of the voltage drops across the two channel types fit with students' understanding that the resting potential is closer to the potassium equilibrium potential than the sodium equilibrium potential because the potassium conductance is high and the sodium conductance is low. The magnitudes are also a solution of the parallel conductance equation.

The tricky part comes when we look at the signs of the voltage drops on each resistor. The sign of the voltage drop on each channel type is revealed by the arithmetic. The inward current on the sodium branch produces a voltage drop on the sodium channels, whose signs indicate that the inside is negative with respect to the outside. Likewise, on the potassium branch the sign on the voltage drop indicates that the inside is positive with respect to the outside. In other words, the inward sodium current produces a voltage drop across the sodium channels where the inside is negative with respect to the outside (the direction of hyperpolarization or repolarization) and the outward potassium current produces a voltage drop across the potassium channels where the inside is positive with respect to the outside (the direction of depolarization). Obviously, an isolation of channels in this way never occurs in biological membranes. Channels are scattered in the lipid bilayer, so there is no condition in which one can measure potential changes on any set of ''isolated'' channels. Such an experiment can, however, be done on paper, permitting us to really isolate the current components. This is by far the biggest leap of faith anyone has to make. Its value is that the directions of current flows are absolutely clear for each limb of the circuit and that the directions of the voltage changes associated with each of these currents are now also clear. Changes in the sizes of the currents or the conductances are easily correlated with changes in the sizes of the voltage drops on our ''isolated'' channels. The directions of the voltage drops (i.e., their hyperpolarizing or depolarizing effects on the membrane) will only change if the direction of the current flow changes. Because we are not controlling the membrane potential (e.g., in a voltage-clamp arrangement), nothing will change the *direction* of current flow.

We do everything again for the peak of the action potential. A common summary of the regenerative steps involved in action potential generation that students bring to the lecture is *1*) sodium channels open, *2*) sodium enters the cell, and *3*) the cell depolarizes to open more sodium channels. To look at this set of events with the model, we set the potential across the capacitor to the peak of the action potential. Students do the arithmetic to get the magnitude and sign of the voltage drops across the sodium and potassium channels (resistors). The arithmetic reveals that the magnitude of the voltage drop across the sodium channel is small but still negative inside and positive outside. Students understand that its smaller size is due to the higher conductance of the activated sodium channels. From the model or the parallel conductance equation, this point is reinforced as students see that, if the conductance for sodium becomes very high, the sodium conductance gets to be simply a wire (i.e., the resistance goes to zero) and V_m approaches E_{Na}. In comparison, the voltage drop across the potassium channel is relatively large. From the lower total resistance, we can confirm that the magnitude of the current in the circuit is increased. I remind them that the small size of the voltage drop across the sodium channels is also why, even though the effect of the inward current is in the hyperpolarizing direction, its direct contribution to the membrane potential is small. The real ''action'' takes place along the return paths.

The final step is to let current in one set of parallel branches leave for another set of parallel branches to model local circuit currents in an axon. This is where I talk about net inward or outward currents in particular patches of membrane. The outward current can be examined on each branch in the ''distant'' parallel circuit. I am intentionally *not* making a special distinction between active and passive regions of the membrane. It is not necessary. I explain what the terms ''active'' and ''passive'' mean, but the principles we have developed do not require us to distinguish active from passive current flows. Students can see the current changing the charge on the membrane capacity, or they can add the current to the resting currents on each of the ionic branches and do the arithmetic again. An outward current on the capacitive branch reduces the charge stored on the capacitor. An outward current on the potassium branch adds to the

resting outward potassium current, and the result is a larger voltage drop on the potassium channels. With this drop added to the potassium equilibrium potential, we see that the total potential difference on the potassium branch is less negative than that rest. On the sodium branch, the outward local circuit current adds to the resting inward sodium current to reduce its magnitude. The result of a smaller inward current on the sodium limb is a smaller voltage drop on the sodium conductance and a more positive total potential difference on the sodium limb. Any way you choose, the outward current is depolarizing. In the patch of resting membrane, where there is a net outward current, the membrane of the patch depolarizes. At this point, discussions of differences between action potentials in spherical cells and axons or the mechanism by which extracellular stimulators work serve to support rather than confuse.

Finally, because I raised the issue earlier, I will say a little about chloride channels. These are handled probably most efficiently by adding a term to the parallel conductance equation. In the equivalent circuit model, a chloride branch will look like a potassium branch and will drive current out across the membrane that will return on any or all of the other paths. This will increase the inward sodium current, decrease the outward potassium current, and contribute an inward current to the capacitive branch. Each of these actions works to repolarize the membrane.

ADDRESSING THE NEEDS OF STUDENTS AND FACULTY

In summary, I try to get students thinking in terms of complete circuits everywhere possible. When the current flows and voltage changes are considered in complete circuits, a consistency emerges in the voltage changes associated with current flows. This has been my most effective strategy for developing a general set of principles. It works. If students understand it, they will have a superior foundation in membrane physiology, one without special conditions or cases, and they can go far beyond what gets covered in the lectures.

I admit that it is difficult material for faculty to teach and difficult material for students to learn. Medical students can be very demanding and at the same time very creative. One student had me chasing the effects of single ions on membrane potential before he was satisfied. Equivalent circuits put ion movements into a useful and rigorous context. Used early and often, they can prevent some difficult conceptual problems. By first getting students to stop thinking about ions as simply crossing the membrane and more about circuits, and then by isolating the effects of current across particular channel types, the discussion of local circuit currents in propagating axons and the mechanism for extracellular stimulation will follow nicely from the concepts of ionic gradients and selectively permeable channels.

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