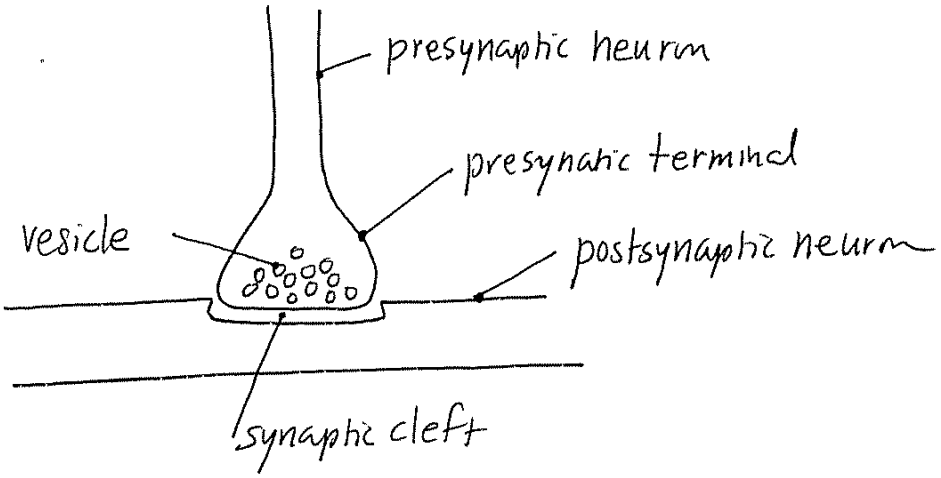
Charles S. Peskin 11/25/91

Synaptic transmission



Synaptic transmission is the mechanism of information transfer from one neuron to another (or from a neuron to a muscle). We consider here only chemical synapses, in which a transmitter substance is involved. The events of synaptic transmission may be summarized as follows:

presynaptic: Increased membrane potential opens Ca^{++} channels. Ca^{++} stimulates fusion of vesicles to presynaptic membrane, spilling transmitter into cleft.

cleft: Transmitter binds to receptors which are ion channels of postsynaptic membrane. Channels with bound transmitter can open. Transmitter action is terminated by enzymatic degradation, uptake into presynaptic terminal, and diffusion out of the cleft.

postsynaptic: Ionic current through open synaptic channels displaces the membrane potential. Depending on the channel type, the change may be either excitatory or inhibitory.

Unlike the all-or-none action potential, synaptic transmission is graded. It is therefore a favorite site of hormonal, pharmacologic, and neural regulation of nervous activity.

We begin the discussion of the synapse with the synaptic cleft itself:

Events in the synaptic cleft:

For now, we regard the rate of release of transmitter from the presynaptic terminal as a given quantity, possibly depending on time. Let

$$r_T = \frac{\text{rate of transmitter release}}{\text{volume of synaptic cleft}}$$

We assume that diffusion is fast, so that transmitter is always uniformly distributed over the volume of the cleft.

The reaction scheme that we consider is as follows:

1) release of transmitter:

$$\longrightarrow T \qquad \text{(rate } r_T \text{ per unit volume)}$$

2) binding of transmitter to receptor and subsequent channel opening:

$$T + R \underset{K_R}{\longrightarrow} TR \underset{\beta_R}{\overset{\alpha_R}{\rightleftharpoons}} (TR)^*$$

3) enzymatic degradation of receptor:

$$T + E \underset{K_E}{\longrightarrow} TE \xrightarrow{\alpha_E} E$$

where

T = transmitter
R = receptor (post-synaptic channel)
E = enzyme that degrades receptor

TR = closed-channel state of transmitter-receptor complex
$(TR)^*$ = open-channel state of transmitter-receptor complex

TE = transmitter-enzyme complex

There are three simplifying assumptions in the foregoing:

i) that diffusion of transmitter is fast,
ii) that each receptor binds only one molecule of transmitter,
iii) that enzymatic degradation is the only important route of transmitter removal.

The symbol [ ] will be used to denote concentration: number of molecules (or moles) per unit volume. When applied to a membrane-bound molecule, the symbol [ ] will denote the effective concentration: the number of molecules (or moles) at the synapse divided by the volume of the synaptic cleft.

The symbol $\overset{K}{\longleftrightarrow}$ denotes a fast reversible reaction with equilibrium constant K. This notation is shorthand for the limit of $\underset{k_2}{\overset{k_1}{\rightleftharpoons}}$

as $k_1 \to \infty$, $k_2 \to \infty$, with $k_2/k_1 = K$.

Our task now is to write differential equations corresponding to the reaction scheme given above. To do this, it is useful to introduce the concept of <u>net rate</u> per unit volume for each of the past reactions. The net rate is finite although the forward and backward rates are infinite. These net rates must be counted as additional unknowns, since they cannot be determined from the concentrations of the reactants themselves. Thus, let

r_R = <u>net</u> rate per unit volume of $T + R \rightarrow TR$

r_E = <u>net</u> rate per unit volume of $T + E \rightarrow TE$

Then

$$\frac{d[T]}{dt} = r_T - r_R - r_E$$

$$\frac{d[TR]}{dt} = r_R - \alpha_R [TR] + \beta_R [(TR)^*]$$

$$\frac{d[(TR)^*]}{dt} = \alpha_R [TR] - \beta_R [(TR)^*]$$

$$\frac{d[R]}{dt} = -r_R$$

$$\frac{d[TE]}{dt} = r_E - \alpha_E [TE]$$

$$\frac{d[E]}{dt} = -r_E + \alpha_E [TE]$$

These are 6 equations in the 8 unknowns:

$$[T],\ [TR],\ [(TR)^*],\ [R],\ [TE],\ [E],\ r_R,\ r_E$$

(recall that r_T is regarded here as a given quantity). The two missing equations are provided by the equilibrium relations

$$[T][R] = K_R\,[TR]$$

$$[T][E] = K_E\,[TE]$$

Note that these relations hold even though the system is not at thermodynamic equilibrium (or even in a steady state) because the reactions in question are infinitely fast.

The number of unknowns in our system can be reduced by the recognition that there are certain conserved quantities. Let

$$[E_0] = [E] + [TE]$$

$$[R_0] = [R] + [TR] + [(TR)^*]$$

Thus E_0 denotes the enzyme molecule in either of its two states, with or without transmitter, and R_0 denotes the receptor molecule in any of its three states: free, closed with transmitter bound,

and open with transmitter bound. Since neither enzyme nor receptor is created or destroyed, we must have

$$\frac{d[E_0]}{dt} = 0$$

$$\frac{d[R_f]}{dt} = 0$$

and this can also be confirmed by adding the relevant differential equations. We now have enough algebraic equations to express both $[E]$ and $[TE]$ in terms of $[T]$. This is done by solving the 2x2 system

$$[T][E] - K_E[TE] = 0$$

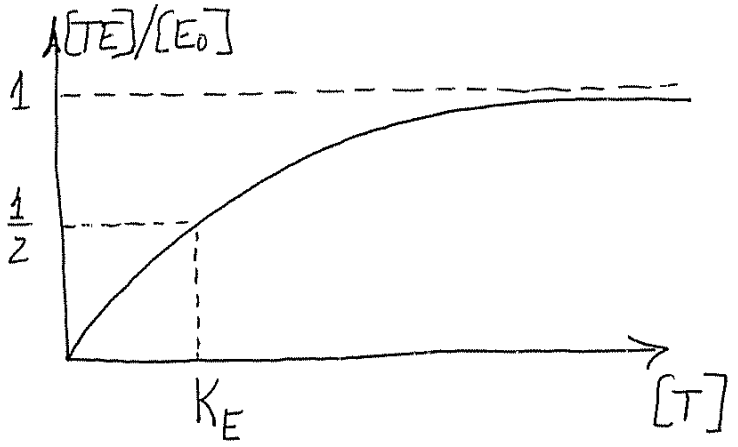
$$[E] + [TE] = [E_0]$$

The result is

$$[E] = \frac{K_E}{K_E + [T]}[E_0]$$

$$[TE] = \frac{[T]}{K_E + [T]}[E_0]$$

This is an important result in enzyme kinetics. It establishes a hyperbolic relationship between the saturation of the enzyme $[TE]/[E_0]$ and the free substrate concentration $[T]$.



Note that the enzyme is half saturated when $[T] = K_E$. This gives an intuitive meaning to the constant K_E (which has units of concentration).

In precisely the same way, we can use the algebraic relations involving R to express $[R]$ and $[TR]$ in terms of $[T]$ and $[(TR)^*]$. Here we solve the pair of equations

$$[T][R] - K_R[TR] = 0$$

$$[R] + [TR] = [R_0] - [(TR)^*]$$

The result is

$$[R] = \frac{K_R}{K_R + [T]}\left([R_0] - [(TR)^*]\right)$$

$$[TR] = \frac{[T]}{K_R + [T]}\left([R_0] - [(TR)^*]\right)$$

We have now reduced the number of unknowns to 4: $[T]$, $[(TR)^*]$, r_R, and r_E. The quantities r_R and r_E can be eliminated* by adding all four differential equations involving T:

$$\frac{d}{dt}\left([T] + [TR] + [(TR)^*] + [TE]\right) = r_T - \alpha_E [TE]$$

In terms of $[T]$ and $[(TR)^*]$ this can be rewritten

$$\frac{d}{dt}\left([T] + \left([R_0] - [(TR)^*]\right)\frac{[T]}{K_R + [T]} + [(TR)^*] + [E_0]\frac{[T]}{K_E + [T]}\right)$$

$$= r_T - \alpha_E [E_0]\frac{[T]}{K_E + [T]}$$

* Think about how you would solve for r_R and r_E if they were of interest.

