Chapter 2
Spines and Long-Term Potentiation

2.1 Introduction to Long-Term Potentiation

In 1949, Donald Hebb proposed a coincidence detection rule in which the strength of association between two neurons would increase if they were simultaneously active [38]. In 1973, such a phenomena was first observed in the dentate gyrus of anesthetized rabbits and was termed long-term potentiation (LTP) [11, 10]. LTP may be expressed as a persistent increase in the size of the synaptic component of the evoked response recorded from individual cells or from populations of neurons [12]. By persistent, we mean a stable increase in synaptic efficacy which lasts at least one hour. This is to be differentiated from shorter changes such as post–tetanic potentiation (PTP), which lasts on the order of seconds, and short–term potentiation (STP), which is a transient increase in synaptic weight that decays back to baseline within the first 30 minutes.

One of the most interesting features of LTP is that it is prominent in all areas of the hippocampus. This brain structure has long been implicated from lesion studies as being important to memory [104]. What is even more intriguing is that stimulation protocols that are capable of inducing LTP in the laboratory are qualitatively similar to those actually seen within the hippocampus during learning.

LTP exhibits three very important features that are expected of any cellular substrate of memory: cooperativity, associativity, and input–specificity [12]. Cooperativity is used
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to describe the requirement that LTP has a threshold wherein weak stimuli are not encoded into an increase in synaptic efficacy. Such a mechanism is required to prevent "spontaneous memory" formation. Associativity means that a subthreshold activation at one synapse may become potentiated if there is strong activity at a nearby synapse. Thus the efficacy of a synapse is dependent upon its coactivity patterns with its neighbors. Input–specificity is the requirement that inactive synapses should not undergo an increase in efficacy. This once again can be viewed as some form of prevention of "spontaneous memory".

2.2 The Coincidence Detector

LTP has garnered a great deal of interest because of its possible linkage with memory and because it has yielded many fascinating biochemical results. One of the first problems to be dealt with was the identification of a coincidence detector. By a coincidence detector, we mean a cellular process which is activated during strong pre– and postsynaptic coactivation and serves as a signal for the induction of LTP [12]. In CA1 hippocampal pyramidal neurons, the coincidence detector is most likely a ligand–gated cation channel, the nicotinic–methyl–D–aspartate receptor (NMDAR), which opens in response to glutamate and NMDA. NMDARs are thought to be capable of functioning as the coincidence detector because they are largely blocked at potentials below -20 mV by Mg$^{2+}$ [73]. Thus in order for a synapse to become potentiated, it has to be active, and the neuron has to be highly depolarized in the vicinity of the NMDAR. As a result, the neuron may be in the firing mode (threshold is typically a potential of $\approx -50$ mV at the axon hillock). In support of this theory, it has consistently been found that NMDAR antagonists block the induction of LTP [12, 73].

The next issue becomes what feature of NMDAR activation is crucial to LTP induction. NMDARs are highly calcium permeable, and Ca$^{2+}$ is a potent second messenger capable of
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initiating many different biochemical cascades involving enzymes such as protein kinases (PKs), which add $\text{PO}_4^{3-}$ to proteins, and protein phosphatases (PPs) which remove $\text{PO}_4^{3-}$ from proteins, and a variety of other proteins such as CaM and actin. Evidence for the importance of calcium in LTP induction comes from experiments which show that calcium chelators are capable of preventing the induction of LTP when administered to the postsynaptic neuron.

Thus, the idea that LTP is a calcium-dependent process depending on NMDAR activation became entrenched within the discipline. This led inevitably to the question about the nature of the calcium transients that are involved in the induction of LTP. It was generally accepted that there must be some sort of threshold relating to some feature of the calcium transient. Above this threshold, LTP would be induced, but below this threshold, only STP or perhaps even PTP could be induced. However, due to the limitations of experimental techniques, there was no known method of actually observing the calcium transients within dendritic spines, the presumed locus for LTP induction. As a result, a number of mathematical models were proposed to help gain insight into the importance of various biophysical systems thought to be important in mediating the calcium transients.

