A model of bidirectional synaptic plasticity: From signaling network to channel conductance

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In many regions of the brain, including the mammalian cortex, the strength of synaptic transmission can be bidirectionally regulated by cortical activity (synaptic plasticity). One line of evidence indicates that long-term synaptic potentiation (LTP) and long-term synaptic depression (LTD), correlate with the phosphorylation/dephosphorylation of sites on the \( \alpha \)-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit protein GluR1. Bidirectional synaptic plasticity can be induced by different frequencies of presynaptic stimulation, but there is considerable evidence indicating that the key variable is calcium influx through postsynaptic \( \text{N}
\text{methyl-D-aspartate (NMDA)} \text{ receptors. Here, we present a biophysical model of bidirectional synaptic plasticity based on } [\text{Ca}^{2+}] \text{-dependent phospho/dephosphorylation of the GluR1 subunit of the AMPA receptor. The primary assumption of the model, for which there is wide experimental support, is that the postsynaptic calcium concentration, and consequent activation of calcium-dependent protein kinases and phosphatases, is the trigger for phosphorylation/dephosphorylation at GluR1 and consequent induction of LTP/LTD. We explore several different mathematical approaches, all of them based on mass-action assumptions. First, we use a first order approach, in which transition rates are functions of an activator, in this case calcium. Second, we adopt the Michaelis-Menten approach with different assumptions about the signal transduction cascades, ranging from abstract to more detailed and biologically plausible models. Despite the different assumptions made in each model, in each case, LTD is induced by a moderate increase in postsynaptic calcium and LTP is induced by high \( \text{Ca}^{2+} \text{ concentration.}

The majority of fast excitatory synaptic transmission in the mammalian central nervous system is mediated by the AMPA (\( \alpha \)-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) subtype of ionotropic glutamate receptor. As such, changes in the conductance of individual AMPA receptors (AMPARs) will have a significant effect on the efficacy of synaptic transmission. Phosphorylation and dephosphorylation of receptors have been shown to be major mechanisms of regulation of synaptic strength (Walaas and Greengard 1991; Mallinow and Malenka 2002). The signal-transduction cascades controlling synaptic phosphorylation/dephosphorylation are complex, and mathematical descriptions of such networks are essential in order to obtain a quantitative understanding of their function. AMPA receptors are tetramers composed of four homologous subunit proteins (GluR1–GluR4) that combine to form different AMPA receptor subtypes (Petralia and Wenthold 1992). The AMPA receptor subunit protein GluR1 is one of the most abundantly expressed subunits in the mammalian hippocampus and neocortex, and in combination with GluR2, is thought to comprise the majority of AMPA receptor complexes in these regions (Wenthold et al. 1996). Several serine residues in the intracellular carboxy-terminal tail of GluR1 have been identified as important sites for activity-dependent regulation of AMPA receptor function by phosphorylation. Protein kinase A (PKA) specifically phosphorylates serine 845 (S845), while protein kinase C (PKC) and CaMKII phosphorylate serine 831 (S831). Phosphorylation of S831 increases the unitary conductance of the AMPAR, and phosphorylation at S845 increases the channel mean open time, in both cases increasing the average channel conductance (Roche et al. 1996; Barria et al. 1997; Benke et al. 1998; Derkach et al. 1999; Banko et al. 2000). Dephosphorylation at each site is mediated by an activity-dependent protein phosphatase cascade. Long term potentiation (LTP), a sustained increase in synaptic strength, is associated with an increased phosphorylation at S831 (Barria et al. 1997; Lee et al. 2000a), whereas long term depression (LTD), a sustained decrease in synaptic strength, has been associated with a decrease in S845 phosphorylation (Kameyama et al. 1998; Lee et al. 1998, 2000a).

A considerable body of evidence indicates that a key variable controlling the sign and magnitude of synaptic plasticity is the amount of integrated postsynaptic NMDA receptor (NMDAR) activation (Dudek and Bear 1992; Mulkey and Malenka 1992; Cummings et al. 1996). Modest NMDAR activation, induced by low-frequency stimulation, results in LTD, while strong, high-frequency activation produces LTP. NMDAR's are permeable to \( \text{Ca}^{2+} \); therefore, it has been proposed that an increase in postsynaptic calcium is a primary signal for the induction of bidirectional synaptic plasticity (Bear et al. 1987; Lisman 1989; Artola and Singer 1993). A robust, transient increase in postsynaptic calcium is observed during high-frequency stimulation (HFS), while a modest prolonged increase in postsynaptic calcium is observed during low-frequency stimulation (LFS). Indeed, chelating postsynaptic calcium with EGTA blocks both high-frequency-induced LTP and low-frequency-induced LTD (Lynch et al. 1983; Brocher et al. 1992). Here, we present a model of bidirectional synaptic plasticity (Fig. 1) based on regulation of AMPAR func-

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A schematic of an excitatory glutamatergic synapse. Action potentials traveling down the presynaptic axon trigger the calcium-dependent release of glutamate. Glutamate binds to two types of postsynaptic ionotropic receptors, NMDARs and AMPARs. The postsynaptic terminal contains a set of enzymes (PP1, PP2b, PKA, CaMKII) that transduce the influx of Ca\(^{2+}\) through the NMDARs into changes in the phosphorylation state of GluR1, thereby regulating AMPAR conductance.