Finally, the equation for $d[(TR)^*]/dt$ can also be rewritten in terms of $[T]$ and $[(TR)^*]$:

$$\frac{d[(TR)^*]}{dt} = \alpha_R\left([R_0]-[(TR)^*]\right)\frac{[T]}{K_R+[T]} - \beta_R[(TR)^*]$$

The last 2 equations constitute a first order system in the unknowns $[T]$ and $[(TR)^*]$.

Next, we consider the linearization of these equations which occurs when r_T is small. Let

$$r_T = \varepsilon r_T'$$

$$[T] = \varepsilon [T]'$$

$$[(TR)^*] = \varepsilon [(TR)^*]'$$

Substitute, divide each equation by ε, let $\varepsilon \to 0$, and drop the primes. The result is

$$\left(1+\frac{[R_0]}{K_R}+\frac{[E_0]}{K_E}\right)\frac{d[T]}{dt} + \frac{d[(TR)^*]}{dt} = r_T - \alpha_E\frac{[E_0]}{K_E}[T]$$

$$\frac{d[(TR)^*]}{dt} = \alpha_R\frac{[R_0]}{K_R}[T] - \beta_R[(TR)^*]$$

let

$$x = [T] \qquad y = [(TR)^*]$$

$$A = 1 + \frac{[R_0]}{K_R} + \frac{[E_0]}{K_E}$$

$$\gamma_E = \alpha_E \frac{[E_0]}{K_E} \qquad \gamma_R = \alpha_R \frac{[R_0]}{K_R}$$

Then

$$A\frac{dx}{dt} + \frac{dy}{dt} = r_T - \gamma_E x$$

$$\frac{dy}{dt} = \gamma_R x - \beta_R y$$

We shall solve these equations for the situation in which $x(t) = y(t) = 0$ for $t < 0$, and $r(t) = q\delta(t)$. This corresponds to the sudden release at $t = 0$ of a small* amount of transmitter into the synaptic cleft. The amount released is q times the volume of the synaptic cleft.

*Recall that we have linearized the equations.

The first step is to find the conditions prevailing just after the release of transmitter. This is done by integrating over the interval $(-\varepsilon, \varepsilon)$ for some $\varepsilon > 0$ and then letting $\varepsilon \to 0$. Since $x(t) = y(t) = 0$ for $t < 0$, we get

$$A x(\varepsilon) + y(\varepsilon) = a_0 - \gamma_E \int_{-\varepsilon}^{\varepsilon} x(t)\,dt$$

$$y(\varepsilon) = \gamma_R \int_{-\varepsilon}^{\varepsilon} x(t)\,dt - \beta_R \int_{-\varepsilon}^{\varepsilon} y(t)\,dt$$

As long as x and y are bounded, we know that

$$\lim_{\varepsilon \to 0} \int_{-\varepsilon}^{\varepsilon} x(t)\,dt = 0$$

$$\lim_{\varepsilon \to 0} \int_{-\varepsilon}^{\varepsilon} y(t)\,dt = 0$$

Thus

$$\left.\begin{aligned} A x(0^+) + y(0^+) &= a_0 \\ y(0^+) &= 0 \end{aligned}\right\} \Rightarrow x(0^+) = a_0/A$$

where $x(0^+)$ is shorthand for $\lim_{\varepsilon \to 0} x(\varepsilon)$ $(\varepsilon > 0)$

and similarly for $y(0^+)$. Since $\frac{dy}{dt} = \gamma_R x - \beta_R y$, we also have

$$\frac{dy}{dt}(0^+) = \gamma_R x(0^+) - \beta_R y(0^+) = \frac{\gamma_R a_0}{A}$$

This condition will be useful in the following.

Remark: One might expect on physical grounds that $x(0^+)$ would be equal to a_0, the amount of transmitter released at $t=0$ divided by the volume of the synaptic cleft. This is not the case because of the assumptions that the binding of transmitter by receptor R or by enzyme E are infinitely fast processes. Because of these reactions, the concentration of free transmitter in the cleft is instantaneously reduced to a_0/A.

We are now ready to consider the time interval $t > 0$, on which $r_T(t) = 0$. One way to proceed is to find a second-order differential equation for $y(t)$, the concentration of open channels. This is done as follows:

$$\frac{d^2y}{dt^2} = \gamma_R \frac{dx}{dt} - \beta_R \frac{dy}{dt} = \gamma_R \frac{1}{A}\left(-\frac{dy}{dt} - \gamma_E x\right) - \beta_R \frac{dy}{dt}$$

$$= -\frac{\gamma_R}{A}\frac{dy}{dt} - \frac{\gamma_E}{A}\left(\frac{dy}{dt} + \beta_R y\right) - \beta_R \frac{dy}{dt}$$

$$\frac{d^2y}{dt^2} + \left(\beta_R + \frac{\gamma_R}{A} + \frac{\gamma_E}{A}\right)\frac{dy}{dt} + \frac{\beta_R \gamma_E}{A} y = 0$$

We seek the solution of this differential equation on $t>0$ with initial conditions

$$y(0^+)=0 \qquad \frac{dy}{dt}(0^+) = \frac{\gamma_R a_0}{A}$$

as derived above. The solution will be of the form

$$y(t) = c_1 \exp(\lambda_1 t) + c_2 \exp(\lambda_2 t)$$

where λ_1 and λ_2 are the roots of

$$\lambda^2 + \left(\beta_R + \frac{\gamma_R}{A} + \frac{\gamma_E}{A}\right)\lambda + \frac{\beta_R \gamma_E}{A} = 0$$

$$\lambda = \frac{-b \pm \sqrt{b^2-4c}}{2}$$

where

$$b = \beta_R + \frac{\gamma_R}{A} + \frac{\gamma_E}{A} > 0 \qquad c = \frac{\beta_R \gamma_E}{A} > 0$$

Note that $b^2-4c = \left(\left(\beta_R + \frac{\gamma_E}{A}\right) + \frac{\gamma_R}{A}\right)^2 - 4\frac{\beta_R \gamma_E}{A}$

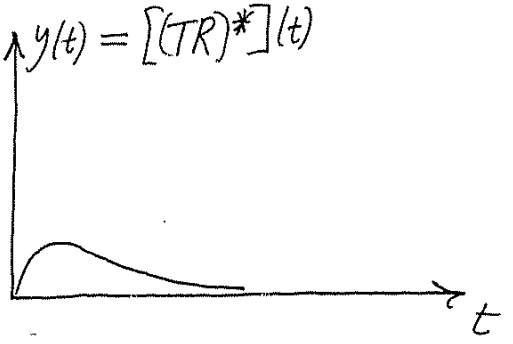
$$= \left(\beta_R + \frac{\gamma_E}{A}\right)^2 + 2\left(\beta_R + \frac{\gamma_E}{A}\right)\frac{\gamma_R}{A} + \frac{\gamma_R^2}{A^2} - 4\frac{\beta_R \gamma_E}{A}$$

$$= \left(\beta_R - \frac{\gamma_E}{A}\right)^2 + 2\left(\beta_R + \frac{\gamma_E}{A}\right)\frac{\gamma_R}{A} + \frac{\gamma_R^2}{A^2} > 0$$

Thus, the roots are real. Also, $\sqrt{b^2-4c} < b$, since $c > 0$, so both roots are negative. From the initial conditions, we find

$$C_1 = -C_2 = \frac{\gamma_R a_0}{A}\left(\frac{1}{\lambda_1 - \lambda_2}\right)$$

Thus, $$y(t) = \frac{\gamma_R a_0}{A}\left(\frac{\exp(\lambda_1 t) - \exp(\lambda_2 t)}{\lambda_1 - \lambda_2}\right)$$



This is the postsynaptic conductance change in response to the sudden release of a small amount (e.g., one vesicle) of transmitter from the presynaptic terminal.

Postsynaptic events

The postsynaptic channel:

At the neuromuscular junction, the channel opened by acetylcholine (the transmitter) has a linear current–voltage relation with a reversal potential (the voltage at which the current is zero) of -15 mv. The channel is selective for positive ions and does not discriminate strongly among different positive ions.

