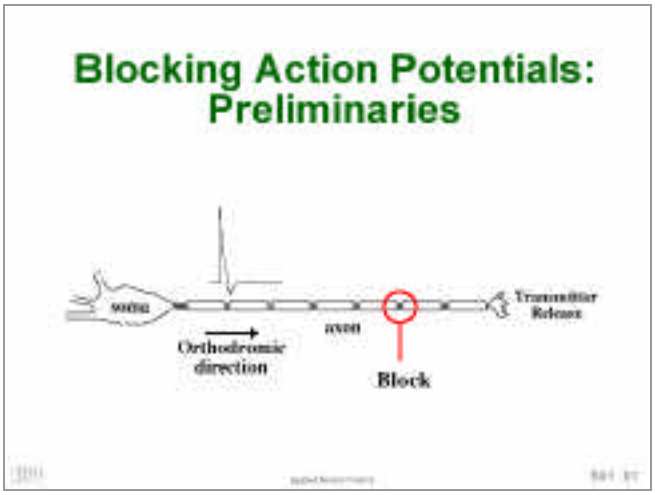
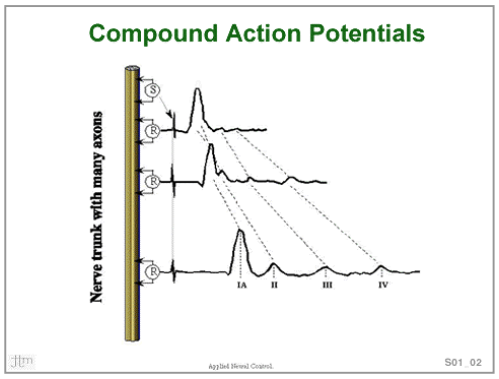


Introduction

In some clinical applications, it is desirable to arrest or block propagating action potentials and eliminate the possibility of neurotransmitter release from the axons. **Action potentials** are propagating signals that are transmitted by neurons and can be initiated by natural or artificial inputs to their neuronal membrane. When the initiating signal causes a change in the local transmembrane potential a self-propagating depolarization signal can be generated. This self-propagating action potential signal, which is an active process by entry of Sodium through **ion channels** in the membrane, travels along the length of the axon.

The conduction of this signal can be prevented by rendering a section of the axon unresponsive to this traveling wave of depolarization. Bio-toxins like Tetradotoxin and some local anesthetics like Lidocaine do this by binding to the Sodium channels. These chemical methods cannot be reversed quickly and can have wider responses on other organ systems. Manipulating the excitability of the membrane ion channels through applied electric fields or altering the temperature may enable one to rapidly induce and reverse block of action potential propagation.

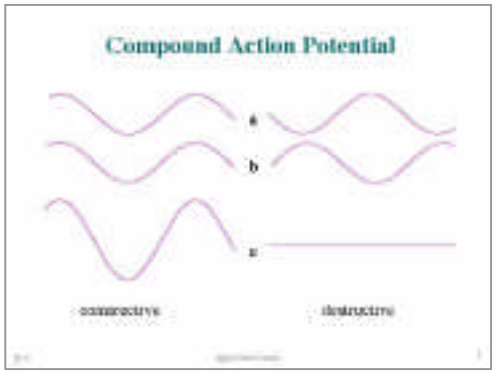
Compound Action Potentials: Problems when Testing for Block



The compound action potential is often use as an indicator of neural transmission and can yield erroneous interpretations. A **compound action potential** (CAP) is a signal recorded from a nerve trunk made up of numerous axons. It is the result of summation of many action potentials from the individual axons in the nerve trunk. A CAP may be initiated on a peripheral nerve by an electrical stimulus applied to the nerve at some point at a distance from the recording site. The latency between the application of the stimulus and the onset of the compound action potential is a function of the distance between the recording site and

the site of stimulation. In the figure, the initial biphasic spikes are the signal artifact from the stimulator (S). As the recording site (R) is moved further away from the stimulation point, the features of the CAP change. The different **conduction velocities** of the axon population results in a shift in time of arrival for the amplitude peaks as the recording site moves further from the stimulation site.

