Cation channels and myelinization in signal processing

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Abstract

Sodium (Na) and potassium (K) channel-proteins in myelinated nerve are clustered in specialized membrane domains around the node of Ranvier. The channels are characterized by free space to store cation charge Q as free electrical potential. As such the channels are channel-capacitors. The cation channel-capacitors are also characterized by voltage sensitive domains, enabling the proteins to flip-flop in the membrane while transferring stored charge as current Q/sec across the membrane. Phase-shifts between potential- and current generation by the enzyme 3Na/2K-ATPase allows the enzymes to generate membrane impedance, Volt/Q.sec\(^{-1}\), or admittance, Q.sec\(^{-1}\)/Volt. The small H\(_2\)O cavity between Schwann cell and neuron is causing a 3-fold increase in K channel-capacitance and concomitant 3-fold increase in membrane potential. This temporary charge buffer is easing the lipid K mobility with a factor 3 when the capacitors release the stored K charge as K current. A model for the enzyme activity supports current- and voltage generation by 2 enzyme subpopulations. The laws of electricity demand that the 2 enzyme subpopulations constitute one array of enzymes with 10\(^{-3}\)C.sec\(^{-1}\)/28mV charge generating capacity. This particular array of enzymes is active in all biological membranes expressing the enzyme 3Na/2K-ATPase. Net antegrade and retrograde axonal fluid transport is generated by K- and Na-current, respectively. Numerical analysis shows that one enzyme array is generating one action potential, being the expression of the non-imaginary capacitance components of membrane admittance and impedance. This result suggests that conventional electrophysiological methods could be inadequate to study membrane admittance and impedance in full detail.

Introduction:

Membrane potential- and excitability are thought to originate in response to altering voltage dependent Na- and K membrane permeability’s of the membrane. It is widely accepted that Nernstian membrane diffusion potentials are set up by the enzyme 3Na-2K-ATPase. The generation of a diffusion potential, or any electrical potential in general, however requires current (Q.sec\(^{-1}\)) and especially this current generation appears to be a weak spot in the dogma of membrane potential difference (PD) generation. To show this we choose the propagation of the action potential at the
node of Ranvier (fig.1a). The interaction between Schwann cell and neuronal axon at
the level of the node of Ranvier is highly complex. The adhesion molecules in the
paranode between Schwann cell and axon allow site-specific expression of Na- and K
channels in the neuronal nodes and internodes, respectively, as is shown by Boyle et
al\textsuperscript{1}. Disruption of this adhesion site causes the K-channels to drift apart (fig.1b) with
concomitant 3-fold reduction in peripheral nerve conduction and excitability. However, the current-leak
model developed by the authors to find an explanation
for these altered electrical properties is inadequate
because the net transmembrane Na cation current is
zero in their model. Their model is based on general,
widespread models for pulse propagation in the axon
having in common the absence of this net axonal cation
current. For example, the results of the authors show
that the neuron develops a 3-fold higher action potential
in response to a given trigger stimulus in neurons with
normal K-channel capacitor distribution (fig.1). This
suggests that the K-channel density plays an important
role in the development of nerve action potential, which
could suggest that the normal axon is able to conduct a
net K over Na current along the length axis of the
neuron and across the membrane of the axon. This net
axonal K cation current- and corresponding net fluid
output at the synapse can be realized by the enzyme
3Na/2K-ATPase. This enzyme stores K-charge
temporary in the axon membrane as free electrical
potential that is subsequently used as K-current for
boosting antegrade fluid pumping in the direction of the
synapse, as is shown in this study.