A model for the AMPAR phosphorylation cycle

A first step toward the construction of a model for the AMPAR phosphorylation cycle (Fig. 2) is to note that the involved kinases and phosphatases can be grouped in phospho–dephospho couples acting on the same substrate. We will refer to these enzymes as K1, P1, K2, and P2, where K1 stands for Enzyme Kinase of type 1, P1 for Enzyme Phosphatase of type 1, and so on. These enzymes act on the GluR1 subunit (A) that can be phosphorylated and dephosphorylated at different sites, with high specificity. We make a crucial assumption, supported by sufficient experimental evidence (Meyer et al. 1992), that these enzymes are differentially activated by intracellular [Ca\(^{2+}\)].

In this section, we assume that the enzymatic activity of each enzyme can be represented as a function of [Ca\(^{2+}\)]. It is known that at lower [Ca\(^{2+}\)], phosphatases are more activated than kinases, while at higher [Ca\(^{2+}\)], kinases are more activated than phosphatases.

The AMPAR phosphorylation cycle shown in Figure 2, which assumes two phosphorylation sites on GluR1, is composed of four reactions:

\[
\begin{align*}
A & \xrightarrow{K_1} A_{p1}, \quad A^{p2} \xrightarrow{K_2} A_{p1}^{p2}, \\
A^{p2} & \xrightarrow{P_1} A_{p1}^{p2}, \quad A_{p1} \xrightarrow{P_2} A_{p1}^{p2}. 
\end{align*}
\]

(1)

Where A, Ap1, Ap2, and Ap1p2 denote, respectively, the AMPAR not phosphorylated, phosphorylated at p1 (s831), at p2 (s845), and double phosphorylated. This kinetic scheme (Fig. 2) can be described mathematically by applying the first order Mass Action approach, which leads to the following system:

The phosphorylation cycle shown in Figure 2, which assumes two phosphorylation sites on GluR1, is composed of four reactions:

\[
\begin{align*}
A & \xrightarrow{K_1} A_{p1}, \quad A^{p2} \xrightarrow{K_2} A_{p1}^{p2}, \\
A^{p2} & \xrightarrow{P_1} A_{p1}^{p2}, \quad A_{p1} \xrightarrow{P_2} A_{p1}^{p2}. 
\end{align*}
\]

(1)
This system, by construction, keeps constant the total amount of protein:

\[
\frac{dA}{dt} + \frac{dA_{p1}}{dt} + \frac{dA_{p2}}{dt} = 0 \Rightarrow A(t) + A_{p1}(t) + A_{p2}(t) = A_0
\]

This approach can be generalized to the case of a nonspecific phosphatase \( P2 = P1 \) and it gives relations similar to equation 6.

We will concentrate on the behavior of the \( A_{p2}(t) \) solution, and how the solution depends on assumptions regarding the calcium dependence of the enzymes involved in the pathway. We compare two quantitatively different sets of assumptions as follows: (1) The activity of each enzyme shows a sigmoidal dependence on \( Ca^{2+} \), but with different sensitivity to the concentration; this type of dependence will be described by a Hill-type function

\[
f(Ca) = \frac{Ca^h}{K^h + Ca^h}
\]

(3B), where \( h > 1 \) (Fig. 3A); (2) the activity of each enzyme shows a Michaelis-Menten dependence on \( [Ca^{2+}] \), but with different affinity constants:

\[
k_{a0}(Ca): v = \frac{k_{a0}E_aCa}{k_{a0} + Ca}
\]

(Fig. 3B), where \( v \) is the velocity of product formation.

The behavior of the solutions (equation 6) in the cases of two phosphatases and two kinases is shown in Figure 4, where we use the Michaelis Menten and the Hill function, respectively. Qualitatively, the two cases have a similar form. At low calcium levels, they exhibit LTD, whereas at higher levels, they exhibit

\[
M1 = P1 \cdot P2 \cdot P2 + K1 \cdot P2 \cdot P1 + P1 \cdot P2 \cdot K1 + K2 \cdot P2 \cdot P1
\]

\[
M2 = K1 \cdot P2 \cdot P2 + P1 \cdot P2 + K1 + P1 \cdot P2 + P2 \cdot K1
\]

\[
M3 = K2 \cdot K2 \cdot P1 + P1 \cdot K2 + P1 \cdot K1 + K2 \cdot P2 + K1 \cdot K2 + P1
\]

\[
M4 = K1 \cdot K2 \cdot K2 + K1 \cdot K2 \cdot K1 + K2 \cdot P2 + K1 \cdot K2 + K1 \cdot K2 + P1 \cdot K2 + K1
\]
LTP. However, quantitative differences are significant. For example, the LTD region resulting from the Michaelis-Menten curves occurs at a significantly lower value of \([Ca^{2+}]_i\), the magnitudes of the \(AP_0\) state increase more gradually and saturate at higher calcium levels. Synaptic strength is related to the phosphorylation state of GluR1, since phosphorylation at both sites increases the average AMPAR conductance level by approximately twofold. If mechanisms for phosphorylation at each site are independent, conductance approximately follows the following equation conductance \(\sim (A + 2^* (A_{P1} + AP_0) + 4AP_0^2)\) (Castellani et al. 2001). We used this equation above to estimate the conductance in arbitrary units.