### 2.3 First Generation Mathematical Models of LTP

The first generation models of LTP focussed heavily on what was biophysically necessary to produce large, spatially localized $\text{Ca}^{2+}$ transients under stimulation protocols similar to ones that experimentally induce LTP. It was thought that memory formation should involve some sort of a biochemical threshold. Below this threshold, there is very weak activation of a critical molecule, and above this threshold there is near maximal activation of this molecule. Thus, some recurring themes in these models were: the limitation of calcium channels to spine heads which acted as the sole source of calcium for the cell, and
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a heterogeneous distribution of calcium pumps and calcium buffers (these are calcium-binding proteins (CaBPs) which generally diffuse slowly and can be seen to function, in some sense, as a capacitance [121]).

The basic physical geometry of the spine was a cylindrical spine head attached via a cylindrical spine stem to a cylindrical dendritic shaft. In some of the models, only a single spine stem was modeled, while in others, many were.

In the electrical part of models, the spine head was modeled as an isopotential compartment which is linked through a resistor to the parent dendrite. The resistor represents the theoretical total resistance conferred by the spine stem. This represents a lumped-sum resistance approximation which gives a good approximation to the actual system. Certainly, the amount of error introduced in this approximation is far smaller than the uncertainty in the electrical parameters used. The voltage in the dendrite was determined from the passive cable equation.

The motion of calcium was based on simple linear diffusion, coupled to nonlinear effects due to the pumps and buffers. The entry of Ca$^{2+}$ is voltage–dependent and occurs only in the distal part of the spine head. Otherwise, the voltage and Ca$^{2+}$ movement equations are decoupled. However, this may not be justified in small compartments such as dendritic spines [86].

The following parameters and variables are common in many of the following models and will be defined here:
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<table>
<thead>
<tr>
<th>Parameter or Variable name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_c$</td>
<td>The synaptic reversal potential of a channel, $c$</td>
</tr>
<tr>
<td>$t_{peak,c}$</td>
<td>The time to peak conductance of a channel, $c$</td>
</tr>
<tr>
<td>$g_c$</td>
<td>peak conductance of a channel, $c$</td>
</tr>
<tr>
<td>$C_m$</td>
<td>specific membrane capacitance</td>
</tr>
<tr>
<td>$R_m$</td>
<td>specific membrane resistance</td>
</tr>
<tr>
<td>$R_i$</td>
<td>specific intracellular membrane resistance</td>
</tr>
<tr>
<td>$R_{cc,sp}$</td>
<td>spine input resistance</td>
</tr>
<tr>
<td>$R_{\infty,d}$</td>
<td>infinite dendrite input resistance</td>
</tr>
<tr>
<td>$D_{Ca}$</td>
<td>calcium diffusion coefficient</td>
</tr>
<tr>
<td>$R_N$</td>
<td>spine neck resistance</td>
</tr>
<tr>
<td>$r_n$</td>
<td>radius of spine neck</td>
</tr>
<tr>
<td>$l_n$</td>
<td>length of spine neck</td>
</tr>
<tr>
<td>$r_{sp}$</td>
<td>radius of spine head</td>
</tr>
<tr>
<td>$l_{sp}$</td>
<td>length of spine head</td>
</tr>
<tr>
<td>$r_d$</td>
<td>radius of dendrite</td>
</tr>
<tr>
<td>$k_{bf}$</td>
<td>forward buffer rate constant</td>
</tr>
<tr>
<td>$k_{bb}$</td>
<td>backwards buffer rate constant</td>
</tr>
<tr>
<td>$k_{pi}$</td>
<td>rate constant for a first-order kinetic Ca(^{2+}) pump</td>
</tr>
<tr>
<td>$K_{\text{max}}$</td>
<td>Ca(^{2+}) turnover rate for a Ca(^{2+}) pump</td>
</tr>
<tr>
<td>$P_s$</td>
<td>surface density of a Ca(^{2+}) pump</td>
</tr>
<tr>
<td>$K_d$</td>
<td>constant of dissociation</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday's constant ($9.6 \times 10^4$ Coulombs/mol)</td>
</tr>
<tr>
<td>$[B]$</td>
<td>concentration of Ca(^{2+})-bound buffer</td>
</tr>
<tr>
<td>$[M]$</td>
<td>concentration of free buffer</td>
</tr>
<tr>
<td>$[Bt]$</td>
<td>total concentration of buffer</td>
</tr>
<tr>
<td>$D_B$</td>
<td>diffusion coefficient of the bound buffer</td>
</tr>
</tbody>
</table>
2.3.1 The First Attempt: Gamble and Koch, 1987