The action potential is thought being related to a
Nernstian diffusion potential difference (PD) as
follows:

\[
PD = R^\circ K/F \cdot \ln(C_1/C_2) \quad \text{Equation 1}
\]

R is 8.313 Joule/mole, °K (Kelvin) is the absolute temperature, F (Faraday) is
96000Coulomb, and C\textsubscript{1} and C\textsubscript{2} are the concentrations of a given ion at either side of
the membrane. The constant R°K/F is 26.7mV per coulomb (C) at 37°C and equation
1 predicts a potential change of 61.4mV for a 10-fold change in C\textsubscript{1} or C\textsubscript{2}. Our
experimental data and theoretical considerations show that these values approach by
chance the real change in PD, 56mV in response to a 10-fold change in C\textsubscript{1} or C\textsubscript{2} when
the enzymes are generating membrane capacitance and current, and not diffusion
potentials\textsuperscript{3}. The often-used value for R°K/F, 26.7mV is close to the potential constant
of 27.8mV (R/300). The latter is obtained when a given population of 3Na/2K-
ATPase enzymes per cm\textsuperscript{2} is rotating 3-fold per second in a membrane over a distance
of 100cm while displacing one mole of water in ion channel-capacitors, as is shown in
the following analysis.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig1.png}
\caption{a K- and Na channel in the Schwann cell myelinated
axon cluster in the internode (i) and node of Ranvier (n),
respectively. The paranodal (p) space is the locus of interaction
between Schwann cell and axon. This physical barrier
assigns the Na- and K channels an activation-specific type of
environment. b Demolition of the paranode increases the
volume of the internodal space, which allows the K-channels
to drift apart.}
\end{figure}
Analysis of the membrane potential

The enzyme 3Na/2K-ATPase binds 3Na at the cytosolic side, and 2K at the interstitial side of a biological lipid membrane. The protein, energized by ATP, is therefore a 2K/2Na-exchanger. However, the molecule is also a current- (Q_{Na}/sec) or potential (Q_{Na}) source because the 3rd Na molecule must stay bound to the protein when it crosses a membrane because it also must return. Kirchoff’s law demands the transported cations are part of a closed electrical circuit, which implies that the sum of charge transported across a membrane must be zero.

The enzyme 3Na/2K-ATPase must be able to generate current and potential for the propagation of an action potential. The physical mechanism to generate these electrical components is unraveled in this section. For reasons that will become clear below we start out with a current of 10^{-3}C/sec/cm^2 (C is one coulomb). This current is generated, according to Faraday’s law, by a cluster of enzymes that extract 10^{-8} charge equivalents Q, per cm^2 and per second, from a solution of one liter. For this analysis we furthermore assume that ion channels are channel-capacitors because channels have free space available to store charge as free electrical potential. Ion conduction in a channel for example proceeds by electrostatic, resistance-free, interaction, which also points to a capacitor characteristic of the protein. The channels furthermore also conduct ions, driven by the concentration gradient on both sides of the channel, against their electrical gradient, which is the 3rd capacitor characteristic of the protein. Thus, ample evidence strongly suggests that the enzymes must be able to store transported charge Q as free electrical potential (Volt) across the membrane in channel-capacitors. The enzyme can store these charges only in a discrete quantity. They store 1 Coulomb.ltr/cm^2.sec charge in the channel-capacitors over a distance of one cm. The value is obtained by conversion of 10^{-3}C/cm^2.sec (see above). Thus, it appears that one mole Na charge (C) is able to ‘push aside’ 1 coulomb H_2O (1 mole) when it is stored in the channel-capacitors (fig.2). The generation of one mole H_2O per cm is equivalent to 10^{-2}mole H_2O over a distance of 1 meter, the definition of current, and therefore, using the gas constant of 8.313Volt/mole, the enzymes generate 83.3mV free electrical potential when they store 10^{-3}C/cm^2 charges in the channel-capacitors. This potential, 83.3mV, is the maximal capacitance that the chosen pool of 10^{-3}C/cm^2.sec charge transporting
enzymes can- and must generate, because one Na replaces one \( \text{H}_2\text{O} \) molecule per channel at this enzyme density.