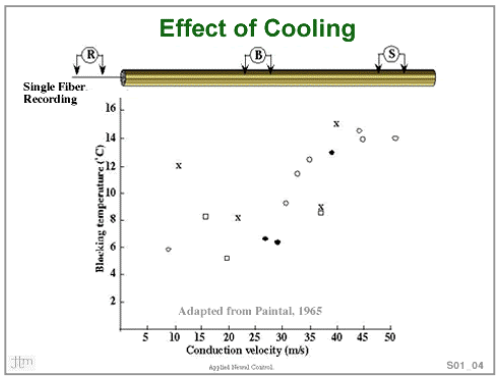
Cooling a nerve and the application of ac and dc electric currents have been reported to effect "block" of large fibers before small nerve fibers. These conclusions were based on the changes in the compound action potential where the high amplitude short latency signal in the compound action potential was observed to diminish, before the long latency signal was affected, as the magnitude of the "blocking" agent was increased. Many investigators interpreted these findings to mean that when a specific peak in the CAP became smaller after cooling, ac or dc potentials were applied that they had effected selective block of a population of fibers, usually large nerve fibers. Subsequent to these observations other investigators reported slowing of the propagation velocity by varying amounts as an action potential passed through the so called "blocking" zone, which gives rise to slight variations in the time of arrival of the action potentials at a recording electrode. Such a slight change can give rise to diminished recorded signal and the illusion that a particular population was blocked.



Summation of Action Potentials

The amplitude of the recorded compound action potential is a summation of the individual action potentials from the different axons. When the waves pass the recording site in phase they add constructively and display a higher peak. On the other hand, when they are out of phase, they add destructively. In the figure, waves 'a' and 'b' add to produce 'c'. In the left panel constructively when in phase, and in the right panel, destructively when out of phase. As a result, action potentials could be traveling on a number of peripheral nerve fibers and no compound action potential be recorded if they sum destructively or if timing differences do not permit constructive summation. This latter point is important to keep in mind when drawing conclusions about 'block' based on the amplitude of a compound action potential.

Effect of localized Nerve Cooling on Compound Action Potentials.



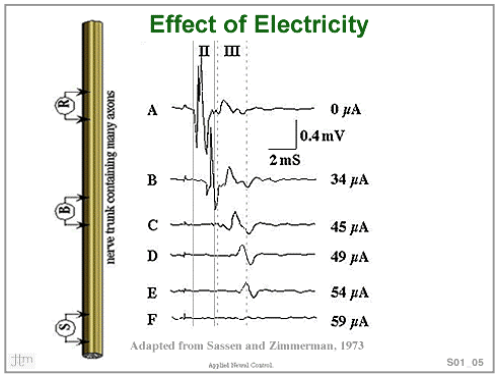
In clinical studies, nerve conduction velocity is considered to decrease by roughly 2 m/s per °C of cooling. Decrease in nerve conduction velocity have been observed following cold water immersion [1]. A positive correlation has been observed between decrease in surface temperature and decrease in nerve conduction velocity of 1.7 m/sec for every 1°C decrease in surface temperature [2].

There have been conflicting results reported on the blocking effect of cooling . Taken together, some of the data suggested that smaller diameter axons were blocked at lower temperatures than larger axons subjected to the same temperature. During cooling, the latencies increase slowly and then rapidly before conduction was blocked. Lowering of conduction velocities destroyed components of the CAP leading to the impression that block was occurring. Single fiber recordings revealed that conduction velocities slowed before block occurred and the blocking temperature was between 3°C and 9°C for all healthy fibers, higher if the nerves were stretched or injured [3].

Lowering the temperature of a nerve might block conductions because gating ion channels involves conformational changes in the protein and the rates at which these conformational changes take place are voltage and temperature sensitive. The Q_{10} for the rate constants are reported to be 2 to 4, meaning that the rate changes by a factor of 2 to 4 for each 10˚C change in temperature [4]. So if ΔT is -25˚C then $Q_{\Delta T} = (Q_{10})^{\Delta T/10} = 0.06$ or about 16 times slower (assuming $Q_{10}=3$).