The first realistic LTP model was put forth by Gamble and Koch (GK) in 1987 [27]. In this model, the dendritic spine head contained synaptic receptors, voltage-gated calcium channels (VGCCs), voltage-gated noninactivating potassium channels, and a leak conductance. The spine head was heterogeneous and contained a specialized area under the outer circular face called the shell which roughly corresponds to the post-synaptic density. The spine neck also was divided into distal and proximal portions, which correspond to the sides furthest and nearest, respectively from the dendrite. The parent dendrite was homogeneous and contained only one leak conductance. Calcineurin and CaM were present as non-mobile, spatially inhomogeneous buffers. The concentrations of calcineurin and CaM were taken to be 10 μM and 50 μM, respectively, in the shell, and 5 μM and 25 μM, respectively, in all other areas. The binding of Ca$^{2+}$ to calcineurin and to each of CaM’s four Ca$^{2+}$ binding sites was taken to be governed by first-order kinetics. There was also a non-saturable adenosine triphosphate (ATP) driven calcium-pump with first-order kinetics, an equilibrium value of 50 nM for [Ca$^{2+}$]$_i$, the intracellular calcium concentration, and a time constant of 2 ms located in a thin compartment apposed to the subsynaptic membrane.

Some of the main discoveries from this model were:

1. HFS is much more important than the total amount of synaptic activity in inducing large increases in [Ca$^{2+}$]$_i$ in small compartments such as dendritic spines.

2. The relative change in [Ca$^{2+}$]$_i$ was much smaller than the relative change in concentration of Ca$_4$$^{2+}$ – CaM. This was due to the fact that the concentration of Ca$_4$$^{2+}$ – CaM depends on the fourth power of [Ca$^{2+}$]$_i$.

3. Synaptic activity at a nearby spine was simulated by the injection of depolarizing current during spike activity at the spine of interest. Depolarizing current more
than doubled the increase in $[\text{Ca}^{2+}]_{sp}$, the concentration of calcium within the spine head. This showed the possible importance of associativity in LTP.

4. The high input resistance of the spine allowed for much larger EPSPs in the spine head than in the parent dendrite. This allowed for the activation of high–threshold VGCCs.

### 2.3.2 The Second Attempt: Holmes and Levy, 1990

Holmes and Levy (HL) developed a model in 1990 looking for insights into how NMDARs might be important for the associative nature of LTP. They based their model on a rat hippocampal dentate granule cell and explicitly included 1–115 dendritic spines in their model. The model also contained buffers, pumps, and diffusion for $\text{Ca}^{2+}$. The model differed from the GK model in that it included other spines and a completely different set of receptors. The HL model included NMDARs and non–NMDARs, which were thought to conduct a mixed $\text{Na}^+/\text{K}^+$ current. The model had no VGCCs since they are not important in the induction of LTP in these cells, and there is little evidence for their existence in significant numbers.

At that point in time there was little quantitative information about the kinetics of the NMDARs. Thus, Holmes and Levy developed a mathematical model involving simple kinetics for receptor binding and for the transitions of receptor to different conductance states for both the NMDARs and non–NMDARs.

The qualitative features that they were trying to build into their model of NMDAR function were that the unbinding of neurotransmitters (NTs) from NMDARs had to be slow, the transition of the NMDAR–NT complex to an open channel state had to be even slower, the NMDAR is blocked by $\text{Mg}^{2+}$ in a voltage–dependent manner, and the average number of NMDARs on a single spine head that were open at any given time had to be small (usually $< 1$). The approach used to calculate $\text{Ca}^{2+}$ influx was simply to
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determine the conductance of the NMDARs and turn it into a $\text{Ca}^{2+}$-flux by assuming that NMDARs were permeable to $\text{Ca}^{2+}$, $\text{Na}^{+}$, and $\text{K}^{+}$ with a relative permeability ratio of $P_{\text{Ca}} : P_{\text{K}} : P_{\text{Na}}$ of 10.6:1.0:1.0. The important qualitative differences between the NMDARs and non-NMDARs were the longer mean open times for NMDARs.