![Diagram](image)

**Fig.3** a 3 ATP-activated enzymes in the soma of a neuron (s1) are active as current source (i-mode) together with 3 enzymes in the soma of another neuron (s2) when the Na cations on the enzymes stay put. The 3 K-current generating enzymes store 3K cations in 3 high-density K channel-capacitors (p paranode) as capacitance. A 6\( \text{H}_2\text{O} \)-substitution volume of 6K cations is left behind in the cell when the protein is conducting the charge as current \( Q_{\text{K}.\text{sec}} \) across the membrane (fig.2b). The generated membrane impedance is defined as volt/current (\( V/i \)). The charge density is \( 3 \times 10^{-3} \text{QK.cm}^{-2}/83.3 \text{mV} \) (see text). b The enzymes of neuron s2 reset the enzymes on neuron s1. Enzyme reset is represented by 1/8 of the total enzyme activity and 3 serial currents of one \( Q_{Na} \) depolarize the membrane capacitance by removal of one K of the stored K current. 1/8 of 6\( \text{H}_2\text{O} \) has to follow (fig.2d). Thus, the 3 Na-cations drag net 3-0.75=2.25\( \text{H}_2\text{O} \) volumes into the soma. The K-potential of the membrane decreases with 1/8 of 83.3mV (10.4mV) to 72.9mV. This potential signals the voltage activation of the Na channel-capacitors. c One Na-capacitance generating (v-mode) enzyme in every 3 K-current generating enzymes (i-mode) depolarizes the membrane to essentially 0Volt. Na in the channels is carried to the other side of the membrane as current (fig.2b) when the channel-protein flip-flops in the membrane. The Na cations cannot enter the channels as capacitance from - to + potential as long as the K-capacitors are activated. However, a retrograde (scavenger) vesicle containing 3\( \text{H}_2\text{O} \) volumes (fig.2b) is generated instead at the synaptic membrane at essentially 0Volt. The Na-current clamps the membrane to zero Volt and the remainder of K capacitance (5K) is discharged as current when an antegrade synaptic vesicle is secreted with the remainder of 5.25 \( \text{H}_2\text{O} \) volume units (fig.3d). The net fluid secretion at the synapse means that the enzymes generate membrane admittance. The admittance of the enzymes is approaching the value \( 9 \times 10^{-3} \text{C}/83.3 \text{mV} \) or \( 10^{-3} \text{C}/9.3 \text{mV} \), which means that the apparent membrane resistance is approaching the value of \( 9.3 \Omega \text{cm}^2 \). The local membrane potential is 0Volt and the presence of admittance must be shown indirectly by measuring axonal \( \text{H}_2\text{O} \) transport (see text). The model is valid also when vesicles are preloaded with transmitter salt. Note that the Na cations do not leave that capacitors under condition current clamp d A neuron consists of synapse, axon, soma, and dendrites. Fluid transport in the dendrite to synapse direction in the neuron is organized by the enzyme 3Na/2K-ATPase. The ratio of retrograde over antegrade fluid pumping is 0.58 in the synapse (see text). Net volume throughput of a neuron is 2.25volume unites per action potential. The neurons (s1-2) are electrotonically coupled.

**Charge neither can be diluted nor compressed in a closed electrical circuit**

The charge density generated by a given enzyme population of active enzymes must be \( 10^{-3} \text{C/cm}^2 \cdot \text{sec} \) for every membrane, the density we have chosen in the foregoing paragraph. The chosen density shows that one charge is replacing one \( \text{H}_2\text{O} \) molecule in a channel-capacitor (Fig.2). However, the 83mV substitution potential would dissipate if not the enzymes in reality were displacing \( 3 \times 10^{-3} \) charge equivalents per \( \text{cm}^2 \) and per second, which lowers the water potential 3-fold from 83.3mV to 28mV per \( 10^{-3} \text{Q.cm}^2 \cdot \text{sec} \). Thus, instead of loading up single channels with 83mV capacitance the enzyme population charges up 3 parallel channel-capacitors with 28mV capacitance each. This binds the charge in the channel-capacitor and its surrounding dissolving water forever to the membrane because it requires 3 times
more potential for the charges to leave the channels and reenter the solution (fig.'s 2c,d). Charge only leaves the capacitors when the channels close. Current cannot be diluted nor compressed (Kirchoff) and therefore the potential must increase when more cations enter the capacitors as current; up to $3.10^{-3}\text{C}/83.3\text{mV}$ can be stored in the capacitors (fig.2d)\(^3\). Furthermore, a $6\text{H}_2\text{O}$ water volume is left behind when 6 additionally K charges enter a set of 3K capacitors as current $Q_{\text{K}.\text{sec}^{-1}}/\text{H}_2\text{O}$ (fig.2d). This volume has to be co-transported with the 6 K charges to the other side of the membrane under current clamp condition because current in a closed electrical circuit (Kirchoff) cannot be compressed nor diluted.