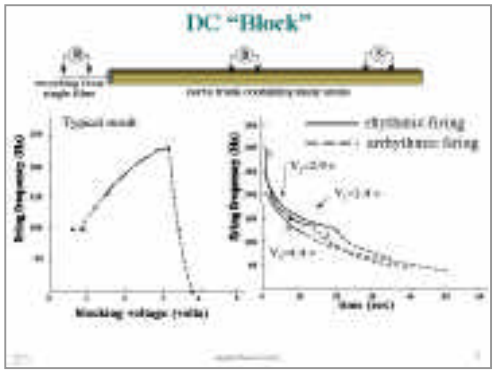
[1.] Abramson DI, Chu LS, Tuck S Jr, Lee SW, Richardson G, Levin M. (1966) Effect of tissue temperatures and blood flow on motor nerve conduction velocity. **JAMA.** 198:1082-1088.
[2.] Halar EM, DeLisa JA, Brozovich FV. (1980) Nerve conduction velocity: relationship of skin, subcutaneous and intramuscular temperatures. **Arch Phys Med Rehabil.** 61(5):199-203.
[3] Paintal, A.S. (1965) Block of conduction in mammalian nerve fibers by low temperatures. **J. Physiol. 180** (1) 1-19.
[4] Hille, B. (2001) **Ion Channels of Excitable Membrane** (3rd Edition). Sunderland, Mass. Sinauer Associates, Inc.

Effect of Currents on Compound Action Potentials.



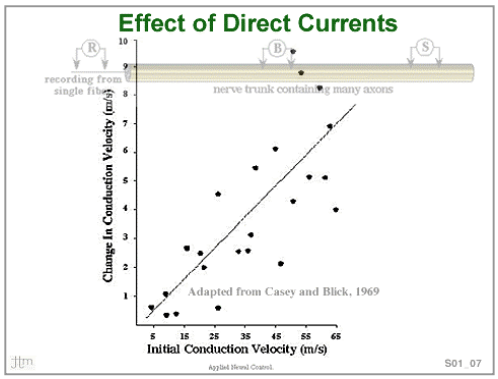
Sassen and Zimmerman [1] recorded (R) compound action potentials during nerve trunk stimulation (S) while applying an electric current at the blocking site (B). The changes in the Compound Action Potential (CAP) with increasing current strengths is shown in the figure. As the current increases, the amplitude of the primary peak diminishes and the arrival of the CAP is delayed. Although a conclusion could be made that some action potentials are blocked, the effect could be due to destructive summation of differentially delayed action potential peaks.

'A' shows the CAP recorded from the Sural Nerve; II and III label the components from the Group II and III fibers. 'B-F'- Recording of the CAP with application of polarizing currents of strength shown on right at the electrodes at B. Conduction distance of 58 mm.



Campbell and Woo [2] explored the effect of imposing a DC "blocking" stimulus (B) between a stimulating electrode (S) and a recording electrode (R). They used two recording sites, one on the peripheral nerve (recording a compound action potential; not shown in the figure) and one on a dorsal root filament (recording a "single" fiber response). They found that fast conducting action potentials were successively reduced in the compound action potential as the DC "block" amplitude was increased. They also noticed the base line of the peripheral nerve recording appeared noisier than when the DC "block" was off. Recordings from the dorsal root filament showed that the effect of the DC "block" was to cause repetitive firing of the nerve fiber that lasted for 10's of seconds, yet single stimuli could still come through the "blocking" region. After some time, 10's of seconds, true block could be effected but the compound action potential was not a reliable indicator of conduction block.

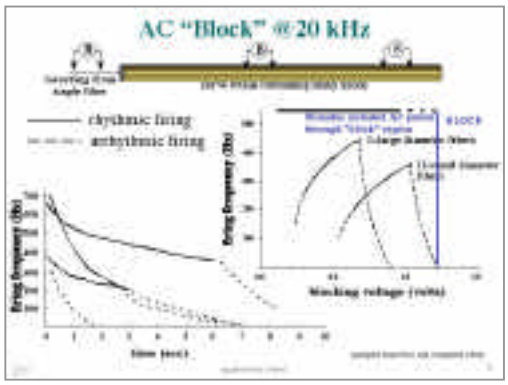
'DC' Block



Casey and Blick [3] have shown that anodal polarization of peripheral nerve causes a decrease in the conduction velocity when action potentials propagate through the "blocked" region. The decrease is fiber diameter dependent and can be fitted to a curve that shows the change is approximately 10% of the original velocity.

