Action Potential Initiation and Conduction in Axons

J H Caldwell, University of Colorado, Aurora, CO, USA

Introduction

Transmission of electrical signals in biological systems operates under constraints imposed by the materials used to build the conducting pathways. The conduction medium in nerves is a membrane-enclosed dilute salt solution rather than a metal wire, for example, as in macroscopic physical systems. Axons have been compared for decades to undersea cables that are surrounded by salt water. However, the axon relies on a salt solution both inside and outside the axon. Since the resistivity of mammalian physiological saline is about 1 ohm-meter and that of copper is about 1.7×10^{-8} ohm-meter, the ease of current flow in 1 mm of an axon (a typical length constant in a large-diameter axon) is equivalent to that of a wire that is 170 km long. The difficulty of passive propagation of signals in an axon is compounded by two factors. First, the signal is carried by cell membranes that have a capacitance ($\sim$1 μF cm^{-2}) that needs to be charged to propagate a voltage change. Second, the membrane is inherently leaky due to a requirement for ion channels that are open to set the negative resting membrane potential. Thus, passive conduction of a signal in neurons is effective over distances of only a few millimeters. For this reason, it does not matter whether you are a worm or a whale: transmission of signals in axons requires a booster mechanism to replenish the decrementing electrical signal. The analogy between undersea cables and axons has other similarities and divergences. Undersea cables use repeater or booster stations that amplify the analog signal and send it on. The nervous system also uses a booster system but transforms the analog signal into a digital one (i.e., the continuously varying voltage signal becomes either 'on' or 'off'). This encoding is a trade-off that neurons accept for the sake of long-distance communication. The amplitude of the graded signal is transformed into the timing and frequency of action potentials.

Ionic Basis of the Action Potential in Axons

Sodium and Potassium Channels

The fundamental ionic mechanism of a propagating, regenerative increase in sodium conductance was described by Hodgkin and Huxley for the squid giant axon, and this process holds in both the central nervous system (CNS) and peripheral nervous system (PNS) of both vertebrates and invertebrates. The action potential is generated by the opening and subsequent inactivation of voltage-gated sodium channels and, with a slight delay, the opening of voltage-gated potassium channels. This ionic interplay of opening and closing of sodium and potassium channels is present in such diverse phyla that it must have evolved hundreds of millions of years ago. This conservation of the molecular foundation of the action potential has additional complexities that are not yet completely understood. In mammals there are 10 voltage-gated sodium channels (Nav1 and Nav2 families), and many of these are expressed in neurons and localized in axons. In addition, there are at least 20 voltage-gated potassium channels in vertebrates (Kv1 to Kv4 and KCNQ families). Thus, the molecular species and combinations of sodium and potassium channels utilized for conduction are potentially quite large. This suggests that characteristics of different axon types (e.g., frequency of firing and ability to maintain conduction) are dependent on the isoforms of sodium and potassium channels present in the axon.

Sodium–Potassium Pump

Although the immediate basis of the action potential is the activation of voltage-gated sodium and potassium channels, long-term support of the action potential requires sodium–potassium pumps to maintain the concentration gradients. It has been often emphasized that if the sodium pump is blocked (e.g., with ouabain) in a squid axon, hundreds of thousands of action potentials can still be generated because few sodium and potassium ions cross the membrane for each action potential. The squid axon illustrates the fact that the energy for ion flux (the concentration gradient of sodium and potassium between the inside and outside of the axon) has already been established by the pump. However, the squid giant axon is 1 mm in diameter, and when considering small-diameter axons that are less than a micrometer in diameter (e.g., pain and temperature fibers in the periphery or parallel fibers in the cerebellar cortex), sodium and potassium fluxes during each action potential are significant. For these small-diameter axons, the ability to maintain action potential firing is highly dependent on sodium pump activity.

Structural and Functional Differences of Vertebrate Axons

Axons fall into two major categories depending on the structure of the glial cells that envelop them. The first category is the unmyelinated axon, which describes all invertebrate axons and small axons of vertebrates, typically axons with a diameter below 1 μm. The unmyelinated axon is usually loosely surrounded by a glial cell or in some cases, such as parallel fibers in the cerebellar molecular layer, is not covered by a glial cell. The speed of conduction of an action potential in an unmyelinated axon is proportional to the square root of the axon diameter. Thus, invertebrates have large-diameter axons for signals that need to be propagated rapidly. The squid giant axon, which is part of a circuit used for rapid propulsion in the escape response, is as large as 1 mm in diameter, conducts at $25\,m\,s^{-1}$ (at 25 °C), and is formed by the fusion of axons of many neurons.