**Enzymes transport fluid in the neuron under condition of current clamp**

An enzyme transfers charge, capacitance, when it transfers 3Na across the membrane. The enzymes furthermore generate admittance ($Q.\text{sec}^{-1}/\text{Volt}$) and impedance ($\text{Volt}/Q.\text{sec}^{-1}$) in cooperation with the channel-capacitors. Admittance describes the special condition that neutral salt can abandon the generated $RC$-circuits. For example rabbit mandibular main duct and human sweat duct main duct\(^3\) reabsorb neutral salt under condition of essentially 0Volt, realized by voltage clamp of the membrane. It means, as has been shown, that Na- and Cl channels clamp the local membrane potential to 0Volt in these tissues (vesicle mediated salt reabsorption) while only net salt (and no net fluid) is transferred across the membrane. Admittance also describes the special condition that net water (fluid) can escape from the $RC$-circuits while, as has been shown for donkey sweating\(^3\), the electrolytes don’t abandon the $RC$-circuits. Fluid (water) secretion therefore is generated under current clamp condition. Water transport across the neuronal synapse is not zero (see below) which means that the neuron transports water under current clamp condition, as will be shown in the next paragraphs.

**Admittance and impedance generation, the action potential**

An enzyme population of one array can be split into 2 subpopulations. 3 in 4 enzymes generate membrane current (fig.3a) and 1 in 4 enzymes generate membrane capacitance (fig.3c)\(^3\). All enzymes in one array generate either $3Q$ capacitance or $3Q$ current. The currents and capacitances are the elements that determine the admittance ($Q.\text{sec}^{-1}/\text{Volt}$) or the impedance ($\text{Volt}/Q.\text{sec}^{-1}$) of the membrane. The sum of admittance and the reciprocal of impedance must be 3 for a given array\(^3\) according to the following equation:
y + 1/y = 3  Equation 2

Equation 2 has a real (impedance) and imaginary (admittance) part. Admittance (y) is the reverse of impedance (1/y). The solution of Eq.2 shows that only 1 in 8 enzymes in an array are not involved per second in the generation of admittance (fig.'s 5a,b). These enzymes generate membrane impedance. The components of membrane impedance, Volt and current, are generated by two subpopulations of one set of enzymes. Furthermore, one in 8 of the capacitance-generating enzymes are not involved in generation of membrane admittance, shown in fig.5b, and these enzymes generate 28mV Na-capacitance. The activity of this set of enzymes is furthermore electrically coupled\(^3\) to the fraction of 1 in 8 enzymes that are responsible for enzyme reset (fig.3b, 4a). Resetting of the enzymes is generating membrane current, which also generates a remittance of the membrane K-potential with a factor 1/8 (fig.'s 3b, 4a). Enzyme reset involves 1/8 of the total enzyme activity and these enzymes decrease the maximal K membrane potential (-83.3mV) with 10.4mV (fig.3b). This 10.4mV K-potential is also known as the action-potential overshoot. The ratio of inversed impedance, 1/y in eq.2, is 1/8\(^3\) or 0.375. The membrane potential of 72.7mV (83.3mV-10.4mV) furthermore is the potential that initiates the voltage-activation of the Na-channel-capacitors (75mV has been calculated for squid neuron\(^7\)).