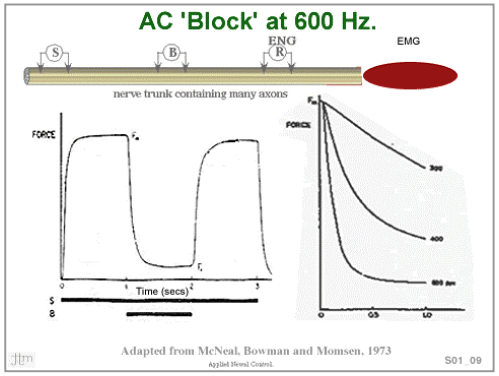
[1] Sassen, M. and Zimmerman, M. 1973 Pflügers Archive **341**, 179-195.
[2] Campbell and Woo, (1966) **Bul. of the Los Angeles Neurological Societies**, Vol 31, No. 2, pp 63-71
[3] Casey and Blick (1969) **Brain Research**, vol 13, pp155-167

Blocking Action Potentials with Alternating Currents.



Woo and Campbell [1] explored the effect of imposing an AC "blocking" stimulus (20 kHz) between a stimulating electrode (S) and a recording electrode. They used two recording sites, one on the peripheral nerve to record compound action potentials (B, not shown in the figure) and one on a dorsal root filament to record "single" fiber responses (R). They found that fast conducting action potentials were successively reduced in the compound action potential as the AC "block" amplitude was increased. They also noticed the base line of the peripheral nerve recording appeared noisier than when the AC "block" off. Recording from the dorsal root filament showed that the effect of the AC "block" was to cause repetitive firing of the nerve fiber and that single stimuli could still come through the "blocking" region. However, after some time true block could be effected but the compound action potential was not a reliable indicator of conduction block.

'AC Block'



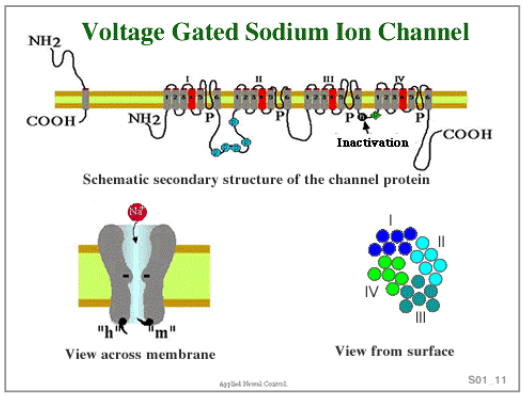
McNeal et al. [2] working on the peripheral motor nerve serving medial gastrocnemius, employed two recording sites, one on the peripheral nerve (recording a compound action potential) and one in the muscle (recording EMG). An "AC "blocking" stimulus was applied to the peripheral nerve distal to the nerve recording electrode and a stimulating electrode was placed proximal to the "blocking" electrode. Their results show that "blocking" stimuli of 600 Hz and greater, yield similar results and that the "block" is occurring at the neuromuscular junction, (probably because of transmitter depletion) and not at the site of the "blocking" electrode. The "block" is not complete because the force does not go to zero. Occasional action potentials must be transmitted across the junction.

Caveat!

Beware that in studies employing maximal muscle force as an indicator before the "block" stimulus is turned on, the true effect of the "block" stimulus may be masked, i.e. a large burst of activity that occurs at the initiation of the "block" cannot cause an increase in muscle force and therefore obscures a transient increase in nerve activity.

[1] Woo and Campbell (1964) **Bul. of the Los Angeles Neurological Societies**, Vol 29, No. 2, pp 87-94
[2] McNeal, Bowman, and Momsen (1973) **Advances in External Control of Human Extremities**, Yugoslav Committee for Electronics and Automation, Belgrade, pp 35 - 46

The Voltage Gated Neuronal Sodium Ion Channel



Sodium Channels play a central role in the transmission of action potentials along a nerve and are prime targets for methods to block propagation. Sodium channels are part of a family of proteins that traverse the cell membrane and form controlled conduits for the exchange of ions between the intra-cellular and extra-cellular fluid compartments. These channels are characterized by their voltage-dependent activation, rapid inactivation, and selective ion conductances.