Vertebrates have evolved an alternative strategy for increasing the speed of action potential conduction. Vertebrate axons larger than about 1 μm are tightly wrapped by many layers of the glial cell, creating the second category, the myelinated axon. Myelination occurs in a repeating pattern, with long wrapped regions (internodes that are up to 1–2 mm in length) interrupted by a very short bare region (the node of Ranvier, 1–2 μm in length). In the PNS the glial cell is a Schwann cell. Each Schwann cell can envelop many unmyelinated axons, but when myelination occurs, one Schwann cell is devoted to the formation of one myelinated internode. Myelin in the CNS is formed by oligodendrocytes, and one oligodendrocyte sends out tens of processes, each one forming an internode on a different axon. Functionally, the myelin acts as an insulator, by reducing the leak of current through the membrane in the internodal regions. The myelin also reduces the effective capacitance of the internodal region, which in turn reduces the capacitive current required to charge the internodal membrane. Consequently, current travels rapidly with little loss in the internodal regions to the node of Ranvier, where the current is boosted or regenerated by voltage-gated sodium channels concentrated at the node (described later). Conduction velocity in myelinated axons is proportional to the axon diameter, and the general rule of thumb is that for axons with an outside diameter greater than 11 μm, the speed of conduction, in meters per second, is about six times the axon diameter, in micrometers. For smaller axons the proportionality factor is 4.5. An axon 20 μm in diameter, which is one of the largest in the mammalian nervous system, conducts at $120\,m\,s^{-1}$, about five times faster than the squid axon, even though it is 50 times smaller. Thus, myelination not only increases speed of conduction, but also does this with an economy of space. This concept of economizing the volume used for conduction is invoked to explain why we have many more unmyelinated axons than myelinated ones: more information can be carried in a given volume with small, unmyelinated axons. In the mammalian nervous system, pain and temperature information is carried by small, unmyelinated axons in the PNS, and the molecular layer of the cerebellum in the CNS is densely packed with parallel fibers that are unmyelinated axons of granule cells.

Initiation of the Action Potential

Stimulation Required for Electrogenesis

A rapid membrane depolarization is necessary to open voltage-gated sodium channels and start the action potential. This depolarization can be achieved artificially by inserting a microelectrode into an axon and injecting current, by extracellularly stimulating an axon with an electrode, or even by mechanically hitting the nerve – for example, when we hit our 'funny bone' (the ulnar nerve near the elbow). Naturally produced depolarizations fall into two categories. For most neurons, synaptic input to the dendrites and cell body provides the required depolarization. Sensory neurons, such as stretch receptors in muscle or cutaneous receptors in the skin, propagate action potentials centrally; a sensory signal in the periphery creates a depolarization called the receptor or generator potential that opens sodium channels to produce action potentials.

Site of Initiation

Unmyelinated axons The simplest treatment of a neuron separates it into three regions: soma, dendrite, and axon. The elementary concept of a passive dendritic tree that simply receives excitatory and inhibitory synaptic inputs and sends these inputs to the cell body, where they are summated, is now known to be an oversimplification. Many neurons have voltage-dependent sodium and calcium channels in dendrites. However, only in rare cases can dendrites initiate action potentials that are propagated orthodromically to the soma. Thus, the integration of excitatory and inhibitory potentials takes place at the soma. The soma contains a variety of voltage-gated channels, but sodium channel density is highest at the axon hillock, an enlargement of the axon at the point it leaves the cell body (Figure 1). For example, sodium channel density is over sevenfold higher on the initial segment of a neurite (presumptive nascent axon),