The one major assumption made was the linearity of conductance addition. That is, the conductance changes due to activation of receptors from temporally separated presynaptic events do not affect each other. This assumption is only valid if the number of receptors bound to NTs is small compared to the number of receptors.

For the $\text{Ca}^{2+}$ part of the model, the spine head was split into four compartments: two just under the outer circular surface of length 50 nm representing the PSD, and two more of length 225 nm. The spine neck was split into three roughly equal sized compartments. To model the $\text{Ca}^{2+}$ in the vicinity of a spine, four dendritic compartments of length 0.5 $\mu$m were used, but adding additional ones didn’t affect the $[\text{Ca}^{2+}]_s$. $\text{Ca}^{2+}$ movement between the different compartments was modeled according to simple linear diffusion between compartments, binding to buffers (based on CaM dynamics as assumed in the ZK model), and elimination by nonhomogeneously distributed pumps.

The main conclusions of this model were:

1. Prior theories about the NMDAR acting as the source of the nonlinearity in LTP are probably not true. It was previously thought that the voltage-dependence of the NMDAR could result in a positive feedback loop which would generate a large $\text{Ca}^{2+}$ influx. However, $\text{Ca}^{2+}$ influx could never be increased more than four-fold over the control.

2. The role of fast buffers is to amplify the nonlinearity in $[\text{Ca}^{2+}]_i$, and buffers play a more important role in modifying the $\text{Ca}^{2+}$ transient than pumps, or diffusion.

3. The associativity of LTP could be seen in the voltage-dependence of the NMDAR.
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as it required a large number of coactive synapses firing at high frequency to result in large changes in Ca$^{2+}$ influx, and [Ca$^{2+}]_{sp}.$

4. Dendritic spines, because of their small volume, "provide a locus for physiologically important transient increases in [Ca$^{2+}]_i," [41].

2.3.3 The Third Attempt: Zador and Koch, 1990

Later in 1990, Zador and Koch (ZK) put forward a model [122], with the same spirit as the HL model. The basic models for the voltage equations were the same although only one spine was explicitly modeled, and the basic Ca$^{2+}$ movement model was the same, incorporating diffusion, pumps, and buffers for Ca$^{2+}$. There were, however, a couple of differences which make this model worth noting.

The most fundamental difference is that the ZK model is based on CA1 hippocampal tissue with pyramidal neurons which is the most thoroughly studied tissue in the field of LTP research. The morphology of the model was similar to the one used in the previous two models with only slight quantitative differences. It should be noted that the value of the input resistance was much larger than in the HL model and result in less stringent requirements for associativity.

In the electrical model, explicit, empirical formulas for the forms of the time-dependent conductances for NMDA, and non-NMDAR (here it was based on AMPARs) were found:

\[
g_{\text{non-NMDA}}(t) = \frac{\bar{g}_{\text{non-NMDA}}}{t_{\text{peak}}} \exp \left( 1 - \frac{t}{t_{\text{peak}}} \right), \tag{2.1}
\]

\[
g_{\text{NMDA}}(t, V) = \frac{\exp \left( -\frac{t}{\tau_1} \right) - \exp \left( -\frac{t}{\tau_2} \right)}{1 + \eta[Mg^{2+}] \exp(\gamma V)}, \tag{2.2}
\]

where $g_{\text{non-NMDA}}$ is an alpha function with $t_{\text{peak}} = 1.5$ms, and a peak conductance of $\bar{g}_{\text{non-NMDA}} = .5$mS. The parameter values for $g_{\text{NMDA}}$ were $\tau_1 = 80$ms, $\tau_2 = .67$ms, $\eta = 0.33$ mM$^{-1}$, $\gamma = 0.06$ mV$^{-1}$ and $\bar{g}_{\text{NMDA}} = 0.2$ nS. The corresponding currents generated

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in the spine head were calculated under the assumption of linear conductance addition as in the HL model.