As a model for such a channel, recall our model of a channel with background negative charge on the walls. We considered such a channel with only one type of positive ion permeating it, but suppose there are two, Na^+ and K^+, and suppose for simplicity that these ions have the same mobility. Then the channel cannot distinguish between these ions at all* and so the current–voltage relation must be given by

$$i = g(v - v_{rev})$$

with

$$v_{rev} = \frac{kT}{q} \log \frac{[Na^+]_{out} + [K^+]_{out}}{[Na^+]_{in} + [K^+]_{in}}$$

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* This argument is due to D. Tranchina

Putting in the known concentrations for muscle (in mM) and recalling that the logarithm is to the base e, we get

$$V_{rev} = (25\,mv)\,\log_e \frac{109 + 2.25}{10.4 + 124}$$

$$= (25\,mv)\,\log_e \frac{111.25}{134.4} = -4.7\,mv$$

which is not too far off. One can elaborate the theory by allowing different mobilities. Then the reversal potential becomes

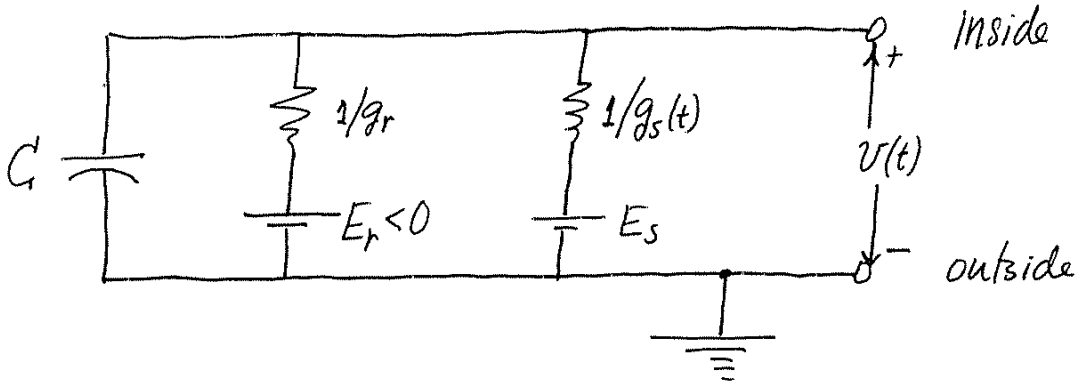
$$V_{rev} = \frac{kT}{q} \log \frac{\mu_{Na}[Na^+]_{out} + \mu_K[K^+]_{out}}{\mu_{Na}[Na^+]_{in} + \mu_K[K^+]_{in}}$$

but the i-V relation becomes nonlinear. We skip the details.

The main point is that the channel has a reversal potential which is well above the rest potential (about -70 mv). Opening this channel therefore causes an increase* in the membrane potential. Such a change is excitatory.

* increase in the algebraic sense: it makes the potential less negative.

The postsynaptic electrical circuit:



The postsynaptic membrane can be modeled by the foregoing circuit, in which

C = membrane capacitance.
E_r = rest potential.
g_r = resting conductance.
E_s = reversal potential of transmitter-sensitive channels.
$g_s(t)$ = overall conductance of transmitter-sensitive channels at time t.
$v(t)$ = transmembrane voltage at time t (inside voltage minus outside voltage).

Note that $g_s(t)$ is proportional to $[(TR)^*]$. Here we regard $g_s(t)$ as given and we seek to determine $v(t)$. The differential

equation for $v(t)$ that corresponds to the foregoing circuit is:

$$C\frac{dv}{dt} + g_r(v - E_r) + g_s(t)(v - E_s) = 0$$

Consider the situation in which $g_s(t)$ is small:

$$g_s(t) = \varepsilon g_s'(t)$$

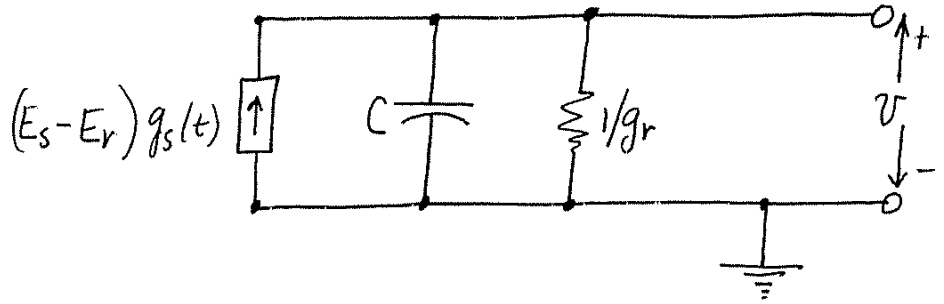
Let v' be defined by

$$v(t) = E_r + \varepsilon v'(t)$$

so that $\varepsilon v'$ is the deviation of the membrane potential from rest. Substitute, divide by ε, let $\varepsilon \to 0$, and drop the primes. The result is

$$C\frac{dv}{dt} + g_r v = (E_s - E_r) g_s(t)$$

This is the equation of the following circuit:



Thus, for small synaptic conductance, the deviation from rest of the membrane potential behaves like the response of a parallel RC circuit to a current source equal to $(E_s - E_r) g_s(t)$. This is excitatory if $E_s > E_r$ and inhibitory if $E_s < E_r$.

We shall solve this equation for the case

$$g_s(t) = g_0 \left(e^{\lambda_1 t} - e^{\lambda_2 t} \right)$$

where $\lambda_2 < \lambda_1 < 0$, with $v(0) = 0$. The solution is of the form

$$v = v_0 e^{-\frac{g_r t}{C}} + v_1 e^{\lambda_1 t} + v_2 e^{\lambda_2 t}$$

where v_1 and v_2 are chosen so that $v_1 e^{\lambda_1 t}$ and $v_2 e^{\lambda_2 t}$ are particular solutions of the equation, and where v_0 is then chosen to satisfy the initial condition. This leads to the equations

$$(C\lambda_1 + g_r) v_1 = (E_s - E_r) g_0$$

$$(C\lambda_2 + g_r) v_2 = -(E_s - E_r) g_0$$

$$0 = v_0 + v_1 + v_2$$

We sketch the solution for the case

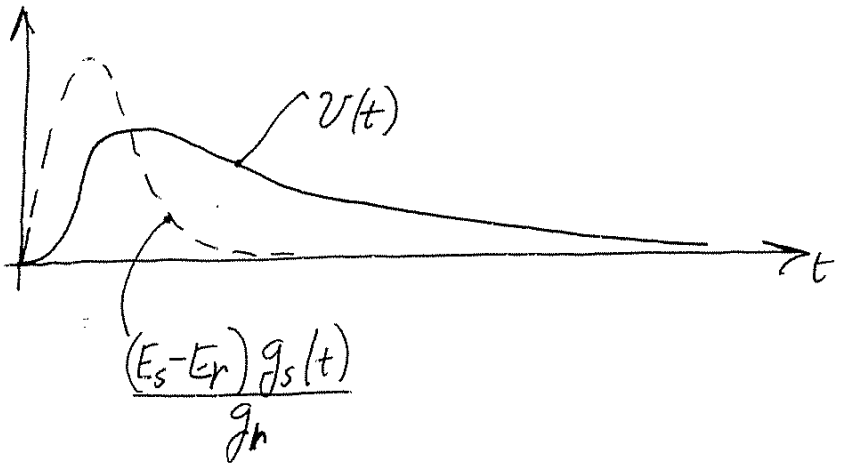
$$\lambda_2 < \lambda_1 < -\frac{g_r}{C} < 0$$

so that the membrane time-constant C/g_r is the longest time-constant in the problem. To make the sketch, it helps to note that

$$C\frac{dv}{dt}(0) + g_r v(0) = (E_s - E_r)\, g_s(0)$$

Thus

$$\frac{dv}{dt}(0) = 0$$



Because of the membrane capacitance, the postsynaptic voltage has a longer tail than the post-synaptic conductance change.

The postsynaptic conductance change is not always small enough for the linear theory to be applicable. When it is not, a new phenomenum comes into play: an increase in conductance can be inhibitory even when its associated reversal potential is above the rest potential. To understand this, it is easiest to consider shunt changes in which the membrane capacitance is effectively zero. Then consider two different synapses acting in parallel on the same cell:

$$g_r (V - E_r) + g_{s_1} (V - E_{s_1}) + g_{s_2} (V - E_{s_2}) = 0$$

with

$$E_r < E_{s_1} < E^* < E_{s_2}$$

where E^* is the threshhold for excitation. Then

$$V = \frac{g_r E_r + g_{s_1} E_{s_1} + g_{s_2} E_{s_2}}{g_r + g_{s_1} + g_{s_2}}$$

Now an increase in g_{s_1} drives the potential towards E_{s_1}. This may be an increase or a decrease in V, depending on the sign of $E_{s_1} - V$. In either case, though, the increase in g_{s_1} is <u>inhibitory</u> because it makes it harder to reach E^*.

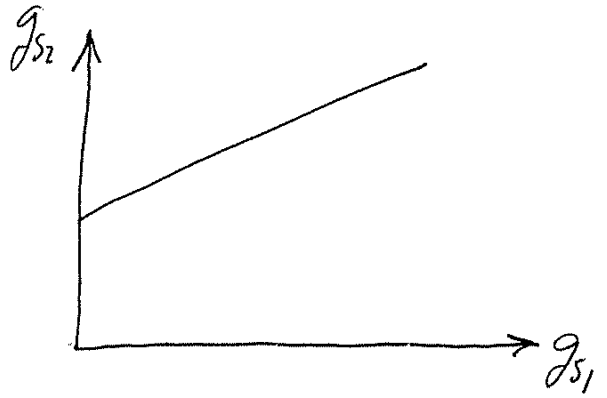
Specifically, $V = E^*$ when

$$E^* = \frac{g_r E_r + g_{s_1} E_{s_1} + g_{s_2} E_{s_2}}{g_r + g_{s_1} + g_{s_2}}$$

or

$$g_r (E^* - E_r) + g_{s_1} (E^* - E_{s_1}) = g_{s_2} (E_{s_2} - E^*)$$

If follows that larger values of g_{s_2} are needed to reach threshhold when g_{s_1} is large. Thus g_{s_1} is <u>inhibitory</u> despite the fact that $E_{s_1} - E_r > 0$.