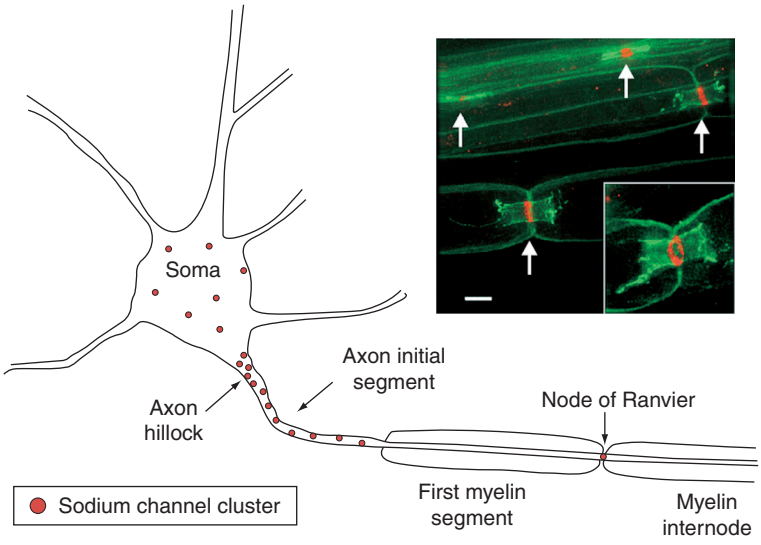

Figure 1 Sites of action potential initiation and sodium channel clustering. Action potentials begin at the point of lowest threshold, which is a function of the balance between sodium, potassium, and leak currents. In general, the action potential originates where sodium channels are clustered at high density, and for both unmyelinated and myelinated axons this is usually at the axon hillock. In myelinated neurons initiation can also occur in the initial segment or at the first node of Ranvier, where sodium channels are highly concentrated. Inset: Four nodes of Ranvier (arrows) in a teased sciatic nerve with nodal labeling by an anti-Nav1.6 antibody (red) and paranodal labeling with an anti-Caspr antibody (green). The node shown at higher power within the inset is tilted such that the labeling of the entire circumference of the nodal membrane can be seen. Scale bar = 5 μm (3.3 μm for the tilted node). (Inset) Reproduced from Caldwell JH, Schaller KL, Lasher RS, et al. (2000) Sodium channel Na(v)1.6 is localized at nodes of Ranvier, dendrites, and synapses. *Proceedings of the National Academy of Sciences of the United States of America* 97: 5616–5620.

compared to the soma in cultured spinal cord neurons. The higher density at the axon hillock has been confirmed with immunolabeling of neurons in many regions of the CNS. The main consequence of the increased sodium channel density at the axon hillock is that the threshold for action potential initiation is lowest there. Thus, for unmyelinated axons, action potential initiation takes place at the axon hillock.

Myelinated axons The site of action potential origination in myelinated axons was also shown to be the axon hillock about 50 years ago. More recently, with improvements in the ability to measure voltage changes optically and with patch clamp electrodes, the precise location of the origin of the action potential has been identified. Action potentials can originate not only at the axon hillock, but also in the axon initial segment, 30–40 μm from the soma and close to the first myelinated segment. In some neurons the action potential even originates at the first node of Ranvier, where sodium channels are highly concentrated (Figure 1). For both myelinated and unmyelinated axons, once the action potential begins in the axon, it not only propagates orthodromically toward the nerve terminals but also propagates antidromically, back into the soma and dendrites.

Conduction in Unmyelinated and Myelinated Axons

Unmyelinated Axons

Before considering a propagating action potential, it is useful to understand the currents that underlie a stationary action potential. It is possible to control the membrane potential experimentally along the length of an axon. In this case, a short stimulus current can be applied to bring the entire length of membrane to threshold, and the whole axon subsequently and simultaneously undergoes an action potential. Once the applied stimulus is over, total membrane current in this artificial situation is zero. Since membrane current is the sum of capacitive and ionic currents, ionic and capacitive currents are equal and opposite. Ionic currents through sodium and potassium channels simply change the membrane potential by charging membrane capacitance. For a propagating action potential, the relationship between ionic and capacitive currents is more complex (Figure 2). The reason for this complexity is that once the action potential is initiated, either by transduction of a sensory stimulus or by summation of postsynaptic potentials, sodium current in the active region not only depolarizes the active region further, but also provides depolarizing