The **Voltage Gated Sodium Channel** protein consists of four homologous domains (I–IV), each with six transmembrane segments (S1–S6, Schematic in upper part of Figure). Single segments and domains are connected by intracellular and extracellular loops. The hydrophobic S4 segment is found in all voltage gated ion channels and is absent in ligand gated channels. The four domains are arrayed in the membrane to form a central pore of ~ 3 to 4 Angstroms (Schematic in lower part of Figure). Loops between the S5 & S6 segments form an ion selective filter. The linker between III & IV domains forms the inactivation gate.

The Voltage Gated Sodium Channel can be in different functional states; (1) a resting state when it can respond to a depolarizing voltage changes (2) activated, when it allows flow of Sodium ions through the channel, (3) Inactivated – when subjected to a suprathreshold potential the channel will not open to allow sodium ions to pass through it.

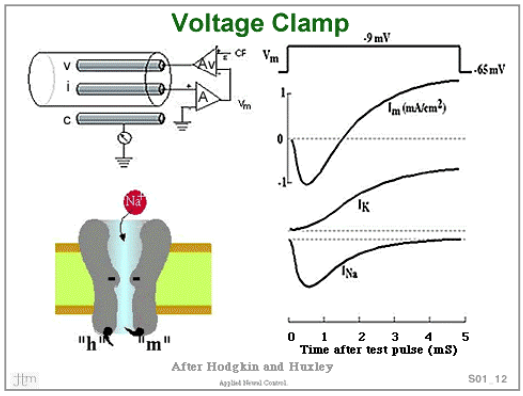
Historical Note: Because the lipid bilayer of the cell membrane creates a barrier for passage of the water-soluble ions, William **Bayliss** and Ernst **Brücke** had postulated the existence of pores in the cell membrane. John **Langley** formulated the idea of 'receptor molecules' on the cell membrane. In the **1940**'s, Kenneth **Cole** developed the 'voltage clamp' technique and studies by Alan **Hodgkin** and Andrew **Huxley** established the role of Sodium and Potassium ions in action potential propagation. This led to the formulation of the Hodgkin-Huxley mathematical models in the **1950**'s. In the next decade, **Tetradotoxin**, a biotoxin was found to prevent Sodium transfer across the membrane, pointing to a ion selective pathway. In the seventies, Hladkey **and Hayden** demonstrated that the antibiotic Gramicidin could form channels in a lipid bilayer, giving rise to step changes in membrane current. **Hille** formulated the idea of a ion selective 'filter' in the channel. The introduction of the 'patch - clamp' technique by **Neher and Sakman** enabled current measurements from single channels. Recombinant DNA technology led to the isolation, cloning and sequencing of the VGSC by **Noda** in 1984. An x-ray crystallographic study of a Potassium ion channel was published by **Doyle** in 1998.

Review Articles:

From Ionic Currents to Molecular Mechanisms: The Structure and Function of Voltage-Gated Sodium Channels. Catterall, W. A. (2000) Neuron, Vol. 26, 13–25.

Structure, Function and Pharmacology of voltage-gated sodium channels. Denac, H. et. al. (2000) Arch. Pharmacol. 362: 453-479.

The 'Sodium' Current.



In an activated state, the Sodium Channel permits Sodium ions to flow into the axon along its electrochemical gradient, giving rise to the 'Sodium' current. These transmembrane currents can be measured by the '**Voltage Clamp**' technique. The membrane potential is monitored with a voltage amplifier (Av) between an electrode inside the axon (v) and ground. The membrane potential (Vm) is supplied to a feedback amplifier (A), which compares it to a 'command' voltage to control a current output to a second electrode inside the axon (i). By monitoring this current from an external electrode (c), a measurement is obtained of the current flowing through the membrane

Hodgkin and Huxley carried out voltage-clamp experiments that led to an understanding of how changes in ionic currents through the membrane gave rise to a propagated action potential. They also formulated a mathematical model to simulate the process. Hodgkin & Huxley dissected the transmembrane current into three components, a capacitive current, an inward current carried by sodium ions and an outward current carried by potassium ions. The sodium current leads to membrane depolarization and the potassium current leads to recovery of the membrane potential to resting levels. There are two distinct phases to the sodium current, the initial portion where the magnitude of the current increases and the second phase where the sodium current decreases. In the last ten to fifteen years the mechanism for this sodium channel behavior has become more understood.