Conductance at synapse 2 that is needed in order to reach threshhold as a function of the conductance at synapse 1.

Presynaptic events

We now consider the events that are responsible for the release of transmitter from the presynaptic terminal. The release is triggered by a Ca^{++} current, so we begin by considering the Ca^{++} channel.

The presynaptic Ca^{++} channel*

As with any channel, there are two separate factors that together determine the current: the number of open channels and the single-channel current. According to the constant-field model, the current through a single Ca^{++} channel should be of the form

$$i_{Ca} = (2g)\,\frac{aD}{l}\left(\frac{2gV}{kT}\right)\frac{[Ca^{++}]_{in}\exp\left(\frac{2gV}{kT}\right) - [Ca^{++}]_{out}}{\exp\left(\frac{2gV}{kT}\right) - 1}$$

*Llinás R, Steinberg IZ, and Walton K: Presynaptic calcium currents and their relation to synaptic transmission: Voltage clamp study in squid giant synapse and theoretical model of the calcium gate. PNAS 73: 2918-2922, 1976

where

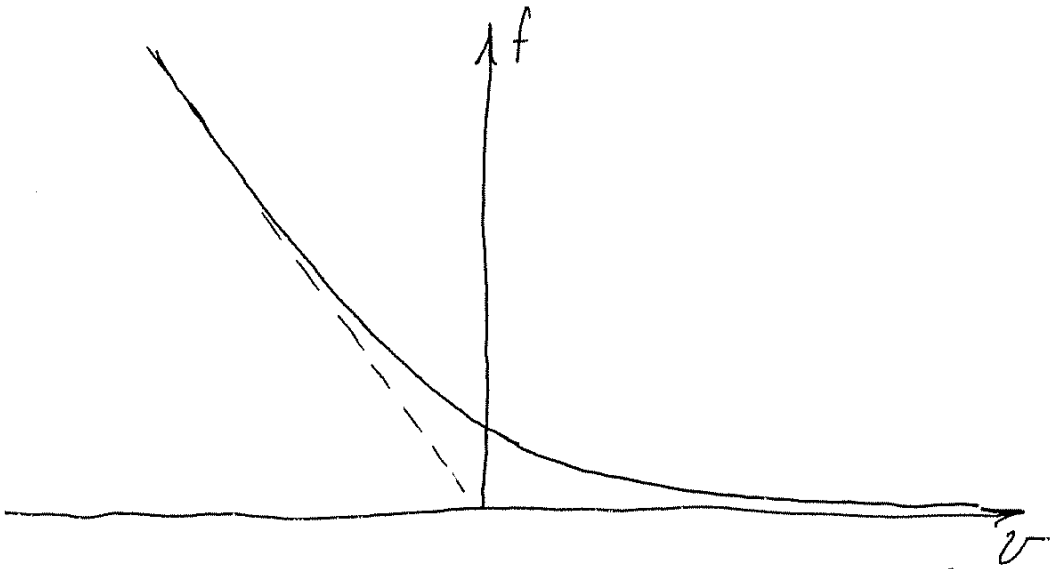
z = elementary charge
a = cross-sectional area of channel
l = channel length
D = diffusion coefficient of Ca^{++}
k = Boltzmann's constant
T = absolute temperature
$[Ca^{++}]_{in}$ = Ca^{++} concentration inside cell
$[Ca^{++}]_{out}$ = Ca^{++} concentration outside cell
v = membrane potential (inside minus outside)
i_{Ca} = Ca^{++} current (considered positive if outward)

Now the free Ca^{++} concentration in cells is very low for three reasons:

i) Ca^{++} is actively pumped out of the cell
ii) Ca^{++} is actively pumped into intracellular organelles
iii) Ca^{++} is heavily buffered

We shall make the approximation that $[Ca^{++}]_{in} = 0$. Also, since we are interested in the flux of Ca^{++} rather than the current, and since that flux is always inward, we define

$$f_{Ca} = -\frac{i_{Ca}}{2\mathcal{F}} = \frac{aD}{l}[Ca^{++}]_{out}\frac{\left(\frac{2\mathcal{F}v}{kT}\right)}{\exp\left(\frac{2\mathcal{F}v}{kT}\right) - 1}$$



Single-channel Ca^{++} flux (positive inward) as a function of membrane potential (inside relative to outside).

We now turn to the process of Ca^{++} channel gating, which has been modeled by Llinás et al. as follows:

i) The Ca^{++} channel has 5 independent subunits

ii) Each subunit has 2 states, with the following transition:

$$S \underset{\beta_s^0}{\overset{\alpha_s^0 \exp\left(\frac{qV}{kT}\right)}{\rightleftarrows}} S^*$$

with $\alpha_s^0 = 2\ ms^{-1}$, $\beta_s^0 = 1\ ms^{-1}$.

iii) The channel is open when all 5 subunits are on the state S^*.

Let $\overline{N}_{Ca}$ be the total number of Ca^{++} channels (assumed large), and let s be the fraction of subunits in the state S^*. Then the number of open channels is given by

$$N_{Ca} = \overline{N}_{Ca}\, s^5$$

and s satisfies

$$\frac{ds}{dt} = \alpha_s^0 \exp\left(\frac{\delta z V}{kT}\right)(1-s) - \beta_s^0 s$$

Multiplying the number of open channels by the flux per channel, we find

$$F_{Ca} = N_{Ca} f_{Ca} = \overline{N}_{Ca}\, s^5\, P_{Ca}\, [Ca^{++}]_{out}\, \frac{\left(\frac{2zV}{kT}\right)}{\exp\left(\frac{2zV}{kT}\right) - 1}$$

where $P_{Ca} = \frac{aD}{l}$ is the single-channel Ca^{++} permeability.

First consider the steady-state Ca^{++} flux. We have

$$S = S_{\infty}(v) = \frac{\alpha_S^0(v)\exp\left(\frac{\delta v}{kT}\right)}{\alpha_S^0(v)\exp\left(\frac{\delta v}{kT}\right) + \beta_S^0(v)}$$

$$= \frac{2\exp\left(\frac{\delta v}{kT}\right)}{2\exp\left(\frac{\delta v}{kT}\right) + 1}$$

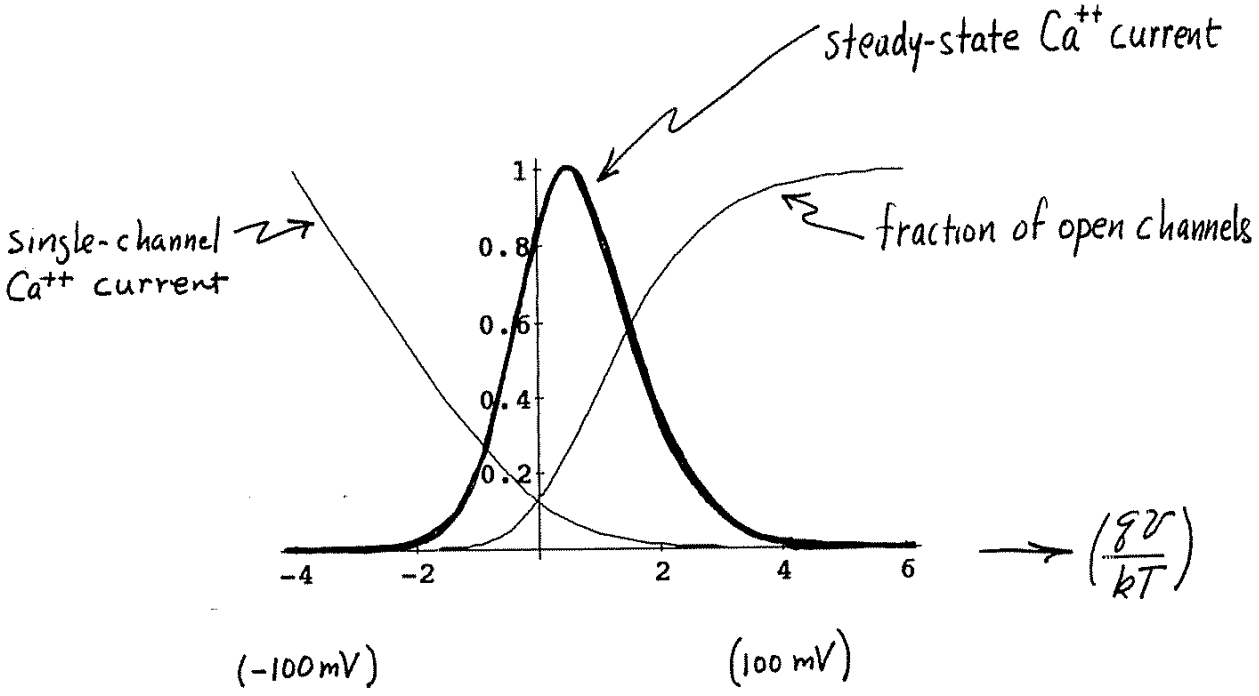
Thus, in the steady state:

$$F_{Ca} = \overline{N}_{Ca}\, p_{Ca}\, [Ca^{++}]_{out} \left(\frac{2\exp\left(\frac{\delta v}{kT}\right)}{2\exp\left(\frac{\delta v}{kT}\right) + 1}\right)^S \left(\frac{\left(\frac{2\delta v}{kT}\right)}{\exp\left(\frac{2\delta v}{kT}\right) - 1}\right)$$

(see plot next page)

In the following, we shall refer to this steady-state flux as $(F_{Ca})_{\infty}(v)$.