The action potential

An action potential (fig.5a) is generated by a set of \(10^{-3}\)C/cm\(^2\) charge generating enzymes. For a moment we may assume that all these enzymes are activated one after the other in one second but it is more realistic to assume that altered channel-properties, like open- and closing probability and channel gating mechanisms, influence the time course of the action potential. The action potential measures 72.9mV (83.3mV-10.4mV) at the beginning (fig.5a), when the voltage sensitive Na channel-capacitors become activated. 3 low-density Na channel-capacitors in the node of Ranvier, acting as current clamp capacitors, and 3 high density K-channel-capacitors, acting as voltage clamp capacitors, are clamping the membrane to essentially 0Volt (fig.3c). It means for the Na channel-capacitors that the Na charges replace a H\(_2\)O volume in the channels before the channels flip-flop (at 0Volt) the charge as current (\(Q_{\text{Na-sec}}\)) across the membrane (fig.2b). A retrograde ‘scavenging’ vesicle endogenizes this volume (fig.'s 3c, 4b). The Na current-clamp is easing the K-current with a factor of 3 when it is enabling K cations to escape from the internode K-channel-capacitors as current and potential (fig.'s 3c, 4b). The remainder of 5.25H\(_2\)O\(_C\) fluid volume is simultaneously transported across the synapse in an antegrade exocytotic vesicle. The fact that the Na channel-proteins are able to flip-flop the Na ions as current explain the apparent sharp increase in Na ‘conductance’ that is measured under experimental condition when the membrane potential depolarizes (fig.5). However, the analysis shows that the Na cations, in contrast with the widely accepted view of massive influx during depolarization\(^1\), are not entering the cytosol through the channels during membrane depolarization. Furthermore, vesicle mediated water transport is the real event that is generated by the enzymes at 0Volt in the neurons. Such cannot be measured with electrophysiological techniques.

The Na channel-capacitor density is low, \(10^{-3}\)/cm\(^2\) (fig.2c), in the non-myelinated node of Ranvier, and the 3 Na channel-capacitors start to express positive, +28mV Na capacitance (fig.4c) when they are not active as current source anymore. The section of
1/8-impedance-generating enzymes in an array (see above) generates this Na membrane potential (+28mV). The same fraction of enzymes also generates the K-current that depolarizes the ‘transient’ supra-threshold K-potential (fig.3b, 4d). The internodal K capacitance is restored after closure of the Na channel-capacitors by antegrade 3K current re-saturating the membrane capacitance with 83mV (fig.’s 2d,3a, 4d) and impedance generation by 7/8 and 1/8 enzymes in an array of 10⁻³C/cm² charge generating enzymes respectively. The action potential having the value of 72.9mV (a) depolarizes completely (d) to 0mV by the local current clamps of the Na- and K channel-capacitors. The potential hyperpolarization further to +28mV is due to low-density Na channel-capacitance after closure of the high-density K channel-capacitors. This potential disappears when the Na channel-capacitors close (e1). The membrane repolarizes (e2) to ~83.3mV when the K-channels are re-saturated by enzymes generating K-current, which is converted into free electrical potential. 1/8 of this potential, the overshoot potential, is removed by enzymes generating impedance. The sum of admittance d/r (y = 72.9mV/27.8mV = 2.63) and impedance e1/r (1/y = 10.4mV/27.8 = .37) is 3 (see text).

**Fluid transport in the neuron**

The admittance of the neuronal membrane cannot be measured directly because alternating opening and closing of the channels mask the presence of these electrical membrane components. Also, different sections in one array of enzymes generate the components. The admittance of the neuronal membrane is an imaginary process that furthermore proceeds at essentially 0Volt because the enzymes transport only water at 0Volt. Admittance can be measured indirectly by measuring axonal fluid transport. The here calculated ratio of retrograde and antegrade vesicle volume transport is 0.58 (fig.3d), and this ratio is obtained by numerical analysis of the enzyme activity in the axon. The ratio is in agreement with the ratio given in the textbook which is ‘between one half and two-third’⁸. The ratio reflects the ratio of vesicle volume turnover in the synaptic membrane, a process that should occur under condition of essentially zero mV. The enzymes generating membrane inward Na current (fig.’s 2b, 3c, 4b) are responsible for the uptake of 3H₂O to the inside of the synapse, the phenomenon itself is known as scavenging. The 3 enzymes that generate K potential export net (1/8*6K) 5.25 volumes of H₂O across the synaptic membrane (fig.3c). Hence, the ratio of axonal retrograde- over antegrade volume output is 0.572 (3/5.25). The model (fig.3d) is valid also when preloaded vesicles are secreted in the synapse.