In the Ca\(^{2+}\) part of the model, it was assumed that all of the Ca\(^{2+}\) influx was mediated by the NMDARs within the spine head. The factors involved in Ca\(^{2+}\) mobility were immobile cytosolic buffers based on CaM, as in previous models, a heterogeneous density of Ca\(^{2+}\) pumps obeying first-order Michaelis–Menten kinetics, and Ca\(^{2+}\) diffusion. The density of pumps in the proximal spine neck, the part closest to the dendrite, was much higher in the spine neck due to the possible presence of the spine apparatus which may be important in calcium sequestration. The method of determining the size of the calcium flux was to convert the current mediated by the NMDAR by using Faraday’s constant, the valence of calcium, and by multiplying by 0.02, into the amount of the current thought to be due to Ca\(^{2+}\).

The basic assumption of the modeling is that the degree of potentiation of a synapse should be a monotonic function of peak [Ca\(_4\)CaM]. Some of the results from these simulations were:

1. The high surface area to volume ratio of the pumps allowed for a great deal of separation between dendritic calcium concentration and spine calcium concentration. Thus spines are to a great extent isolated from each other with respect to calcium fluxes.

2. Ca\(^{2+}\) dynamics in the spine are quite sensitive to spine morphology and this could be an important mechanism in metaplasticity, the plasticity of synaptic plasticity [1] (for a model which explicitly explored this concept see [29]).

3. The associativity nature of LTP arises through the voltage–dependence of the NMDAR. It was found that a weak input which alone resulted in very little elevation of [Ca\(^{2+}\)]\(_i\) in the spine could result in a much greater elevation of [Ca\(^{2+}\)]\(_i\) if there
was enough activity at coactive synapses along the dendrite.

2.3.4 Conclusions about first generation LTP models

At this point, it would seem that these first models had done a credible job of explaining the robust experimental properties of LTP. LTP occurs when there is a sufficient amount of presynaptic activity at a synapse coupled to strong, local depolarization in the neighbourhood of the dendritic spine to alleviate the voltage-dependent Mg$^{2+}$ block of the NMDAR — this explains the associativity and cooperativity properties of LTP. The spine is critical since its morphology allows large local changes in $[\text{Ca}^{2+}]$, due to the presence of buffers, the diffusional restriction of its thin neck, the presence of calcium pumps, and its small volume relative to that of its parent dendrite — this explains the input specificity of LTP. Lastly, the threshold nonlinear type behavior expected of LTP induction can be seen in the activation of CaBPs.

Part of the difficulty with the models as presented is that they are computationally intensive and fail to give much qualitative insight into the nature of calcium dynamics in a dendritic spine. One simplification is to identify a smaller number of lumped parameters whose values would give a good understanding of the nature of the calcium dynamics. If this is not possible, then a more thorough numerical investigation into how buffering, pumping, and diffusion parameters interact with spine shape to determine the shape of calcium transients should be carried out. Work has been done in both of these areas by Woolf and Greer who carried out numerical simulations on diffusion of second messengers in dendritic spines [118], and by Zador and Koch [121] who carried out analytical work (based on the approach used by Wagner and Keizer [110]).
2.4 Numerical Investigation of Second Messenger Diffusion in Dendritic Spines

Woolf and Greer numerically modeled the diffusion of second messengers in the dendritic spines of granule cells of the olfactory bulb. The spine morphologies were based on serial electron microscopy reconstruction [119]. These cells were chosen rather than hippocampal pyramidal cells since their spines have both input and output operations, and their necks are thinner and longer than hippocampal pyramidal cell dendritic spines so the lack of spatial localization of second messengers in the granule cell spines implies the same of the hippocampal pyramidal cell spines.

The numerical results of their simulations suggested the following:

1. In general, longer and thinner spine necks resulted in larger transients of second messengers in the spine heads, and longer times were required to reach peak concentrations in the spine neck.

2. Extrusion mechanisms often can be inefficient in spatial sequestration of second messenger signals at physiologically plausible density levels.

3. Binding mechanisms can profoundly affect the shape of second messenger transients and the ability of the signals to reach the base of the spine.