Presynaptic Ca++ Current



(Vertical scale is arbitrary except in the case of the fraction of open channels.)

Next, consider the response of the Ca^{++} flux to a voltage step. Let the step go from $v_1 \to v_2$ and occur at $t=0$. Prior to $t=0$, the system has been in a constant state for a long time, so $s = s_\infty(v_1)$ and $F_{Ca} = (F_{Ca})_\infty(v_1)$. For $t>0$, we have

$$\tau_s(v_2)\,\frac{ds}{dt} = s_\infty(v_2) - s$$

with $s(0) = s_\infty(v_1)$. This has the solution

$$s(t) = s_\infty(v_2) + \left(s_\infty(v_1) - s_\infty(v_2)\right)\exp\left(-t/\tau_s(v_2)\right)$$

Then, for $t>0$,

$$F_{Ca}(t) = \bar{N}_{Ca}\,P_{Ca}\,[Ca^{++}]_{out} \cdot \left[s_\infty(v_2) + \left(s_\infty(v_1) - s_\infty(v_2)\right)\exp\left(-\frac{t}{\tau_s(v_2)}\right)\right]^5 \cdot \frac{(2fv_2/kT)}{\exp(2fv_2/kT) - 1}$$

$$= \bar{N}_{Ca}\,P_{Ca}\,[Ca^{++}]_{out}\left\{s_\infty(v_2) + \left(s_\infty(v_1) - s_\infty(v_2)\right)\exp\left(-\frac{t}{\tau_s(v_2)}\right)\right\}^5 \cdot A\left(\frac{2fv_2}{kT}\right)$$

where

$$A(\theta) = \frac{\theta}{\exp(\theta) - 1}$$

We remark that $F_{Ca}(t)$ is <u>not</u> continuous at $t=0$. When the voltage jumps, the number of open Ca^{++} channels does not change instantaneously ($s(t)$ <u>is</u> continuous), but the flux of Ca^{++} through each open channel suffers a jump. Thus

$$F_{Ca}(0^-) = (F_{Ca})_\infty (V_1)$$

but

$$F_{Ca}(0^+) = \bar{N}_{Ca} P_{Ca} [Ca^{++}]_{out} (s_\infty(V_1))^5 A\left(\frac{2gV_2}{kT}\right)$$

$$= (F_{Ca})_\infty (V_1) \frac{A(2gV_2/kT)}{A(2gV_1/kT)}$$

Thus the Ca^{++} flux is suddenly multiplied by the factor

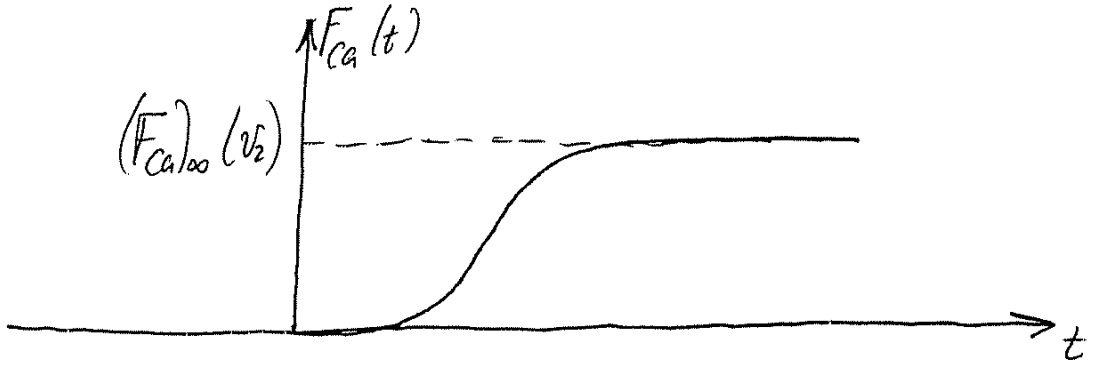
$$\frac{A(2gV_2/kT)}{A(2gV_1/kT)}$$

This factor is less than 1 when $V_2 > V_1$ and more than 1 when $V_2 < V_1$.

For ease of interpretation, we consider the special cases in which $S_\infty(v_1)$ or $S_\infty(v_2)$ is essentially zero. That is, essentially all Ca^{++} channels are closed at the beginning or at the end of the experiment.

If $S_\infty(v_1)=0$, we have

$$F_{Ca}(t) = (F_{Ca})_\infty(v_2)\left(1-\exp\left(-\frac{t}{\tau_s(v_2)}\right)\right)^5$$

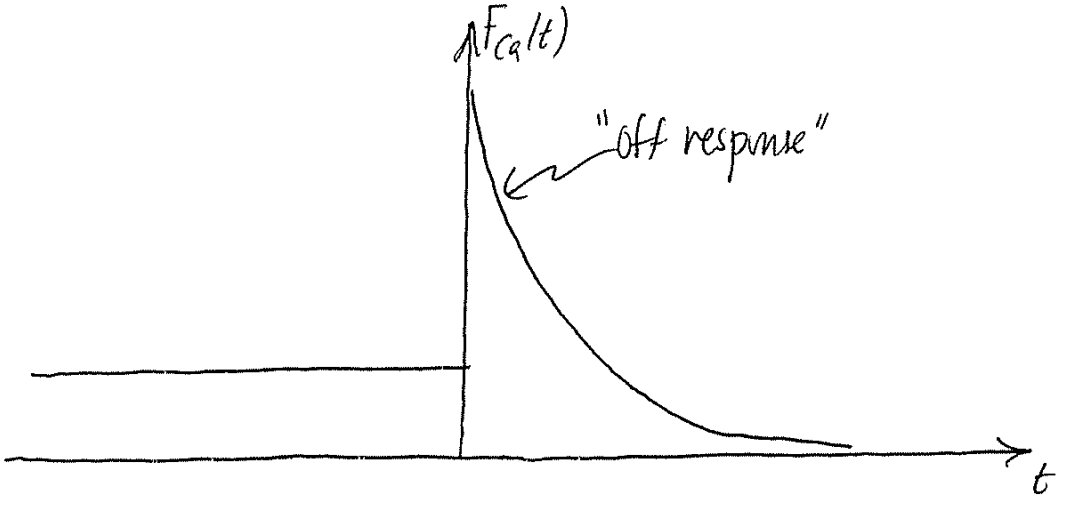


Note the appearance of a delay, which results from the $\sim t^5$ behavior when t is small. (In this special case the Ca^{++} flux is continuous at $t=0$.)

Next, suppose $s_\infty(v_2) = 0$ Then

$$F_{Ca}(t) = \bar{N}_{Ca}\, P_{Ca}\, [Ca^{++}]_{out}\, (s_\infty(v_1))^5 \exp\left(\frac{-5t}{\tau_s(v_2)}\right) A\left(\frac{z_g v_2}{kT}\right)$$

$$= (F_{Ca})_\infty(v_1)\, \frac{A(z_g v_2/kT)}{A(z_g v_1/kT)} \exp\left(\frac{-5t}{\tau_s(v_2)}\right)$$



Thus, there is a large burst of Ca^{++} current following a downward step in voltage that ultimately turns the Ca^{++} current off.

Homework: Derive an approximate formula for the total amount of Ca^{++} that enters the presynaptic terminal during an "off" transient in which the voltage steps down from a large positive voltage to the rest potential (which you may take to be given by $\frac{z_g v}{kT} = -3$)

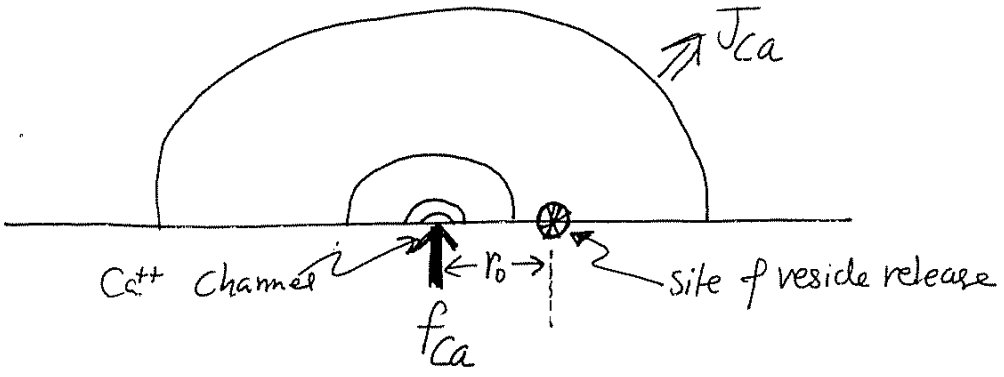
Ca^{++} diffusion within the presynaptic terminal*

The transmitter-laden vesicles of the presynaptic terminal release their contents into the synaptic cleft at specialized membrane sites. Release involves fusion of the vesicle membrane and the cell membrane; this process requires the presence of Ca^{++} within the presynaptic terminal. The vesicle release sites are close to the Ca^{++} channels, however, so the relevant Ca^{++} concentration for vesicle release is the local concentration at some small distance from the channel. This local concentration can be much higher than the general concentration of free Ca^{++} within the presynaptic terminal.