Absence of net Na- and K current is observed when the paranodal cavity is disrupted (fig.1b). The maneuver increases the distance (Δx, fig.4e) between axon and myelin sheet with concomitant increase in internodal volume, which allows the K-channel-capacitors to drift apart. Now the enzymes each generate low-density K channel-capacitance and a 3-fold decrease in neuron activity in response to a given trigger stimulus. However, opposing Na- and K cation gradients still are able to increase the absolute passive voltage sensitive channel-capacitance in normal neurons.
to -112mV (4*28mV) when the enzymes are deprived of ATP\(^7\). The enzymes are electrically uncoupled in such condition and each of the enzymes starts its own mini RC-circuit in the membrane (fig.4e). This amplifies the absolute passive potential in the membrane with a factor 2 for the given enzyme population. For example, inactive enzymes in squid axon still generate uncoupled positive (+56mV) or negative (-56mV) Na or K capacitance (112mV absolute) respectively in the presence of appropriate cation gradients\(^7\). It shows that each enzyme displaces two charges and it is therefore concluded that the active enzyme density must be 5.10\(^{-4}\)/cm\(^2\) in whatever biological membrane. The impedance of the uncoupled enzyme is -56mV(Q\(_{\text{K}}\))/10\(^{-3}\)Q\(_{\text{Na}}\).sec\(^{-1}\) or +56mV(Q\(_{\text{Na}}\))/10\(^{-3}\)Q\(_{\text{K}}\).sec\(^{-1}\) (fig.4e)\(^3\).

Fig.6 The combination can also be active as salt pump when the combination of enzyme and capacitor is voltage-clamping the membrane, for example in human sweat duct and rabbit mandibular duct\(^3\), as shown here. a The enzymes generate high density Na- and K capacitance in respectively the apical and basolateral (not shown) membranes, and antegrade vesicle mediated basolateral ‘H\(_2\)O’ transport generated by K-current generating enzymes. b, c The total concentration of apical and basolateral electrolyte capacitance becomes (1,66C.cm\(^{-2}\), fig.2c,d) when the Na-capacitance flip-flops as current in the membrane. The Na and Cl capacitors are voltage-clamping the membrane to essentially 0 Volt in this condition. 0.83mole/ltr NaCl is transported retrograde in vesicles across the membrane while simultaneously the Na and Cl capacitance dissipates\(^3\). The net sum of transported charge is zero as is demanded by the second law of electricity. Salt uptake is realized by RC-circuits scavenging NaCl from a 2.7um thick water layer on top of the apical membrane. The layer is salt-deprived because electrogenic salt uptake exceeds free diffusion of salt by a factor 3 (www.bijman.info).

Discussion

A formal description of the generation of the action potential by the enzyme 3Na/2K-ATPase has been presented. 7/8 of the enzymes in a defined array generate membrane admittance. Admittance, defined as Q/sec\(^{-1}\)/Volt, is generated by a combination of enzymes generating membrane capacitance (Volt), and membrane current (Q/sec\(^{-1}\)). These enzymes generate net H\(_2\)O transport across the synapse membrane. The electrolytes recirculate (fig.3d) and it is therefore safe to conclude that 87.5% of the enzymes are transporting solely water at 0mV across the neuronal membrane. This process cannot be measured with conventional electrophysiological methods. The remainder of the enzymes (12.5%) in that particular array generates membrane impedance, which can be measured with standard electrophysiological techniques. The laws of electricity furthermore demand that one array of enzymes must be able to generate a charge density of 10\(^{-3}\)C/cm\(^2\) in order to function appropriately. This charge density means that each charge replaces one H\(_2\)O molecule when it moves to the other side of the membrane in the channel of a membrane channel-capacitor. The required number of enzymes for such an array, 5.10\(^{-4}\)cm\(^2\) is rather high, which suggests that many cells function group wise only in a given tissue, as has been shown for rabbit mandibular epithelial cells\(^3\), and neuron (this report) The enzymes generate RC-circuits together with channel-capacitor proteins. The maximal capacitance of an enzyme is 83.3mV because one molecule H\(_2\)O in channel is displaced by one charged particle.
The enzyme is either a net salt pump or a fluid pump (fig.6). The switch between net fluid pumping and fluid and electrolyte pumping depends on membrane specialization. For example human sweat duct transports net salt, and the neuron transports net fluid (fig.6). However, the sequence of enzyme activation is the same in salt transporting epithelia and in neurons. 1 enzyme generates capacitance and subsequently 3 enzymes generate current. Fluid and current furthermore cannot be compressed (Kirchoff) and therefore the enzyme starts to build up electrical potential when little space is available. This stored electrical potential subsequently can be used to generate net fluid transport like in the neurons, or net salt transport like in human sweat duct or rabbit salivary duct. Examples of curbed areas for temporary potential storage areas are the internodal space (fig.1a) and the lateral space and lumen of epithelial cells lining small ductal cavities. Also cochlea, cornea, synapse and intestinal crypt may have similar function in fluid and electrolyte secretion.