**Analysis of the AMPAR cycle by Michaelis-Menten kinetics**

For a more accurate description of AMPAR phosphorylation/dephosphorylation cycle (Fig. 2), we use the Michaelis-Menten approach. Here, the effect of each enzyme is described as a combination of reversible and nonreversible reactions, making the standard pseudo-steady state assumptions (Segel 1975). Hence, with two phosphorylation sites and four different enzymes, this cycle takes the following form:

\[
\begin{align*}
K1 + A & \xrightleftharpoons[k1_A^r]{k1_A} K1A \rightarrow K1 + A_{p1} ; \\
K1A + Ap1 & \xrightleftharpoons[k2_A]{k2_A} K2A_{p1} \rightarrow K2 + AP_{p1}^2 ; \\
P1 + AP_{p1} & \xrightleftharpoons[k3_A]{k3_A} P1A_{p1} \rightarrow P1 + A \\
P1 + AP_{p1}^2 & \xrightleftharpoons[k4_A]{k4_A} P1A_{p1}^2 \rightarrow P2 + A \\
K2 + A_{p1} & \xrightleftharpoons[k5_A]{k5_A} K2A_{p1} \rightarrow K2 + AP_{p1}^2 ; \\
P2 + AP_{p1}^2 & \xrightleftharpoons[k6_A]{k6_A} P2A_{p1}^2 \rightarrow P2 + A_{p1} \\
K1 + AP_{p1} & \xrightleftharpoons[k7_A]{k7_A} K1A_{p1} \rightarrow K1 + AP_{p1}^2 ; \\
P1 + AP_{p1}^2 & \xrightleftharpoons[k8_A]{k8_A} P1A_{p1}^2 \rightarrow P1 + AP_{p1}^2. 
\end{align*}
\]

The constants \(k1_A, k2_A, k3_A, i = 1, . . . , 8\) are the rate constants for the forward, backward, and irreversible steps, respectively. According to the Michaelis-Menten analysis, we also define the Michaelis-Menten constants

\[
k_{irr} = k1_A + k_{irr}, i = 1, . . . , 8
\]

The pseudo-steady state hypothesis sets zero the derivatives of all the enzyme-substrate complexes as follows: \(K1A = P1A_{p1} = K2A_{p1} = P2A_{p1}^2 = K1A_{p1} = P1A_{p1}^2 = 0\). The conservation law for each enzyme sets the total amount of each enzyme as a constant as follows: \(K1 + K1A + K1A_{p1} = K1; P1 + P1A_{p1} + P1A_{p1}^2 = P1; K2 + K2A + K2A_{p1} = K2; P2 + P2A_{p1} + P2A_{p1}^2 = P2\).

This approach allows us to write a compact representation for the concentrations of the different fractions of AMPAR:

\[
\bar{\omega} = \bar{\mathbf{R}} \bar{\omega};
\]

where \(\Omega = (A, A_{P1}, AP_0, AP_0^2)\), and \(\mathbf{R}\) is a “coefficients matrix”:

\[
\mathbf{R} = \begin{pmatrix} -c_1 F_{K1} - c_2 F_{K2} & c_1 F_{P1} & c_1 F_{P2} & 0 \\
-c_1 F_{K1} & -c_2 F_{K2} + c_1 F_{P1} & 0 & c_2 F_{P2} \\
c_2 F_{K2} & 0 & -c_2 F_{K2} + c_1 F_{P2} & c_1 F_{P1} \\
0 & c_2 F_{K2} & c_1 F_{K1} & -c_2 F_{P1} + c_1 F_{P2} \end{pmatrix}
\]

(9)

with:

\[
\begin{align*}
c_1 &= k1_A \cdot k_{irr} \cdot c2 \cdot k_{irr} \cdot c3 = k_{irr} \cdot k_{irr} \cdot k_{irr} \cdot k_{irr} \cdot k_{irr} \cdot k_{irr} = k_{irr} \cdot k_{irr} \\
c_2 &= k_{irr} \cdot k_{irr} \cdot c4 = k_{irr} \cdot k_{irr} \cdot c5 = k_{irr} \cdot k_{irr} \cdot k_{irr} \cdot k_{irr} \cdot k_{irr} = k_{irr} \cdot k_{irr}
\end{align*}
\]

and the four “fluxes” have the form

\[
\begin{align*}
\mathcal{F}_{K1} &= \frac{K1_A}{k_{irr} k_{irr} + k_{irr} k_{irr} + k_{irr} + k_{irr}} \cdot \mathcal{F}_{P1} = \frac{P1_A}{k_{irr} k_{irr} + k_{irr} + k_{irr} + k_{irr}} \\
\mathcal{F}_{K2} &= \frac{K2_A}{k_{irr} k_{irr} + k_{irr} + k_{irr} + k_{irr}} \cdot \mathcal{F}_{P2} = \frac{P2_A}{k_{irr} k_{irr} + k_{irr} + k_{irr} + k_{irr}}
\end{align*}
\]

(11)

Note that equation 8 has a form similar to the first order mass action equation (equation 2); however, there is a significant difference in that the “fluxes” are not constant, but are functions of the dynamic variables. Thus, this equation is nonlinear and a full solution of the dynamics has not been analytically obtained. In order to characterize the fixed points, we observe that the matrix \(\mathbf{R}\) has a nontrivial kernel, hence, a vector basis of \(\text{Ker}(\mathbf{R})\) is:

\[
\mathbf{b}_{\text{Ker}(\mathbf{R})} = \begin{pmatrix} \frac{3}{4} A_{P1} + \frac{3}{4} A_{P1}^2 + \frac{3}{4} A_{P1} + \frac{3}{4} A_{P1}^2 \end{pmatrix}
\]