We make the following assumptions:

1) Each release site has its own Ca^{++} channel. (The other channels are too far away to have any influence on the site in question.)
2) The presynaptic terminal looks to each channel like a half-space with plane boundary (the membrane) containing a source at one point on the boundary (the channel).
3) The Ca^{++} concentration approaches zero with increasing distance from the channel.
4) The Ca^{++} concentration profile near the channel rapidly achieves a steady state

* Simon SM and Llinás R: Compartmentalization of the submembrane calcium activity during calcium influx and its significance in transmitter release. Biophys. J. 48: 485-498, 1985



Let the flux through the channel be f_{Ca}. For an open channel, we previously found

$$f_{Ca} = \frac{aD}{l}[Ca^{++}]_{out}\,\frac{\left(\frac{2qV}{kT}\right)}{\exp\left(\frac{2qV}{kT}\right) - 1}$$

(see p. 25). For a closed channel $f_{Ca} = 0$.

We seek a <u>spherically symmetric</u> solution to the steady-state diffusion problem because such a solution is consistent with the boundary condition of no flux through the membrane (except at the channel). Let $J_{Ca}(r)$ be the flux per unit area at radius r. According to Fick's law of diffusion

$$J_{Ca} = -D\frac{\partial}{\partial r}[Ca^{++}]$$

In the steady state, the flux through an arbitrary hemisphere at radius r must equal the flux through the channel. Thus

$$f_{Ca} = 2\pi r^2 J_{Ca} = -2\pi r^2 D \frac{\partial [Ca^{++}]}{\partial r}$$

It follows that

$$[Ca^{++}] = \int_r^\infty \frac{f_{Ca}\, dr'}{D 2\pi (r')^2} = \frac{f_{Ca}}{D 2\pi r}$$

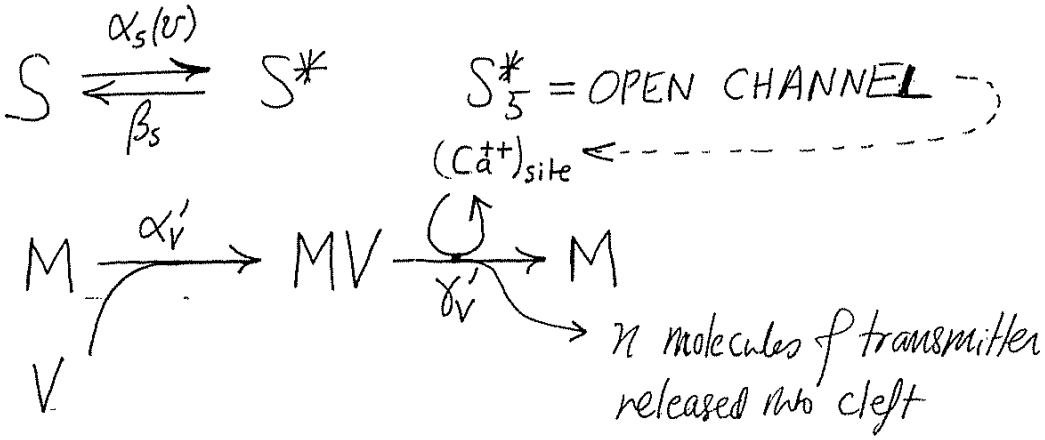
Thus the Ca^{++} concentration at a distance r_0 from the open channel is given by

$$[Ca^{++}]_{site} = [Ca^{++}]_{out} \frac{a}{2\pi r_0 l} \frac{\left(\frac{2gV}{kT}\right)}{\exp\left(\frac{2gV}{kT}\right) - 1}$$

Note how the diffusion coefficient D drops out, and we get simple proportionality between the external Ca^{++} concentration and the concentration at the site of vesicle release (when the channel is open). This proportionality involves the dimensionless geometric parameter $\left(\frac{a}{r_0 l}\right)$ and the dimensionless membrane potential parameter $\left(\frac{2gV}{kT}\right)$. When the channel is closed, $[Ca^{++}]_{site} = 0$.

Release of vesicles from the presynaptic terminal

We have assumed in the foregoing that each membrane site for vesicle release has its own Ca^{++} channel and that the Ca^{++} concentration at the release site has a switch-like character, being zero when the channel is closed and instantaneously determined by the solution of the steady-state diffusion equation when the channel is open. Thus, the membrane site at the Ca^{++} channel form a complex, and the kinetics of vesicle release can only be described by considering all possible states of that complex. The reactions in question are as follows:



Here S denotes one of the 5 subunits of the Ca^{++} channel; when all 5 subunits are in the state S^*, the channel is open and Ca^{++} appears at the vesicle release site, which is denoted by M. The free vesicles are denoted V, and MV represents a site with a vesicle bound, ready for release. When release occurs, the vesicle is destroyed, the

free membrane site M is regenerated, and n molecules of transmitter (a so-called "quantum" of transmitter) are dumped into the synaptic cleft.

Let

$$\alpha_V = \alpha_V' [V]$$

(we assume that the vesicle concentration $[V]$ in the presynaptic terminal is effectively constant). Then α is the first-order rate constant (probability per unit time) for the reaction $M \rightarrow MV$. Similarly, let

$$\gamma_V = \gamma_V' \left[Ca^{++}\right]_{\text{site}}^{\text{OPEN CHANNEL}}$$

$$= \gamma_V' \left[Ca^{++}\right]_{out} \frac{a_0}{2\pi r_0 l} \frac{\left(\frac{2qV}{kT}\right)}{\exp\left(\frac{2qV}{kT}\right) - 1}$$

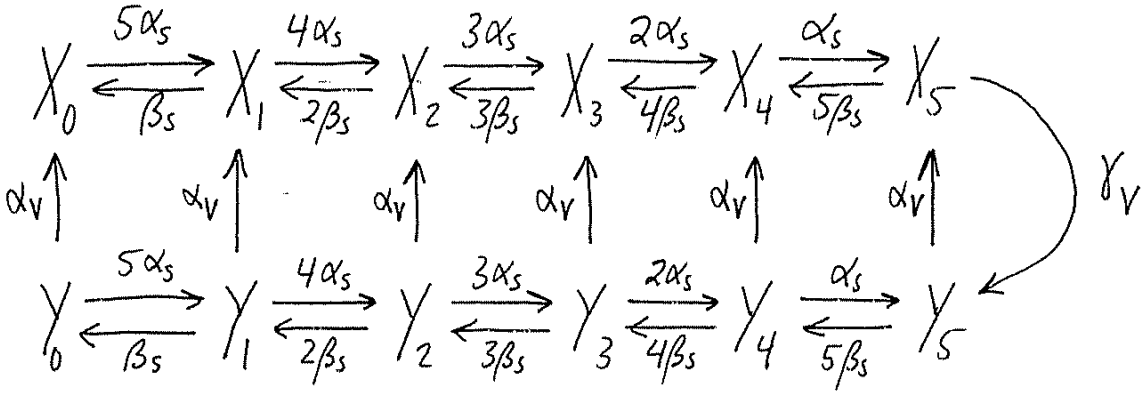
Then γ_V is the first-order rate constant (probability per unit time) for the reaction $MV \rightarrow M$ <u>when the Ca^{++} channel is open</u>. When the Ca^{++} channel is closed, the reaction $MV \rightarrow M$ cannot occur.

We are now in a position to write down a complete state diagram for the (channel) – (release site) complex. To simplify the notation, let

$$X_k = S_{5-k}\, S^*_k\, MV$$

$$Y_k = S_{5-k}\, S^*_k\, M$$

where $k \in \{0,1,2,3,4,5\}$. Thus X_k denotes a state in which k channel subunits are in the state S^*, $5-k$ channel subunits are in the state S, and the membrane release site has a vesicle bound. The state Y_k is the same as X_k except that the membrane release site is empty. The possible states and transitions are as follows:



Note that the entire diagram is determined by 4 rate constants: $\alpha_s, \beta_s, \alpha_v$, and γ_v. Of these, α_s and γ_v are voltage-dependent, whereas β_s and α_v are constants.

The numerical factors multiplying α_s and β_s in the foregoing are explained as follows. Consider, for example, the reaction

$$X_1 \underset{2\beta_s}{\overset{4\alpha_s}{\rightleftharpoons}} X_2$$

In the state X_1, there are $5-1=4$ subunits in the state S which are therefore available for the transition

$$S \xrightarrow{\alpha_s} S^*$$

Hence the rate constant for $X_1 \rightarrow X_2$ is $4\alpha_s$. Similarly, in state X_2, there are 2 subunits in the state S^* which are therefore available for the reaction

$$S \xleftarrow{\beta_s} S^*$$

so the **rate constant** for $X_1 \leftarrow X_2$ is $2\beta_s$.