A given array of enzymes, generating current and potential, is involved in a very complex electrical circuit with components expressing admittance and impedance. As soon as the first enzyme in such an array is activated it has to await activation of all other enzymes before it can be activated again. Rapid cooling of the active enzyme in rabbit mandibular main duct epithelium unmasked the existence of an enzyme-channel density with $10^{-3}$C.sec$^{-1}$/28mV impedance. This study shows that neither impedance, nor admittance (see above) can be demonstrated in full detail with conventional electrophysiological methods, because different sections in a single enzyme array generate voltage and current up to $9.10^{-3}$C/83mV. Electrical measurements fail to miss the increase in admittance at essentially 0Volt membrane potential. This notion is underscored by the observations of Cole and Curtis (1938) who showed that the imaginary capacitive- and inductive components of squid axon admittance did not change during the action potential. The enzyme activity furthermore is complex. Enzymes are involved in several circuitries and each circuit contains 2 enzymes per unit of time. Sometimes the enzymes must be reset for renewed activation because ATP can only activate the enzymes from inside a cell. Even the passive, non-activated, enzyme is therefore electrically not uncoupled as long as it is part of a circuit. All enzymes are activated once per array and the enzyme pool generating admittance ($y$) or reciprocal impedance (1/$y$) must be recruited from one and the same pool as shown by equation 2.

The array can execute very complex tasks in combination with channel-capacitors when the latter are endowed with additional mg$^{++}$ blocks, ATP-binding sites, phosphorylation sites or other regulatory gadgets. These features, functioning as temporary charge buffers or temporary charge buffering intermediates, will only affect the time course for an enzyme array to complete its supposed activity. For example, we showed that one array of enzymes is able to generate a complete action potential, which requires i) converting Na-potential into Na current ii) conversion of...
K current into K potential iii) easing of the lipid K mobility, and iv) generation of an additional Na-voltage and K-current impedance. Such an action potential can be slowed down. But the enzymes can perform their complex tasks only because the sequence of activation of the individual enzymes within a given array responsible for the generation of that action potential cannot be influenced; each enzyme/channel combination has its own specialized task in that array.

![Diagram of action potential and ion fluxes](image)

**Fig.8 A factor 10.** Admittance is easing the electrotonic coupling at, for example, the neuromuscular junction or calyx with a factor 3. Ca supports the coupling between the pre-end postsynaptic potentials. The measured absolute Ca current of $10^{-12}$Amp yields in the calyx of Held a density of $6.6 \times 10^{-7}$C/cm$^2$, assuming the calyx has a diameter of 15um (Meinrenken et al., 2003, J Physiol 547: 665). By crossing the membrane through the Ca-channel-capacitors this current is converted to $6.6 \times 10^{-4}$molCa/cm/l. Hence, a very local volume current $6.6 \times 10^{-6}$ mol Ca.mtr/l (Faraday) is running along the inner surface of the caliceal membrane (an external device measures the inward Ca current only). The net zero Ca-current supports the efflux of transmitter salt, (3*, see below) 6.7umol/l Glutamate salt, from docked vesicles. Vesicle fluid transport is driven by admittance, generated by the enzyme 3Na/2K-ATPase. The fluid generates zero net Ca-transport at 0Volt transmembrane potential. The Ca-fluxes, starting at depolarized, 0Volt membrane potential, clamp the local membrane at the vesicle pore for prolonged time to essentially zero Volt. Ion-channels behave like current sources at this potential. At non-zero potentials ion-channels behave like channel-capacitors. The Ca-channel-capacitors represent a small Ca-buffering capacity; Ca-buffers inside the calyx are not required. The enzyme 3Na/2K-ATPase appears to be a salt pump in the neuron. See fig.9 for further evaluation of quantal vesicle uptake and secretion. The Calcium-paradox: the fluid transports cations in the pore actively to a higher potential and anions actively to a lower potential. Ca outside the pore is running passively to a lower potential as passive inward current as the channel flip-flops in the membrane (charge included), and to a higher potential as outward capacitance. The total calcium cation circuit is an electrical RC-circuit and as such must be closed (Kirchoff). Hence, an ammeter measures only the passive inward current. Note that opening- and closing of the calcium channel-capacitors are important events in RC-circuit generation at 0Volt.