(12)

where:

\[
\begin{align*}
\mathbf{b}_{1} &= c_1 c_2 c_5 A_{p1} F_{P1} F_{P2} F_{K1} + c_1 c_2 c_4 A_{p1} F_{P1} F_{P2} F_{K2} + c_1 c_2 c_3 A_{p1} F_{P1} F_{P2} F_{K3} \\
\mathbf{b}_{2} &= c_1 c_2 c_5 A_{p1} F_{P1} F_{P2} F_{K1} + c_1 c_2 c_4 A_{p1} F_{P1} F_{P2} F_{K2} + c_1 c_2 c_3 A_{p1} F_{P1} F_{P2} F_{K3} \\
\mathbf{b}_{3} &= c_1 c_2 c_5 A_{p1} F_{P1} F_{P2} F_{K1} + c_1 c_2 c_4 A_{p1} F_{P1} F_{P2} F_{K2} + c_1 c_2 c_3 A_{p1} F_{P1} F_{P2} F_{K3} \\
\mathbf{b}_{4} &= c_1 c_2 c_5 A_{p1} F_{P1} F_{P2} F_{K1} + c_1 c_2 c_4 A_{p1} F_{P1} F_{P2} F_{K2} + c_1 c_2 c_3 A_{p1} F_{P1} F_{P2} F_{K3}
\end{align*}
\]

This permits us to write the equilibrium solutions, in a form that is similar to equation 6.

\[
\begin{pmatrix} A(x) \\ A_{P1}(x) \\ AP_0(x) \\ AP_0^2(x) \end{pmatrix} = \begin{pmatrix} 3_1 \cdot \mathbf{b}_{1} \\ 3_2 \cdot \mathbf{b}_{1} + 3_3 \cdot \mathbf{b}_{2} + 3_4 \cdot \mathbf{b}_{3} \\ 3_1 \cdot \mathbf{b}_{1} + 3_3 \cdot \mathbf{b}_{2} + 3_4 \cdot \mathbf{b}_{3} + 3_4 \cdot \mathbf{b}_{4} \\ 3_1 \cdot \mathbf{b}_{1} + 3_3 \cdot \mathbf{b}_{2} + 3_4 \cdot \mathbf{b}_{3} + 3_4 \cdot \mathbf{b}_{4} \end{pmatrix}
\]

(13)
This relation allows us to compute the “fluxes” in various cases, even when two specific kinases (K1, K2) and only one phosphatase P1 are present. Here, we can calculate the constants c1, c2, ..., c6 and define three “fluxes” \( F_{P1}, F_{K2}, F_{P3} \).

From these solutions, we can characterize the phosphorylation state of the various AMPAR fractions as a function of enzymatic activity. When the phosphatases (P1, P2) are activated above basal levels, the equilibrium will shift toward a state in which the fraction of A is greater than the others. When the kinases (K1, K2) are activated above basal levels, the AP fraction will be predominant. An asymmetric situation occurs when there is activation of one kinase and one phosphatase (K1 P2) or (K2 P1), in which an increase of A or P fractions takes place.

The synchronous activation of all of the AMPAR enzymes to the same level of activity will generate a state in which all of the AMPAR fractions are at the same concentration.

**Calcium dependence of enzymatic activity**

The Michaelis-Menten approach can be extended to account for the calcium dependence of the enzymes. We consider again four enzymes, K1, P1, K2, and P2, that are activated by calcium with the reactions

\[ E_{Ca_i} + Ca \rightarrow \frac{K_i}{K_{i+1}} E_{Ca_{i+1}} \]

where the subscript s denotes the number of Ca molecules bound to E, here s = 0, ..., 3, and where \( K_i, K_{i+1} \) and \( \sigma \) are the association, the dissociation, and the equilibrium constant for each reaction. With these assumptions (see Appendix), we can define a saturation curve \( \sigma \) that relates \([Ca^{2+}]\) to the activity of each enzyme (see Appendix). These functions are sigmoidal (Hill-like functions) because the process is cooperative. Previous derivations assumed that the enzymes become fully activated by binding four molecules of calcium, and we will adopt the same assumptions. The approach of equations 6–13 can be applied here to obtain the calcium dependent fluxes:

\[ F_{P1} = \frac{K_{P1}}{K_{P2}} \frac{P1 \sigma_{P1}(Ca)}{1 + \sigma_{P1}(Ca) \cdot A_{P1}} \]

\[ F_{P2} = \frac{K_{P2}}{K_{P3}} \frac{P2 \sigma_{P2}(Ca)}{1 + \sigma_{P2}(Ca) \cdot A_{P2}} \]

\[ F_{K1} = \frac{K_{K1}}{K_{K2}} \frac{K1 \sigma_{K1}(Ca)}{1 + \sigma_{K1}(Ca) \cdot A_{K1}} \]

\[ F_{K2} = \frac{K_{K2}}{K_{K3}} \frac{K2 \sigma_{K2}(Ca)}{1 + \sigma_{K2}(Ca) \cdot A_{K2}} \]

where the \( \sigma_i, i \in \{K1, K2, P1, P2\} \) are the saturation functions for the calcium binding of each enzyme:

\[ \sigma_i = \frac{1}{1 + \left(K_{i+1}\right)^{\sigma_i}(Ca)} \]