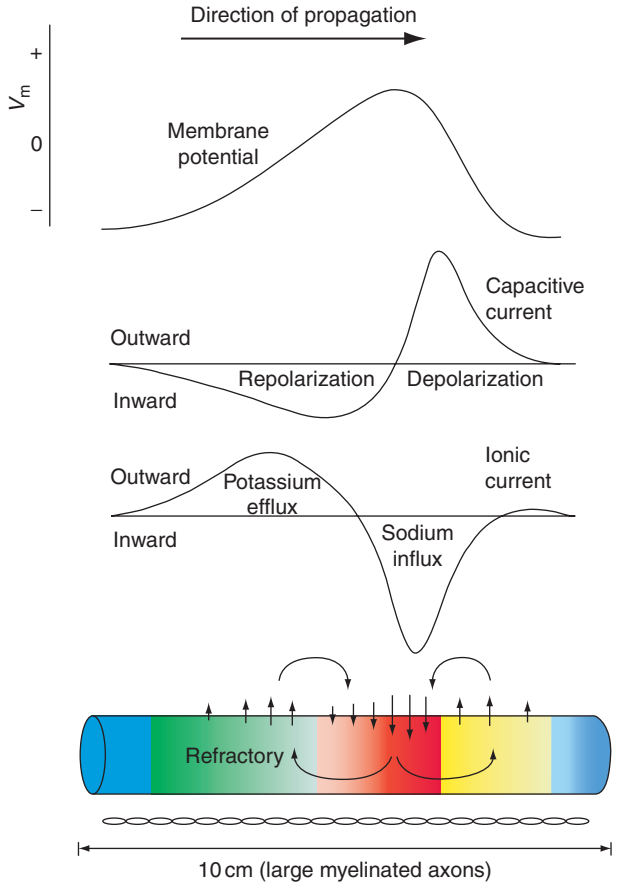

Figure 2 Ionic and capacitive currents that underlie a propagating action potential. The membrane potential change of an action potential propagating from left to right is illustrated at the top. Capacitive and ionic currents are schematically shown below the membrane potential and are drawn to illustrate the major relationships. Since capacitive current is proportional to the first derivative of the membrane potential, peak capacitive currents occur at the maximum slopes of depolarization and repolarization, and capacitive current is zero at the peak of the action potential. Total membrane current (the sum of capacitive and ionic currents) is proportional to the second derivative of the membrane potential and is zero at the maxima of the capacitive current where total current changes between inward and outward (yellow to red boundary and red to green boundary in the schematic of the axon at the bottom). For an action potential propagating at a constant velocity, the scale bar at the bottom can be thought of as time at a fixed point on the axon (typical action potential duration is 1 ms) or as distance over which the action potential is occurring at one instant of time (10 cm for an action potential with a duration of 1 ms and conduction velocity of 100 m s^{-1}). A series of myelinated nodes is shown above the scale bar to illustrate that many nodes participate at any given time. For the fastest conducting axons, there would be five times as many nodes as are illustrated here.

current to the adjacent region of the axon at the leading edge of the action potential to bring it beyond threshold (Figure 2). The depolarization at the leading edge of the action potential is primarily a capacitive current until threshold is reached. Current also spreads longitudinally behind the action potential, but an action potential is not created in the retrograde or backward direction because of the residual changes in the state of potassium and sodium channels. Potassium channels are still activated and are holding the membrane potential near the resting potential while sodium channels in this region are still recovering from the depolarization; they are inactivated, and this part of the axon is temporarily refractory to action potential generation.

The conduction velocity of unmyelinated axons depends on how much current is injected into the axon by the sodium channels, how far the current can spread longitudinally, and how quickly the adjacent membrane can be brought to threshold. The amount of current depends on the density of sodium channels. Since more sodium channels provide more current, one might think that an increase in channel density will always increase conduction velocity. This proportionality is valid only for low to moderate sodium channel densities because the channels act as dipoles (the source of their voltage sensitivity) and add additional capacitance to the membrane. The time required to charge the membrane is the product of the specific membrane resistance and capacitance. At very high channel density, the effect of the added capacitance outweighs the additional current provided because it takes longer to charge the membrane and conduction velocity is decreased. Typical sodium channel density of unmyelinated axons is 50–500 channels μm^{-2}, with potassium channel density about tenfold lower. An especially low density (2–3 channels μm^{-2}) has been reported in garfish olfactory nerve and neonatal rat optic nerve.