Historical Note:

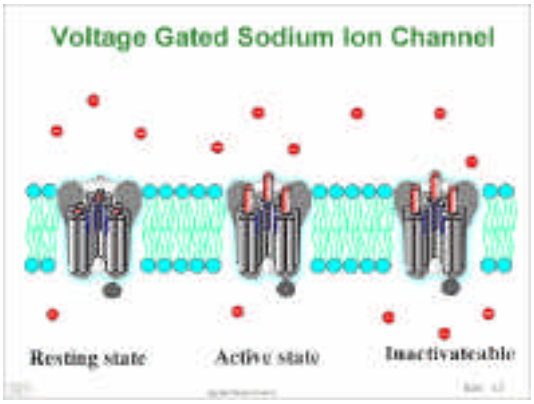
John Z. **Young** (~1930) introduced the use of the giant squid axon with ~ 1mm diameter for study of ion currents. **Hodgkin and Katz** showed that Sodium ions were basic to AP generation by making changes in the fluid bathing the axon. In the forties, **Cole** developed the '**voltage clamp**'. The insertion of a long electrode inside the axon removed the variable due to AP propagation, maintaining equipotentiality inside the axon, a '**space clamp**'. In 1952 Hodgkin and Huxley published results of their numerical model for action potential initiation and propagation.

REFERENCES:

Hodgkin, A.L., and Huxley, A.F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. 117, 500–544.

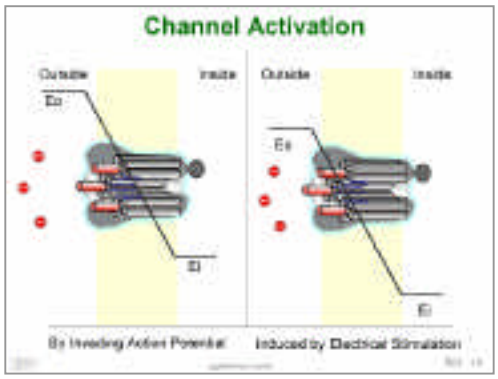
Armstrong, C.M. (1981). Sodium channels and gating currents. Physiol. Rev. 61, 644–682.

Sodium Channel Activation and Inactivation



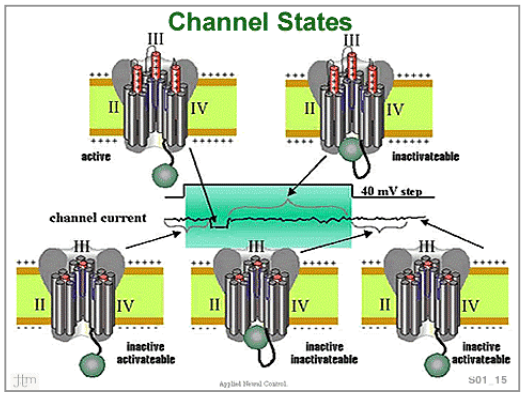
Results from more recent experiments have shown that the mechanism that controls the sodium ion current lies in the voltage sensitive sodium channel protein. A propagating action potential gives rise to positive charge moving inside of the axon, which causes a decrease in the transmembrane potential. This change in transmembrane potential results in a conformation change in the sodium ion channel. The four S4 segment alpha helices translocate, which leads to an opening of the channel pore. Sodium ions flow down the concentration gradient and the voltage gradient, causing the potential inside of the axon to become more positive. Thereafter, a second mechanism comes to play. The linker loop between the S4 & S5 domains alters its conformation to move into the channel and block further sodium ion flow. The blocking effect of the linker is often likened to that of ball, on a chain, moving in to block ion movement through the channel. It is this second phenomenon that results in inactivation state of the channel.

Electrical Stimulation Can Activate the Sodium Channels.