With this formalism, we can rewrite the system as

\[ \dot{q} = \mathbf{F} \cdot q \]

where the matrix \( \mathbf{F} \) is formally similar to the matrix \( \mathbf{R} \) of equation 9, but the fluxes \( F_i \) are substituted by the calcium-dependent fluxes \( F_i^{Ca} \). The structure of these equations is similar to that of equations 2 and 8 because, again, the total amount of AMPAR protein is conserved (\( A + A_{P1} + A_{P2} = A_1 \)). The matrix formed by the “calcium-dependent fluxes” has a nontrivial kernel, thus, again, we can apply the fixed point analysis. In this case, it is not possible to find a closed form solution, and we will therefore examine the behavior of the solutions by numerical integration of equation 15. Our interest is to characterize the fixed point as a function of \([Ca^{2+}]\), and therefore, we numerically integrate the system with different levels until it reaches the stable fixed point. The results are illustrated in Figure 5. In comparison to Figure 4, this approach results in a solution with larger LTD and a sharper transition between LTP and LTD. Therefore, it seems that the Michaelis-Menten approach results in more robust synaptic plasticity. The results depicted here in Figures 4, 5, and 7, below, are the steady-state solutions for a prolonged elevation of calcium level. These results cannot be easily compared with experimental results in which LTP and LTD are induced by protocols that produce transient changes in calcium concentration. In a previous work (Shouval et al. 2002b), we obtained a result that can be applied to induction of plasticity with calcium transients. The rule extracted by Shouval et al. (2002b) gives the derivative of the synaptic weight as a function of instantaneous calcium. This seems qualitatively similar to results predicted by BCM theory (Bienenstock et al. 1982), but depends on calcium rather than on pre- and postsynaptic activity.

We have previously translated the biophysical model to be a function of pre- and postsynaptic activity (Castellani et al. 2001; Shouval et al. 2002b); there, however, we find that, unlike BCM, the plasticity equations are no longer linear in presynaptic activity. These differences from the BCM theory, which result from this more detailed biophysical modeling, are to be expected, and the consequences of these detailed differences are currently under investigation (Yeung et al. 2004).

**A detailed enzymatic model**

In the previous section, we have shown that our biophysical model can qualitatively account for the calcium dependence of the phosphorylation state of AMPAR under a set of mild assumptions. In this section, we ask whether this set of assumptions is applicable to real cortical neurons. To do this, we introduce a more detailed model of the \([Ca^{2+}]\) dependence of each enzyme (Fig. 6). In addition, we ask whether the more detailed enzymatic model results in more specific physiological and biophysical consequences. This scheme differs from one described in the previous section, in that we have named specific enzymes as responsible for phosphorylation and dephosphorylation of GluR1.

![Figure 5. Levels of the \([A_{P2}^{Ca}]\) using the Michaelis-Menten approach.](image-url)
After making these assumptions, the remaining kinetic coefficients (such as $k_{1,2}$) for the $[Ca^{2+}]$ activation of each enzyme were extracted from the literature. Some kinetic coefficients were determined by a direct measurement or control of calcium (at high levels of CaM), while other experiments measured Ca–CaM (Calcium Calmodulin complex), by manipulating CaM levels (at a given high calcium concentration).

In order to translate the results obtained using the Ca–CaM approach, we use the result of Persechini and Cronk (1999), who measured the dependence of Ca–CaM on intracellular calcium concentration. They have shown that the level of Ca–Cam can be expressed as a function of Ca and the total amount of Calmodulin in the cell

$$Ca – CaM = K \frac{Ca^{2+}}{k_{1,2} + Ca^{2+}}$$

where $K$ is a parameter related to total CaM in the spine, which is regarded as an isolated compartment. It is reasonable to assume biologically realistic parameters, in which the maximum concentration of Ca–CaM is $45 \text{ nM}$, the constant $k_{1,2}$ is 1 $\text{mM}$, the Hill coefficient is -2.6, and $K$ can range from 10 to 40 $\text{nM}$. In Table 1, we list our assumptions about each kinetic coefficient. The experimental data often give good estimates for $k_{1,2}$ and sometimes for the Hill coefficient $h$; moreover, the results are typically not sensitive to the value of $h$. The values of the maximal activity level of each enzyme listed in Table 1 ($V_{max}$), and the basal activity level ($V_{base}$) cannot be extracted from the experimental data, as these depend critically on total enzyme concentration in vivo. We have chosen the parameters similar to that observed in vivo and to obtain reasonable results. In Figure 7, we display results using kinetic coefficients for direct calcium dependence (left) and Ca–CaM dependence (right). In Figure 7, A and D, the activity levels of the three directly interacting enzymes CaM KII, PKA, and PPI are displayed relative to intracellular concentration of calcium (left) or calcium–calmodulin (right). In Figure 7, B and E, the fraction of AMPARs in each of the different phosphorylation states is displayed, and in Figure 7, C and F, an estimate of the total conductance is shown. The conductance is estimated, as above by: $\text{conductance} = A + 2(A_{p1} + A_{p2}) + 4A_{p3}$.