These results have some important implications. If there is a calcium–induced calcium release (CICR) mechanism in spine heads, then the effect may simply prolong the elevation of calcium in the spine head. However, if the release mechanisms are present in the spine neck and throughout the dendrite, then there could be active propagation of calcium signals from one spine head to another. There is also a question about the diffusion of activated CaBPs such as CaM. An important factor to consider here is the off–rate, or the rate of unbinding. CaM has a relatively rapid off–rate, so activated CaM levels will quickly decay once the calcium transient begins to die. Thus the degree of
localization will be strongly influenced by calcium-clearance mechanisms. The picture is a bit different for membrane-bound second messengers such as DAG and PKC. They will probably be restricted to the spine head and theoretically allow a much better spatial localization of the effects of synaptic activity than cytoplasmic diffusible second messengers such as \( \text{Ca}^{2+} \). This suggests that modeling the effect of buffering on calcium transients by scaling the diffusion coefficient of calcium may lead to qualitatively incorrect results.

2.5 Linearizing Calcium Dynamics through Asymptotic Expansions

To obtain a better understanding of the non-linear calcium dynamics, Zador and Koch [121] show how it is possible in certain limiting cases to reduce the dynamics to one linear PDE with constant coefficients in cylindrical domains. This equation is formally identical to the cable equation in the low \([\text{Ca}^{2+}]_i\) range, and simple linear diffusion in the high \([\text{Ca}^{2+}]_i\) range. Their method used asymptotics for cases in which \([\text{Ca}^{2+}]_i\) is expected to be small and large relative to certain parameters and then to determine a leading order equation.

The calcium dynamics involve diffusion, saturable pumps obeying Michaelis–Menton dynamics, and diffusible buffers. The governing system of equations are

\[
\frac{\partial [Ca]}{\partial t} = D_{Ca} \frac{\partial^2 [Ca]}{\partial x^2} - P([Ca]) - k_{bf}[Ca][M] + k_{bb}[B] + \frac{2}{r}f(x,t), \quad (2.3)
\]

\[
\frac{\partial [B]}{\partial t} = D_{b} \frac{\partial^2 [B]}{\partial x^2} + k_{bf}[M][Ca] - k_{bb}[B], \quad (2.4)
\]

\[
[Bt] = [M](x,t) + [B](x,t), \quad (2.5)
\]

where \( P[Ca] \) is the contribution of the pump term, \( D_B \) is the diffusion coefficient of bound buffer, \( r \) is the radius of the cylinder, and \( \frac{2}{r}f(x,t) \) is a source term. Here we have made the implicit assumption that \([Bt]\) is a constant which is tantamount to a spatially homogeneous distribution of buffers and equivalent diffusion coefficients for bound and
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free buffers.

There are two assumptions that are critical in the reduction. The first is to assume that the time scale of diffusion is much longer than the time scale for buffering (i.e. for a length $l$, we require $\frac{r^2}{D_B} \gg \tau_B$ where $\tau_B = \frac{1}{k_{b} + k_{b}([Ca])}$). If we follow the methods of Zador and Koch, we can obtain a single equation for the $Ca^{2+}$ dynamics

$$(1 + \beta[Ca]) \frac{\partial[Ca]}{\partial t} = \frac{\partial}{\partial x} \left[ (D_{Ca} + D_B\beta([Ca])) \frac{\partial[Ca]}{\partial x} \right] - P([Ca]) + \frac{2}{r} f(x, t). \quad (2.6)$$

The second assumption has to do with looking at high and low $Ca^{2+}$ limits of the terms in equation (2.6).

First, let us consider the low calcium limit. If we assume that $[Ca] \ll K_{d,B}$, and $[Ca] \ll K_{d,P}$ the leading order equation becomes

$$(1 + \beta) \frac{\partial[Ca]}{\partial t} = (D_{Ca} + \beta D_B) \frac{\partial^2[Ca]}{\partial x^2} - \frac{2K_{max}P_s}{r} [Ca] + \frac{2}{r} f(x, t) \quad (2.7)$$

where $\beta = \frac{[Ba]}{K_{d,B}}$. If we multiply both sides by $r/2$ we can rewrite the above equation as:

$$\frac{r(1 + \beta)}{2} \frac{\partial[Ca]}{\partial t} = \frac{r(D_{Ca} + \beta D_B)}{2} \frac{\partial^2[Ca]}{\partial x^2} - P_sK_{max}[Ca] + K_{\infty}P_sK_{max}I(x, t), \quad (2.8)$$

$$I(x, t) = \frac{f(x, t)}{P_sK_{max}K_{\infty}}. \quad (2.9)$$

Now notice that if $I(x, t) = I_0$ is a constant and we look for steady state solutions of (2.8), we get

$$K_{\infty} = \lim_{t \to \infty} \frac{[Ca](x, 0)}{I_0}. \quad (2.10)$$

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If we make the following substitutions:

\[ R_m = P_s K_{\text{max}}, \]  \hspace{1cm} (2.11)
\[ C_m = \frac{r(1 + \beta)}{2}, \]  \hspace{1cm} (2.12)
\[ R_i = \frac{1}{D_{Cu} + \beta D_B}, \]  \hspace{1cm} (2.13)
\[ R_\infty = K_\infty, \]  \hspace{1cm} (2.14)
\[ V = [Ca], \]  \hspace{1cm} (2.15)

into (2.8), we obtain

\[ C_m \frac{\partial V}{\partial t} = \frac{r}{2R_i} \frac{\partial^2 V}{\partial x^2} - \frac{1}{R_m} V + \frac{R_\infty}{R_m} I(x, t), \]  \hspace{1cm} (2.16)

which is readily recognized as the cable equation with a normalized current source term. The value of doing this is that the cable equation has been very thoroughly studied (see [43]). In the framework of the cable equation, we can see the pump acting like a membrane resistance, the buffers contributing to the capacitance, and the diffusion terms acting like intracellular resistances. More quantitatively, we can immediately define quantities such as the space constant, \( \lambda \), the time constant, \( \tau \), and the input resistance of an infinite cable, \( R_\infty \). In cable theory, these quantities are determined by the following formulas

\[ \lambda = \sqrt{\frac{r R_m}{2R_i}}, \]  \hspace{1cm} (2.17)
\[ \tau = R_m C_m, \]  \hspace{1cm} (2.18)
\[ R_\infty = \frac{(2r)^{-\frac{3}{2}}}{\pi} \sqrt{\frac{R_m}{R_i}}. \]  \hspace{1cm} (2.19)

If we now substitute (2.11)–(2.14) into the expressions listed above, we get

\[ \lambda_C = \sqrt{\frac{r(1 + \beta)}{2P_s K_{\text{max}}}}, \]  \hspace{1cm} (2.20)
\[ \tau_C = \sqrt{\frac{r(D_{Ca} + \beta D_B)}{2P_s K_{\text{max}}}}, \]  \hspace{1cm} (2.21)
\[ K_\infty = \frac{(2r)^{-\frac{3}{2}}}{\pi \sqrt{(D_{Ca} + \beta D_B)P_s K_{\text{max}}}}. \]  \hspace{1cm} (2.22)
where $\lambda_C$ is the chemical space constant, $\tau_C$ is the chemical time constant, and $K_\infty$ is the chemical input resistance of an infinite cylinder. Now notice that $\tau_C$ is dependent on the radius of the cylinder, but $\tau$ is not. Thus the time required for equilibration is going to be much larger for a larger cylinder. In particular, the time constant is expected to be small for a structure such as a dendritic spine suggesting that $[\text{Ca}^{2+}]_{sp}$ could rise quickly during stimulation and return to normal levels quickly at the end of stimulation. Also notice that just as in the electrical case, the space constant scales like the square root of the radius. However, the one big difference is that for reasonable physiological values of the given parameters, $\lambda_C \ll \lambda$. It can, in fact, be up to three orders of magnitude shorter than the electrical space constant. Thus, it is expected that the localization of chemical signals is going to be much more efficient than the localization of electrical signals within neurons.

A similar reduction can be performed in the high calcium limit. If one applied this assumption as we did with the low calcium limit, one could reduce the calcium dynamics to a simple linear diffusion equation with a source. In this case, the buffers and pumps are totally saturated, and all the essential nonlinearities are removed.