Statistics of vesicle release during an off-transient

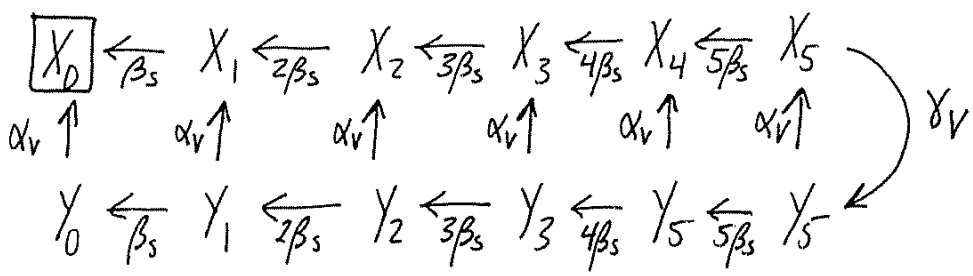
Consider the following experimental manipulation of the presynaptic membrane potential:

1) let the voltage stay at rest $\left(\frac{qV}{kT}\right) = -3$ for a long time.

2) Step the voltage up to a level where $\frac{qV}{kT} >> 1$ and hold it there for a while (long enough to open all channels, see below)

3) Step the voltage back down to rest, hold it there, and count the number of vesicles released. (Each release event causes a blip (EPSP or IPSP) in the postsynaptic membrane potential.

During step (1) α_s is negligible, since $\alpha_s = \alpha_s^0 \exp\left(\frac{qV}{kT}\right)$ with $\alpha_s^0 = 2\ ms^{-1}$; thus at $\frac{qV}{kT} = -3$, $\alpha_s \approx 0.1\ ms^{-1}$ whereas $\beta_s = 1\ ms^{-1}$. Thus the state diagram is effectively this:

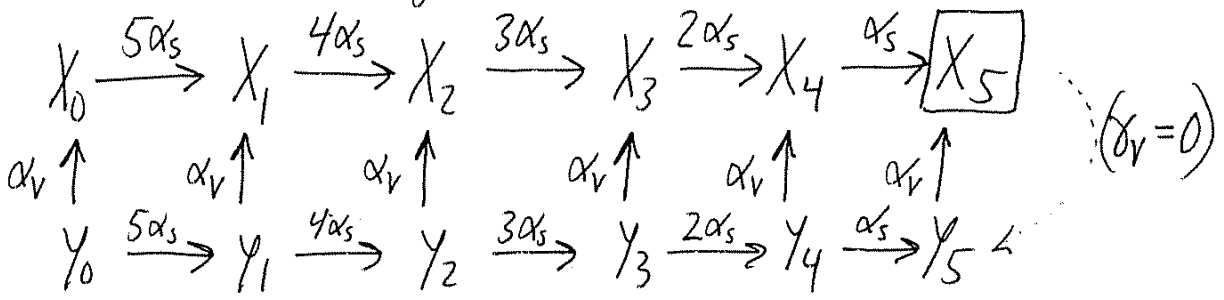


The system moves to the absorbing state X_0 and stays there. Thus the channel is closed with all subunits in state S and the membrane release site is occupied by a vesicle.

During step (2), α_s becomes very large and all channels open. There is essentially no vesicle release, however, because γ_V becomes very small: recall that γ_V is proportional to

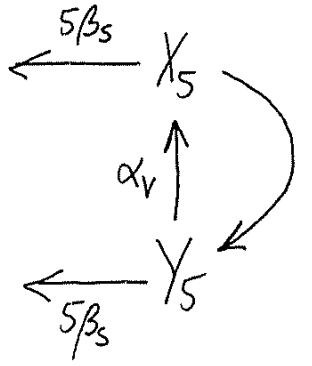
$$\frac{\left(\frac{2qV}{kT}\right)}{\exp\left(\frac{2qV}{kT}\right) - 1}$$

Thus, the state diagram is effectively this:



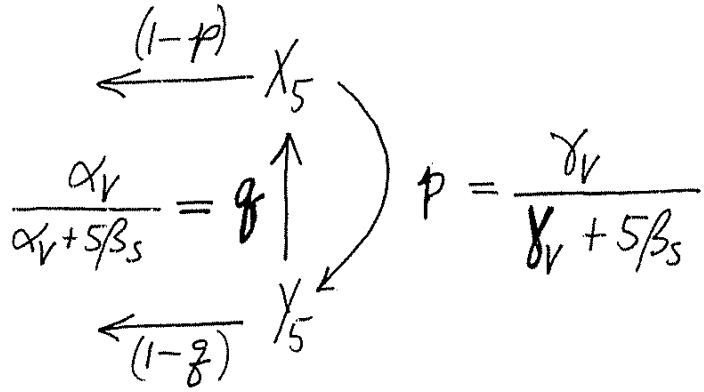
The system moves to the absorbing state X_5 and stays there. In the state X_5, the channel is open (all 5 subunits are in the state S^*) and the membrane site has a vesicle bound and ready for release. Release does not actually occur, however, because the Ca^{++} current through the open channel is negligible at such high membrane potential.

Finally, when the voltage jumps back to the rest potential (step 3) we can analyze the transient in terms of the following diagram:



$$\gamma_V = [Ca^{++}]_{out} \frac{a}{2\pi r_0 \ell} \quad (6)$$

in which the system is known to be in the state X_5 at $t=0$. We are interested in the number of vesicle release events which occur before the system exits to the left. (Each release event is a transition along the arrow labeled γ_V.) To find the probability distribution of this number, we relabel the foregoing diagram in terms of probabilities



(transitions labeled by probabilities)

Let n_V = number of vesicles released (from a single site) during the off-transient.

First, there is only one way that n_V can be zero: if the first transition away from X_5 is to the left. Thus

$$P(n_V = 0) = 1 - p$$

For $k > 0$, there are two separate ways to have $n_V = k$.

$$P(n_V = k \text{ and } (\text{exit is from } X_5)) = (pf)^k (1-p)$$

$$P(n_V = k \text{ and } (\text{exit is from } X_5)) = (pf)^{k-1} p (1-f)$$

Thus

$$\begin{aligned} P(n_V = k) &= (pf)^k (1-p) + (pf)^{k-1} p (1-f) \\ &= p^k f^{k-1} [f(1-p) + 1 - f] \\ &= p^k f^{k-1} (1 - pf) \\ &= (pf)^{k-1} p (1-pf) \qquad , \quad k \geq 1 \end{aligned}$$

As a check, we compute

$$P(n_V \geq 1) = \sum_{k=1}^{\infty} (pf)^{k-1} \; p(1-pf)$$

$$= \frac{1}{1-(pf)} \; p(1-pf) \quad = p$$

This is the correct result, since $P(n_V = 0) = 1-p$.

Note that:

$$P(n_V = 0) \;=\; 1-p$$

$$P(n_V = 1) \;=\; p - p^2 f$$

$$P(n_V \geq 2) \;=\; p^2 f$$

When p or f (or both) are small, the only possibilities that require serious consideration are the release of zero vesicles or one, and these events have probability $(1-p)$ and p, respectively.

Next we derive formulae for the mean and variance of the number of vesicles released from a single site. To do this we need the identities

$$\sum_{k=1}^{\infty} k x^{k-1} = \frac{1}{(1-x)^2}$$

$$\sum_{k=1}^{\infty} k^2 x^{k-1} = \frac{1+x}{(1-x)^3}$$

(These can be derived from $\sum_{k=0}^{\infty} x^k = \frac{1}{1-x}$ by successive differentiation and other algebraic manipulation. We leave the details to the reader.) Then

$$E[n_V] = \sum_{k=0}^{\infty} k\, P(n_V = k) = \sum_{k=1}^{\infty} k (pq)^{k-1} p(1-pq) = \frac{p}{1-pq}$$

$$E[n_V^2] = \sum_{k=0}^{\infty} k^2 P(n_V = k) = \sum_{k=1}^{\infty} k^2 (pq)^{k-1} p(1-pq) = \frac{(1+pq)p}{(1-pq)^2}$$

$$Var[n_V] = E[n_V^2] - (E[n_V])^2 = \frac{p - p^2(1-q)}{(1-pq)^2}$$

The limiting cases $f=0$ and $f=1$ are of interest:

When $f=0$, there is no replenishing of vesicles during the off-transient: at most one vesicle can be released from each site

$$Pr(n_V=0) = 1-p$$

$$Pr(n_V=1) = p$$

$$Pr(n_V \geq 2) = 0$$

$$E[n_V] = p$$

$$Var[n_V] = p-p^2 = p(1-p)$$

When $f=1$, vesicles are replenished immediately and there is the possibility that many vesicles can be released from a single site during an off-transient

$$Pr(n_V=k) = (1-p)\,p^k \qquad (\text{all } k, \text{ including } k=0)$$

$$E[n_V] = \frac{p}{1-p} \qquad Var[n_V] = \frac{p}{(1-p)^2}$$

Up to now, our considerations have refered to a single site. Let the total number of vesicle release sites of the presynaptic terminal be N_S and let N_V be the total number of vesicles released in the experimental protocol described above. Then

$$N_V = \sum_{i=1}^{N_S} (n_V)_i$$

where the index i labels the site. The random variables $(n_V)_i$ are independent and identically distributed.