Channel subtypes In general, all neurons express multiple subtypes of sodium and potassium channels. All channel subtypes in the Nav1 sodium channel family have the basic features described by Hodgkin and Huxley over 50 years ago; they are activated by depolarization, with subsequent inactivation that is removed when the membrane is repolarized. Ion selectivity seems to be the same for all the subtypes, but the details of voltage dependence, the kinetics of opening and closing, and the modulation of these gating properties vary from one subtype to another. The multiplicity of potassium channel subtypes is much greater than that of sodium channels, and their properties are also more variable.

There is evidence that neurons use different channel subtypes in subcellular regions of the cell. This would allow the cell to fine-tune the excitability of the cell in different regions. The expression and targeting of different sodium and potassium channel

proteins to the unmyelinated axon remain an active area of research. Some sodium channel subtypes in the PNS, such as Nav1.8 and Nav1.9, seem to be predominantly expressed in small dorsal root ganglion neurons and are targeted to unmyelinated axons. Parallel fibers in the cerebellar cortex utilize Nav1.6.

Myelinated Axons

The current available for depolarizing the next axonal segment to threshold is dependent on the loss of current through the membrane (its leakiness) and the decrease due to capacitive current required to charge the membrane and change the membrane potential. The number of wraps of the axon by the myelin and the length of the myelin internode have important electrical consequences for both the ionic and the capacitive currents. Since the extracellular fluid between each wrap is squeezed to a negligible volume and since the cytoplasm is also squeezed out of the glial wraps, the axonal membrane is essentially increased in thickness by the myelin membranes. The number of wraps by myelin varies from a low of about 10 to as many as 150, with each wrap consisting of a pair of membranes. For example, a large myelinated axon with 150 wraps will decrease ionic current loss through the internodal membrane by a factor of 300. Because capacitance is inversely related to the distance between the charged surfaces (in this case the thickness of a membrane), capacitance and capacitive current will also be reduced 300-fold. The current thus moves rapidly in the internode, with little loss through the membrane or in charging the membrane, essentially jumping from one node to the next. This is described as saltatory conduction. Each node acts as a booster station to ensure propagation to the next node, and to accomplish this regeneration of the signal, sodium channels are highly concentrated at each node (2000–3000 channels μm^{-2}) and are about 100-fold lower in density in the internodal membrane.

Increasing the internodal distance increases the speed of conduction because the current is jumping farther. However, there is an optimal internodal length. If internodal distances were to become very large, conduction velocity is predicted to decrease. This decrease in velocity is due to the loss of current in the internodal region, slowing the rate of rise of depolarization at the next node. Internodal distances are found to be about 100-fold greater than the axon diameter (in agreement with internodal distances predicted to optimize the conduction speed) and range from a few hundred micrometers to 1–2 mm.

The mental image of an action potential occupying a single node and hopping from one node to the next is a common misconception. Although the action potential is jumping from one node to the next at the leading edge, many nodes are simultaneously participating. The extent of axons actively involved in the action potential is dependent on the speed of conduction. The fastest conducting myelinated fibers have a speed of 100–120 $m\,s^{-1}$. If the action potential duration is 1 ms, an action potential traveling at 100 $m\,s^{-1}$ will, at a given instant of time, occupy 10 cm of the axon, or approximately 100 nodes, since the internodal length is on the order of 1 mm for large-diameter axons (Figure 2).

A measure of the reliability of conduction is called the safety factor, which is defined as the current in excess of that required to reach threshold and maintain propagation. A safety factor of 2 means that the current generated by the sodium channels is twice the minimum needed for conduction. Axons have a safety factor of about 5, and this excess is important because it speeds conduction (allowing the membrane to reach threshold faster) and provides the extra current needed at branch points. Axons branch hundreds of times, each branch imposing an increased load on the current provided by the upstream axonal membrane. If several branches occur close together, conduction can fail in some branches, especially during high-frequency firing. For similar reasons, additional current is also needed at the synaptic terminal where additional membrane must be depolarized. The internodal distances in motor axons decrease as the synaptic terminal is approached, and in some cases are as short as 10–20 μm. The effect of decreasing internodal distance is to concentrate nodes of Ranvier near the synaptic terminal, to provide the necessary current for terminal depolarization. It is not known if the terminals have sodium channels, since immunolabeling with antibodies specific for voltage-gated sodium channels have failed to show this.