Neurons generate net fluid transport in the absence of net electrolyte transport and previously we have shown that exocrine epithelia generate net salt transport in the absence of net H$_2$O transport. The sequence of enzyme activation furthermore appears identical for the 2 different transport mechanisms. The neurons can transport preloaded vesicles with determined volume content but vesicles must contain 0.83 mole/ltr of a given salt in a salt transporting epithelium (fig.6). The transferred charge therefore is concentrated 30-fold by the enzyme/channel combination. Thus, net charge transport always has a final concentration of $3 \times 10^{-5}$C/moleH$_2$O=1.66C/ltr. By concentrating the salt the enzyme/channel combination is scavenging the aqueous surface layer on top of salt transporting epithelia. This generates a very stirred but often called unstirred aqueous fluid column with 2.7um height on top of the epithelial cells when salt uptake is maximal. (fig. 6,7). This unstirred water layer is absent in cystic fibrosis (CF), caused by the impaired Cl capacitance of CF epithelia, which blocks the endocytosis and exocytosis of a tissue (fig.6). The reduced or absent unstirred water layer is the primary defect of the disease, causing the pulmonary and intestinal manifestations of the disease (see also fig.7 at www.bijman.info).
Fluid transport generated by the enzyme 3Na/2K-ATPase most likely is the driving force behind transmitter release in the calyx of Held or neuromuscular junction (fig.8). Vesicles fuse with the membrane under condition of 0Volt transmembrane potential and the neurons exploit the existing high Ca gradient across the membrane for driving out (non-ionic) transmitter salt into the cleft. A model for Ca mediated transmitter release is shown in fig.8. Furthermore, the membrane admittance generation of the enzymes can be disturbed by the generation of membrane impedance. An example might be epilepsy but also low- and high frequency voltage sources may disturb vesicle transport across the membrane. These stimulus generators generate membrane impedance in combination with the 3Na/2K-ATPases in the membranes because the latter permanently are converting current into capacitance and vice versa (fig.9). Impedance hops from one cell to another in a population of neurons once it is introduced in a given cell. Neuronal impedance can be monitored electrically because it is not an essentially 0Volt event. Therefore LTD and LTP most likely reflect the altering osmotic steady state of a set of neurons once impedance starts to invade (infect) the population (fig.9).
Enzymes transport net zero charge (e.g. NaCl) or net fluid across a membrane by generating closed electrical circuits. The laws of electricity show that neither water nor current can be dissociated or compressed in these circuits. The law therefore holds for such diverse manifestations as salt or fluid transport across salivary epithelia or, as shown here, across the neuronal neuron membrane. The shown electrical cell coupling within a given population of cells could provide a rationale for successful stem therapy in clusters of cells sharing electrical activity. Also vacuolar proton transport (0.83mol/l) should obey the rules of electricity. The binding of H2O to the cell membrane by the channel capacitance’s furthermore could provide a physical rationale behind osmotic balancing of euryhaline fish, cell division, muscular motor control, osmotic balancing in the intestine, synaptic strength (memory) and much more. It suggests that a cellular organism apparently tightly controls not only its milieu interior (Claude Bernard) but, as shown here, also its milieu exterior.

**Literature**