Thus

$$E[N_V] = \sum_{i=1}^{N_S} E[(n_V)_i] = N_S \frac{p}{1-pq}$$

$$Var[N_V] = \sum_{i=1}^{N_S} Var[(n_V)_i] = N_S \frac{p - p^2(1-q)}{(1-pq)^2}$$

Homework (analysis of vesicle depletion):

1) In the off-transient described above, what is the probability that the vesicle release site is occupied when the Ca^{++} channel closes?

2) While the presynaptic terminal is resting after the experiment, how does this probability increase with time?

3) Suppose the experiment is repeated after some finite interval of time, so the release site is not certain to be occupied. Let r_0 be the probability that it is occupied at the moment the voltage steps down and the off-transient begins. Compute $P(n_f = k)$ as a function of r_0, p, and q. Hint: consider separately the two cases in which the system starts in X_5 (which has probability r_0) or in Y_5 (which has probability $1-r_0$).

4) What is the probability that the release site is occupied at the end of this experiment when the Ca^{++} channel closes?

5) How could these results be used to analyze a series of off-transients separated by finite intervals of time?

Differential equations for the expected rate of vesicle release

In this section we do not consider fluctuations, but we do consider arbitrary voltage waveforms $v(t)$. Recall the state diagram for the (channel)–(release site) complex (page 39). Let x_k be the probability of finding the system in the state X_k and let y_k be the probability of finding the system in the state Y_k. Then x_k and y_k change with time as follows:

$$\frac{dx_k}{dt} = \alpha_s(5-k-1)x_{k-1} + \beta_s(k+1)x_{k+1} + \alpha_V y_k$$
$$- \alpha_s(5-k)x_k - \beta_s k x_k - \gamma_V \delta_{k5} x_k$$

$$\frac{dy_k}{dt} = \alpha_s(5-(k-1))y_{k-1} + \beta_s(k+1)y_{k+1} + \gamma_V \delta_{k5} y_k$$
$$- \alpha_s(5-k)y_k - \beta_s k y_k - \alpha_V y_k$$

where

$k = 0, 1, 2, 3, 4, 5$

with

$$x_{-1} = x_6 = 0 \qquad y_{-1} = y_6 = 0$$

and where

$$\delta_{k5} = \begin{cases} 1 & k=5 \\ 0 & k \neq 5 \end{cases}$$

These equations can be simplified somewhat by looking for a solution of the form

$$x_k = p_k \binom{5}{k} s^k (1-s)^{5-k}$$

$$y_k = (1-p_k)\binom{5}{k} s^k (1-s)^{5-k}$$

where

$$\binom{5}{k} = \frac{5!}{k!\,(5-k)!} \qquad \text{(binomial coefficient)}$$

and where s satisfies the Hodgkin-Huxley kinetics

$$\frac{ds}{dt} = \alpha_s (1-s) - \beta_s s$$

We seek differential equations for p_k which will ensure that the differential equations for x_k and y_k are satisfied. Note that

$$x_{k+1} = p_{k+1}\binom{5}{k+1} s^{k+1}(1-s)^{5-k-1} = p_{k+1}\,\frac{5-k}{k+1}\,\frac{s}{1-s}\binom{5}{k} s^k (1-s)^{5-k}$$

$$x_{k-1} = p_{k-1}\binom{5}{k-1} s^{k-1}(1-s)^{5-k+1} = p_{k-1}\,\frac{k}{5-(k-1)}\,\frac{1-s}{s}\binom{5}{k} s^k (1-s)^{5-k}$$

$$\frac{dx_k}{dt} = \frac{dp_k}{dt}\binom{5}{k} s^k (1-s)^{5-k} + p_k \binom{5}{k}\left[k s^{k-1}(1-s)^{5-k} - (5-k) s^k (1-s)^{5-k-1}\right]\frac{ds}{dt}$$

$$= \binom{5}{k} s^k (1-s)^{5-k}\left[\frac{dp_k}{dt} + p_k\left(\frac{k}{s} - \frac{5-k}{1-s}\right)\left(\alpha_s(1-s) - \beta_s s\right)\right]$$

Therefore,

$$\frac{dp_k}{dt} + \left(\alpha_s k \frac{1-s}{s} + \beta_s (5-k)\frac{s}{1-s}\right) p_k$$

$$= \alpha_s k \frac{1-s}{s} p_{k-1} + \beta_s (5-k) \frac{s}{1-s} p_{k+1}$$

$$+ \alpha_v (1-p_k) - \gamma_v \delta_{5k} p_k$$

or

$$\frac{dp_k}{dt} = \alpha_s k \left(\frac{1-s}{s}\right)(p_{k-1} - p_k) + \beta_s (5-k)\left(\frac{s}{1-s}\right)(p_{k+1} - p_k)$$

$$+ \alpha_v (1-p_k) - \gamma_v \delta_{5k} p_k$$

(This was obtained by substitution in the equation for dx_k/dt, but substitution in the equation for dy_k/dt yields the same result.) In summary, the mean release rate for arbitrary $v(t)$ can be found by solving the system:

$$\frac{ds}{dt} = \alpha_s (1-s) - \beta_s s$$

$$\frac{dp_k}{dt} = \alpha_s k \left(\frac{1-s}{s}\right)(p_{k-1} - p_k) + \beta_s (5-k)\left(\frac{s}{1-s}\right)(p_{k+1} - p_k)$$

$$+ \alpha_v (1-p_k) - \gamma_v \delta_{5k} p_k$$

$k = 0, 1, 2, 3, 4, 5$

(recall that α_s and γ_v depend on v)

The mean release rate (per site) is then given by $\gamma_v p_5 s^5$

For example, the steady-state vesicle release rate may be found as follows.

$$\frac{dS}{dt} = 0 \Rightarrow \quad \alpha_s (1-S) = \beta_s S$$

Hence, the steady equations for P_k are:

$$0 = \beta_s\, k\,(P_{k-1} - P_k) + \alpha_s (5-k)(P_{k+1} - P_k)$$

$$+ \alpha_v (1 - P_k) - \gamma_v \delta_{5k} P_k$$

Let $\phi_k = 1 - P_k$ Then

$$\beta_s k(\phi_{k-1} - \phi_k) + \alpha_s (5-k)(\phi_{k+1} - \phi_k) - (\alpha_v + \gamma_v \delta_{5k})\phi_k = -\gamma_v \delta_{5k}$$

This tridiagonal system can be solved as follows. Let

$$\phi_{k-1} = \lambda_k \phi_k \quad , \quad k = 1, 2, 3, 4, 5$$

Then $\phi_k = \lambda_{k+1} \phi_{k+1}$

or $\dfrac{\phi_k}{\lambda_{k+1}} = \phi_{k+1}$ $\Bigg\}$ $k = 0, 1, 2, 3, 4$

For $k<5$, $\delta_{5k}=0$, and we have

$$\beta_s k\left(\lambda_k - 1\right) + \alpha_s (5-k)\left(\frac{1}{\lambda_{k+1}} - 1\right) = \alpha_V$$

$$\frac{1}{\lambda_{k+1}} - 1 = \frac{\alpha_V + \beta_s k(1-\lambda_k)}{\alpha_s (5-k)}$$

$$\frac{1}{\lambda_{k+1}} = 1 + \frac{\alpha_V + \beta_s k(1-\lambda_k)}{\alpha_s (5-k)}$$

$$\lambda_{k+1} = \frac{\alpha_s (5-k)}{\alpha_V + \alpha_s (5-k) + \beta_s k(1-\lambda_k)}$$

Note that this formula works even for $k=0$, which yields

$$\lambda_1 = \frac{\alpha_s 5}{\alpha_V + \alpha_s 5}$$

Starting from λ_1 we can find $\lambda_2, \lambda_3, \lambda_4, \lambda_5$. Note that these are all independent of γ_V. Finally, we use the equation for $k=5$:

$$5\beta_s (\lambda_5 - 1)\phi_5 - (\alpha_V + \gamma_V)\phi_5 = -\gamma_V$$

$$\phi_5 = \frac{\gamma_V}{\gamma_V + \alpha_V + 5\beta_5(1-\lambda_5)}$$

Let

$$\gamma = \alpha_V + 5\beta_5(1-\lambda_5)$$

Then

$$\phi_5 = \frac{\gamma_V}{\gamma_V + \gamma}$$

$$p_5 = 1 - \phi_5 = \frac{\gamma}{\gamma_V + \gamma}$$

and the steady-state rate of vesicle release (per site) is given by

$$\gamma_V p_5 s_\infty^5 = \frac{s_\infty^5 \gamma_V \gamma}{\gamma_V + \gamma}$$

Where γ is a function of α_5, β_5, and α_V, but <u>not</u> of γ_V.

(To find γ explicitly, one must apply the recursive definition of λ_k on the previous page.)

Since γ_V is proportional to $[Ca^{++}]_{out}$ and since all of the other parameters are independent of $[Ca^{++}]_{out}$, we have found that the mean rate of vesicle release in the steady state is related to $[Ca^{++}]_{out}$ by an equation of the form

$$\text{release rate} = \frac{[Ca^{++}]_{out}}{[Ca^{++}]_{out} + K(\gamma)}$$

As long as we are on the linear part of this curve, $\gamma_V << \gamma$ and

$$\gamma_V P_5 S_\infty^5 \approx S_\infty^5 \gamma_V$$

which is proportional to the Ca^{++} flux into the presynaptic terminal.