Channel subtypes Many subtypes of sodium channels can be targeted to nodes of Ranvier, and during development several subtypes are found at neonatal nodes. In the adult mammal almost all nodes of Ranvier in the PNS and CNS contain predominantly one subtype, Nav1.6. The switch between neonatal and adult subtypes at the node coincides with the formation of compact myelin. Three types of potassium channels have been identified pharmacologically (inward rectifier, slow outward rectifier, and 4-aminopyridine-sensitive channels) and attributed primarily to internodal membrane. Subtype-specific antibodies have shown that Kv1.1, Kv1.2, and Kv1.4 are present in the internodal region, with the highest density in the juxtaparanodal region at the boundary

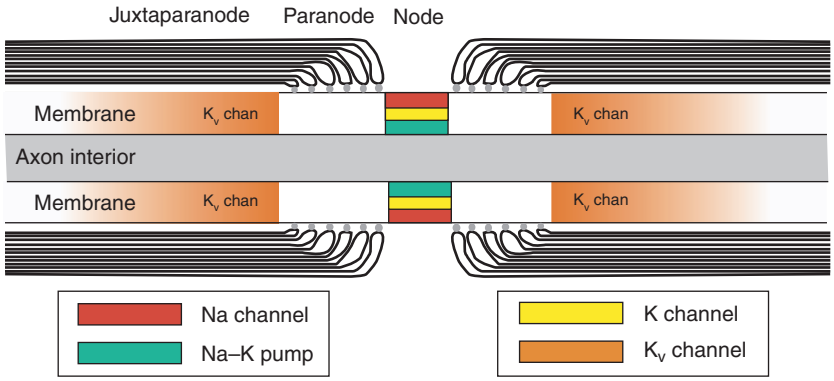

Figure 3 Ion channels and pumps concentrated in the vicinity of the node of Ranvier. Saltatory conduction in myelinated nerves is dependent on a high concentration of sodium channels at the node of Ranvier, to provide the inward current needed for depolarization of the next node. Repolarization is accomplished not only by inactivation of the sodium channels but also by a high resting potassium conductance at the node (KCNQ potassium channels) and by voltage-gated potassium channels excluded from the node and concentrated in the juxtaparanodal region near the node. Sodium–potassium pumps are concentrated at the node to maintain the concentration gradients. The thickness of the membrane (5 nm) is highly exaggerated relative to the axon diameter (>1 μm).

of the paranode. In addition, there is a high resting potassium conductance at some nodes, and a high concentration of KCNQ2 and possibly KCNQ3 potassium channels is co-extensive with the high concentration of sodium channels at the node. These separate highly aggregated clusters of channels are illustrated in Figure 3. As mentioned earlier, the flux of sodium and potassium ions needed to charge and discharge the membrane for each action potential is small, but maintenance of the ion gradients is dependent on sodium–potassium pumps. These pumps are highly concentrated in the nodal membrane.

Summary

The essential features of action potential initiation and propagation were determined over 50 years ago. Research into the electrical excitability of neurons is, however, far from moribund. Recent advances in molecular biology have revealed a multiplicity of sodium and potassium channel subtypes in neurons. Subtle changes in the activation, inactivation, and kinetics of voltage-gated sodium and potassium channels are predicted to have large effects on action potential threshold and rate of firing. The subcellular placement of specific isoforms and the modulation of these isoforms are also critical parameters of neuronal excitability. Many basic questions at the cellular and subcellular level remain. What is the lifetime of these channels in different regions, such as the axon hillock, initial segment, or node of Ranvier? Are there intracellular pools of the channels that can be rapidly inserted to provide plasticity at the level of conduction? What are the signals that target channels to the nodal region and adjacent paranodal and juxtaparanodal regions of myelinated axons? How does the cell achieve a balance between channel synthesis and degradation in the cell body, as well as insertion and retrieval at specific sites such as the node of Ranvier? How is the distribution of channels established during development? Maintenance of electrical excitability during adulthood is a process of continual remodeling that requires constant feedback with signals from target cells and interactions with glial cells. These molecular signals and interactions remain unknown.

See also: Demyelinating Diseases; Demyelination and Demyelinating Antibodies; Ion Channel Localization in Axons; Myelin: Molecular Architecture of CNS and PNS Myelin Sheath; Schwann Cells and Axon Relationship; Sodium Channels; Voltage Gated Potassium Channels: Structure and Function of Kv1 to Kv9 Subfamilies; Voltage-Gated Potassium Channels (Kv10–Kv12).

Further Reading

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