Although the detailed assumptions about enzymatic activity are quantitatively quite different, both approaches produce an LTP/LTD curve with depression at moderate calcium levels and potentiation at high calcium levels. A critical parameter in determining the conductance at basal calcium levels is $V_{base}$, the basal activity level of each enzyme. For each enzyme, the value of $V_{base}$ is low; however, at such low basal levels, small differences in calcium concentration can have a large effect on the results. We have chosen values of $V_{base}$ to attain intermediate values of AMPAR conductance at baseline. In addition, we have chosen the values of $V_{base}$ so that at baseline, S845 would be more highly phosphorylated than the S831, consistent with experimental reports (Lee et al. 2000b). In general, the phosphorylation curves (Fig. 7B,E) are more constraining than the conductance plots (Fig. 7C,F), since
Table 1. Kinetic coefficients for relevant enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Ca–CaM dependence</th>
<th>Direct Ca dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaMKII</td>
<td>$k_{i,2} = 45 \text{nM}$ (Meyer et al. 1992)</td>
<td>$\rightarrow k_{i,2} = 1.5 \text{µM}$</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{max}} = 10$</td>
<td>$V_{\text{max}} = 1$</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{base}} = 0.005$</td>
<td>$V_{\text{base}} = 0.005$</td>
</tr>
<tr>
<td></td>
<td>$h = 4$</td>
<td>$h = 4$</td>
</tr>
<tr>
<td>PP2b</td>
<td>$k_{i,2} = 0.1 - 1 \text{nM}$ (Stemmer and Klee 1994; Wang and Kelly 1997)</td>
<td>$\rightarrow k_{i,2} = 0.1-0.25 \text{µM}$</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{max}} = 0.01$</td>
<td>$V_{\text{max}} = 0.01$</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{base}} = 0.01$</td>
<td>$V_{\text{base}} = 0.01$</td>
</tr>
<tr>
<td></td>
<td>$h = 3$</td>
<td>$h = 3$</td>
</tr>
<tr>
<td>PDE</td>
<td>$k_{i,2} = 0.1 \text{nM}$ (Klee and Cohen 1988)</td>
<td>$k_{i,2} = 2.5 \text{µM}$ (Gu and Cooper 2000)</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{max}} = 1$</td>
<td>$V_{\text{max}} = 1 \text{µM}$</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{base}} = 0.01$</td>
<td>$V_{\text{base}} = 1$</td>
</tr>
<tr>
<td></td>
<td>$h = 2$</td>
<td>$h = 2$</td>
</tr>
<tr>
<td>AC</td>
<td>$k_{i,2} = 15 \text{nM}$ (Klee and Cohen 1988)</td>
<td>$k_{i,2} = 0.38 \text{µM}$ $k_i = 132 \text{µM}^*$ (Gu and Cooper 2000)</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{max}} = 1$</td>
<td>$V_{\text{max}} = 1$</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{base}} = 0.01$</td>
<td>$V_{\text{base}} = 0.01$</td>
</tr>
<tr>
<td></td>
<td>$h = 3$</td>
<td>$h = 4$</td>
</tr>
<tr>
<td>PKA</td>
<td>$k_{i,2} = 1 \text{µM}^*$ (AMP) (Huang and Taylor 1998)</td>
<td>$k_{i,2} = 1 \text{µM}^* (\text{AMP})$</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{max}} = 5$</td>
<td>$V_{\text{max}} = 1$</td>
</tr>
<tr>
<td></td>
<td>$V_{\text{base}} = 0.01$</td>
<td>$V_{\text{base}} = 0.015$</td>
</tr>
<tr>
<td></td>
<td>$h = 2$</td>
<td>$h = 2$</td>
</tr>
</tbody>
</table>

Two approaches were used to extract kinetic coefficients from the literature to produce the calcium-dependence curves of each enzyme. Additional theoretical inferences for extracting the PP1 and cAMP dependence are described in the text. These enzymatic reactions typically have the form $V = V_{\text{max}} + V_{\text{base}} x^h k_i x$, where $x$ is the activating factor, either Ca, or Ca–CaM. Notation: $(-)$ The data was translated into the Ca column from the Ca–CaM column using the relation $CoCaM = CaM Ca^{2+}/Ca^{2+}$ (Perschini and Cronk 1999); $(h)_i$, inactivation constant.

*PKA as a function of AMP. All the velocities $V_{\text{max}}, V_{\text{base}}$ and $V$ are expressed in mol/min.

Multiple configurations of the phosphorylation curves can produce the same conductance curves. A significant factor in both of the detailed models is that the PP1 activity curve is not a monotonic sigmoid, but is approximately bell shaped. This differs from the assumptions made for the abstract qualitative models. LTD occurs at low calcium levels, due to the peak in PP1 activity. Eliminating this peak in PP1 activity would abolish LTD. The shape of the PP1 curve depends on both PP2b and PKA activity. Thus, inhibiting PK1 directly or indirectly through PP2b inactivation would reduce the level of LTD, consistent with experimental observations (Mulkey et al. 1993, 1994). Our models are consistent with Lee et al. (2000b), who found that LTD, from baseline, produces dephosphorylation of $S845$ (PKA-site), and that LTD produces phosphorylation of $S831$ (CaMKII-site).

Both of our models exhibit phosphorylation of AMPAR receptors at the $S845$ site at basal calcium levels, due to the assumption that PKA activity at basal calcium levels is greater than activity of PP1, PP2b, or CaMKII. Examining this key assumption experimentally is a test for the validity of our detailed model.