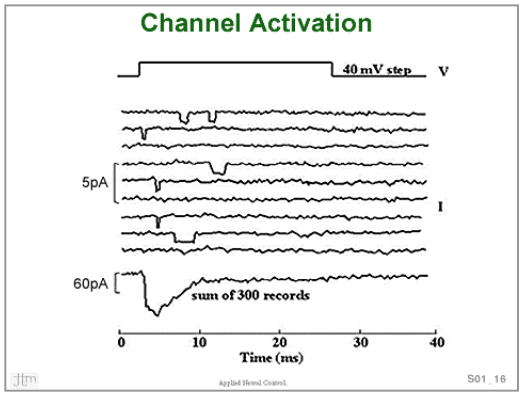
An extracellular electrical stimulus can alter the transmembrane potential in a way that is similar to the way a propagating action potential alters the transmembrane potential. The process of normal action potential propagation involves sequentially gating open sodium ion channels in a timed, proximal to distal, fashion. When the upstream sodium ion channels open the potential inside the nerve membrane becomes more positive. This increase in potential causes the transmembrane potential downstream, distally, to rise and to cause those ion channels to go from a resting state to an active state. This process gives rise to a propagated action potential. When an extracellular current is applied, as when electrically activating a nerve, the potential in the vicinity of the axon decreases, giving rise to a decrease in the transmembrane potential at the site containing the voltage gated sodium ion channels. This change in voltage results in opening of the channels in the resting state and initiates the same sequence of events that occur when a propagating action potential invades the axon membrane.

Channel States

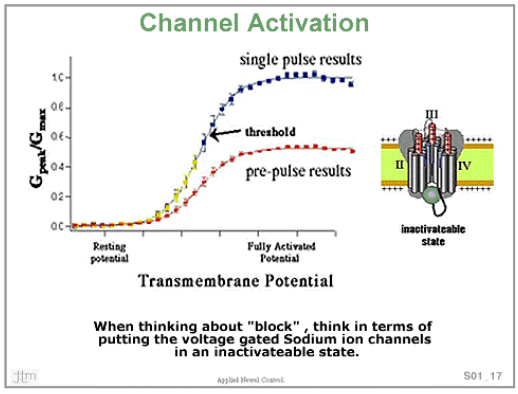


In the center of this picture is shown the recorded current flowing through a single sodium ion channel in response to a step change in transmembrane potential. Surrounding the current-time trace are states of the of the sodium ion channel. Prior to the step change in transmembrane potential, the ion channel is in the inactive-activateable state. When the membrane potential decreases, the channel will begin conduction, followed by channel blockage by the ball and chain representation of the III-IV linker. Immediately after the membrane potential is returned to the resting state, the channel remains blocked for a finite period of time. Eventually the channel returns to the inactive-activateable state. The magnitude of the current change is constant while the channel is open and the magnitude is dependent on the driving force (V_m - V_{na}). Keep in mind the finite inactivateable period after the channel has been activated as this state may be employed to manipulate axon excitability. The inactivatable period corresponds to the refractory period, the time that an axon cannot be reactivated following stimulation induced depolarization.

Patch Clamp



When many channels are activated, channel opening is a stochastic process and the total current recorded is the sum of the currents flowing through the individual channels. Once a channel opens there is a very high probability that it will go into the inactivateable state. 'v' applied step voltage, 'I' individual channel currents and below summated currents. The membrane currents recorded by Hodgkin & Huxley can be seen to form when many channels are activated. The current recorded at a single node of Ranvier represents the contribution of many thousands of ion channels.



The probability that a channel is in the active, conducting state is a function of the transmembrane potential. When step changes in the membrane potential are applied and peak sodium currents are measured and plotted as a function of the magnitude of the step change in membrane potential, the curve shown in blue is recorded when the ion channels in the test patch of membrane were in the inactive/activateable state .

When an intermediate potential is applied, only a fraction of the channels are opened, yellow line, too few to achieve threshold for generating an action potential. If these channels are given time to become inactivateable, and, a second stimulus pulse applied at value that is capable of activating all activatable channels (and before the inactivatable channels have recovered), only those activateable channels are activated leaving the patch unable to develop sufficient depolarization to initiate a propagated action potential. This concept may find utility in the creation of virtual excitation sites that are separated some distance from an actual electrode site, i.e. axons close to the electrode will be incapable of generating a propagating action potential because too many sodium ion channels are temporarily placed in the inactivatable state.

. . . . .