Discussion

Changes in synaptic efficacy underlie many fundamental properties of nervous system function, such as developmental refinement of receptive fields, learning, and memory. As such, the molecular mechanisms underlying the regulation of synaptic strength have been an area of intense investigation. Here, we use experimentally derived properties of intracellular signaling cascades and postsynaptic glutamate receptor phosphorylation to model bidirectional regulation of synaptic strength. First, we demonstrate that knowledge of the activation coefficients of $[Ca^{2+}]_i$-dependent protein kinases and protein phosphatases is sufficient to predict activity-dependent phosphorylation/dephosphorylation of GluR1 and corresponding changes in the conductance of the AMPAR. We show that regulation of AMPAR phosphorylation can indeed qualitatively account for LTD/LTP of the signal-transduction cascade leading from calcium influx in the postsynaptic neuron to AMPAR phosphorylation/dephosphorylation. This has produced LTD/LTP curves that are consistent with experimental results and consistent with our previous model. The quantitative aspects of our results stem from detailed assumptions about the calcium dependence of enzymatic activity, assumptions that, at this point, are not fully substantiated. The significance of these assumptions are revealed in Figure 7, in which the phosphorylation of GluR1 and the conductance of the AMPAR differ greatly, depending on whether direct $[Ca^{2+}]_i$ dependence or $CaM–CaM$ dependence is used to derive the enzymatic activity. Despite the different assumptions made in each model, we report that in all cases, LTD is induced by a moderate calcium increase and LTP is induced by high calcium concentrations.

Recent experimental results show that LTD is correlated with the phosphorylation of $S845$, whereas dephosphorylation (the reversal of LTD with low frequency stimulation) is correlated with dephosphorylation of $S831$ (Lee et al. 2000b). This could imply that $S845$ can only be dephosphorylated if $S831$ is not phosphorylated, thus, phosphorylation at these sites is not independent. Our model, which assumes two independent phosphorylation sites, points to an alternative explanation. If, as we assume, PKA activity levels are high at basal calcium levels, but CaMKII levels are not, then at baseline, $S845$ would tend to be phosphorylated. Hence, LTD from baseline would dephosphorylate $S845$. In contrast, after LTD, $S831$ would be phosphorylated, thus, the same paradigm that induces LTD from baseline, would induce LTD from baseline, would be phosphorylated, thus, the same paradigm that induces LTD from baseline, would induce LTD from baseline, would be phosphorylated, thus, the same paradigm that induces LTD from baseline, would induce LTD from baseline, would be phosphorylated, thus, the same paradigm that induces LTD from baseline, would induce.
Figure 7. Levels of enzyme activity, GluR1 phosphorylation and AMPAR conductance as a function of postsynaptic calcium concentration. (A,E) Calculated from biochemical data with fixed calmodulin concentrations and varied calcium concentrations. (B) The resulting phosphorylation state (expressed as percentage of the total) of the GluR1 subunit of the AMPAR as a function of postsynaptic calcium concentration. The shape of the four curves in each graph is qualitatively similar. The most significant difference is that the range over which A is dominant is broader in the Ca-CaM-based case. (C,F) Translating changes in AMPAR phosphorylation to AMPAR conductance (in arbitrary unit) gives qualitatively similar results when considering the dependence on \([Ca^{2+}]\). AMPAR conductance is lower than baseline levels at low concentrations of intracellular Ca or Ca-CaM, and reaches a maximal plateau level at ~1 μM. The interesting differences are in the shapes of the curves, with a gradual increase in conductance when considering only the Ca dependence and a less gradual increase when considering Ca-CaM.

The long-term maintenance of synaptic potentiation (but, see, Otmakhov et al. 1997), we did not include this in our models, since, here, we focus on early postsynaptic signaling events involved in the induction of LTP/LTD.

In our approach, we have made several methodological assumptions. It is hypothesized that the fraction of phosphorylated AMPA receptors can fluctuate significantly within each spine; however, we assume a quantity of AMPARs averaged across a population of synapses. This assumption may be invalid if the total number of AMPARs is small, resulting in fluctuations that are of the same order of magnitude of the mean value. Furthermore, we assume a “well-stirred” system in which all enzymes have equal access to AMPA receptors, which may be invalid if different enzymes are compartmentalized within the synapse. The validity of some of these assumptions was addressed in a recently introduced model based on Monte-Carlo simulations (MCCell); however, it remains to be determined whether such issues are fundamental to theoretical modeling of synaptic plasticity (Franks et al. 2002).

We only consider plasticity that is maintained by phosphorylation of the AMPA receptors. Therefore, throughout the model, we assume that the shape of the LTP/LTD curves are equivalent to changes in the conductance of the AMPA receptors. There is strong evidence that additional processes, such as insertion of additional postsynaptic AMPA receptors and/or changes in presynaptic probability of release, are also associated with synaptic regulation (Malinow and Malenka 2002). Such additional mechanisms will modify, but not negate, our results.

The central properties of our model support the postulates of the BCM theory (Bienenstock et al. 1982; Intrator and Cooper 1992; Blais et al. 1999), but our molecular model suggests some modifications. The results depicted here are the steady-state conductance of AMPARs, resulting from a prolonged elevation of intracellular calcium. In a previous study (Shouval et al. 2002b), we obtained a rule for the derivative of the synaptic weight, which seems similar to BCM, but depends on calcium rather than on pre- and postsynaptic activity. We have previously translated our biophysical model into a function of pre- and postsynaptic activity (Castellani et al. 2001; Shouval et al. 2002b); however, we find that unlike BCM, our plasticity equations are no longer linear in presynaptic activity. These differences from the BCM theory are currently under investigation (Yeung et al. 2004). We have recently studied the dynamics of these equations, and have approximated the dynamics of AMPAR’s conductance by a simpler dynamical equation that depends directly on calcium (Shouval et al. 2002b). We have also used this equation, which we call the “calcium control hypothesis”, as the basis for a unified model of synaptic plasticity. We have shown that this unified model can account for various induction paradigms of synaptic plasticity, including rate-based and spike timing-based protocols (Shouval et al. 2002a). In addition, we have also shown that changes in the properties of NMDAR conductance have the effect of changing the form of the LTP/LTD curves (Castellani et al. 2001; Shouval et al. 2002a,b). Thus, activity-dependent plasticity of NMDAR, which has been observed experimentally (Carmignoto and Vicini 1992; Quinlan et al. 1999; Watt et al. 2000), can serve as a mechanism for metaplasticity (Bienenstock et al. 1982; Abraham and Bear 1996).

Acknowledgments
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Appendix
Derivation of the calcium-dependent “Flux”
In this section, we derive the calcium-dependent flux for the kinase-1 (K1). We report only one case, because all of the other cases can be obtained following the same procedure.

The relations (equation 14) lead to the following expressions:

\[
K1Ca = \frac{K1 \cdot C}{k_{e0}}, \quad K1Ca_2 = \frac{K1Ca \cdot C}{k_{e1}}, \quad K1Ca_3 = \frac{K1Ca_2 \cdot C}{k_{e2}}, \quad K1Ca_4 = \frac{K1Ca_3 \cdot C}{k_{e3}}
\]

\[
K1Ca = \frac{k_{e0} \cdot k_{e1} \cdot k_{e2} \cdot k_{e3}}{C} \cdot K1Ca_4, \quad K1Ca_2 = \frac{k_{e1} \cdot k_{e2} \cdot k_{e3}}{C} \cdot K1Ca_4, \quad K1Ca_3 = \frac{k_{e2} \cdot K1Ca_4}{C}, \quad K1Ca_4 = \frac{k_{e3} \cdot K1Ca_4}{C}
\]

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where $K_0 = k_4 \cdot k_{5a}$. The phosphorylation reactions catalyzed by the fully activated enzyme $K_1Ca_A$, are, as usual:

$$K_1Ca_A + A^2 \xrightarrow{k_{i_1}^P} K_1Ca_AA$$

$$K_1Ca_A + A^2 \xrightarrow{k_{i_2}^P} K_1Ca_AA$$

These reactions, those for the calcium activation of $K_1$, and the enzyme conservation condition give:

$$K_1 + K_1Ca + K_1Ca_A + K_1Ca_A + K_1Ca_A^2 \xrightarrow{k_{i_1}^P} K_1$$

By substitution we obtain:

$$K_1Ca_4 = \left(\begin{array}{c}
K_1Ca_4 - K_1Ca_A - K_1Ca_A^2 \cdot \sigma(Ca) \\
0
\end{array}\right) = K_1 \cdot \sigma(Ca) \cdot A$$

$\sigma(Ca)$ is a sigmoidal (Hill) function that expresses the degree of binding of $Ca$ with $K_1$. The steady-state hypothesis for the above reactions gives us the following equations:

$$K_1Ca_4 + K_1Ca_A^2 = 0$$

$$K_1Ca_4 + K_1Ca_A^2 = 0$$

After substitution for $K_1Ca_4$, we obtain a linear system in the unknown $K_1Ca_A, K_1Ca_A^2$:

$$\left\{ \begin{array}{l}
\sigma(Ca) \cdot A + k_{m_1} \cdot K_1Ca_A = 0 \\
\sigma(Ca) \cdot A^2 \cdot K_1Ca_A + (\sigma(Ca) \cdot A + k_{m_2}) \cdot K_1Ca_A^2 = 0
\end{array} \right.$$  

whose solutions:

$$K_1Ca_A = \frac{K_1 \cdot k_{m_1} \cdot A}{k_m + k_{m_1} \cdot \sigma(Ca) + k_m \cdot \sigma(Ca) \cdot A}$$

$$K_1Ca_A^2 = \frac{K_1 \cdot k_{m_2} \cdot \sigma(Ca) \cdot A^2}{k_m + k_{m_1} \cdot \sigma(Ca) \cdot A^2 + k_m \cdot \sigma(Ca) \cdot A}$$

allow us to define the “Calcium-dependent flux” associated with the kinase $K_1$:

$$f_{K_1} = \frac{k_m \cdot \sigma(Ca)}{k_m + k_{m_1} \cdot \sigma(Ca) \cdot A + k_m \cdot \sigma(Ca) \cdot A}$$

The same procedure, if applied to the other enzymes, gives us the other “fluxes” that are necessary to write the matrix $f$ (equation 15). We observe that this procedure also holds if we change the number of enzymes so that we can obtain the expression used in the case where there is only one nonspecific phosphatase.

References


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