Zador and Koch then applied this linearization in the low Ca$^{2+}$ limit to their ZK model. One of their observations was that the chemical input resistance of the spine was 10 times larger than that of the parent dendrite. Thus, the same source located on the spine will produce a much larger transient than if it were located on the parent dendrite. Furthermore, the chemical space constant of the spine neck was 0.27 $\mu$m making the spine neck length $\approx 4\lambda_C$ which means that steady state $[\text{Ca}^{2+}]_{sp}$ would decay by a factor of $\approx 55$ by the time it reached the parent dendrite. Furthermore, the small radius of the spine neck and head also mean that spines have much smaller chemical time constants than the parent dendrite. This will only further amplify the concentration gradient between spine head and parent dendrite during calcium transients. This result shows
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how important the morphology of the spine is in both allowing for the development of a large, transient increase in $[\text{Ca}^{2+}]_{sp}$ while simultaneously limiting the extent to which it will spread throughout a dendrite.

Another interesting facet of the linearized $\text{Ca}^{2+}$ dynamics can be seen in the morpho-electrotonic transform [123]. Not only are local $\text{Ca}^{2+}$ transients in active spines isolated from the dendritic shaft, but $\text{Ca}^{2+}$ transients in the dendritic shaft are to a large extent isolated from the spine heads. This is in contrast to the tremendous voltage attenuation from spine head to dendrite when there is an input to the spine head, but when there is an input to the dendrite then there is very little voltage attenuation from dendrite to spine head [88].

This gives us a good understanding of calcium dynamics and how spines provide a microenvironment for large changes in $[\text{Ca}^{2+}]_{sp}$. However, LTP cannot be explained simply as a result of $\text{Ca}^{2+}$ influx through NMDARs when strong presynaptic activity is coupled to strong depolarization in the neighbourhood of the spine, with the resulting large non-linear increase $[\text{Ca}^{2+}]_i$ in the spine, and the activation of CaBPs and $\text{Ca}^{2+}$-dependent enzymes. Thus, the modeling cannot end here.

### 2.6 The Spatial Nature of $\text{Ca}^{2+}$ Transients in LTP

Due to the input–specificity requirements of LTP, the $\text{Ca}^{2+}$ transient must be severely restricted spatially in order to prevent the induction of LTP at adjacent synapses. Thus, it has been suggested that the dendritic spine may act as a separate compartment from the parent dendrite which acts to amplify the $[\text{Ca}^{2+}]_i$ for a given level of stimulation [81]. This idea was built into the models reviewed above in the form of calcium pumps and buffers within the spine. However, only in the last few years have experimental techniques to measure $[\text{Ca}^{2+}]_i$ in dendritic spines been developed.
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It has been found in a number of experiments that the $[\text{Ca}^{2+}]_{sp}$, the concentration of Ca$^{2+}$ in the spine head, is significantly higher than $[\text{Ca}^{2+}]_d$, the concentration of Ca$^{2+}$ in the parent dendrite, during synaptic stimulation [84, 75]. This difference could largely be negated by application of the NMDAR antagonist, D–APV. Furthermore, bath application of NMDA resulted in values of $[\text{Ca}^{2+}]_{sp}$ greater than $[\text{Ca}^{2+}]_d$ in a manner depending on $[\text{Ca}^{2+}]_o$, the extracellular calcium concentration [101]. Lastly, depolarization of the soma to -20 mV results in a larger increase in $[\text{Ca}^{2+}]_{sp}$ than in $[\text{Ca}^{2+}]_d$ [101, 99]. This difference could be blocked by application of L–type voltage gated calcium channel (VGCC) blockers, but not by either a P–type VGCC blocker or an N–type VGCC blocker. This suggests that spines have a hyperconcentration of NMDARs and L–type VGCCs compared to the parent dendrites, or a smaller concentration of CaBPs as assumed in the mathematical models of LTP. For example, a recent experiment [32, 33] followed the dispersal of Ca$^{2+}$ in a dendrite that was temporarily made permeable to Ca$^{2+}$. It was found that there was a significant lag in the change in $[\text{Ca}^{2+}]_{sp}$ compared to $[\text{Ca}^{2+}]_d$ in one-third of all spines. This difference was more frequently found in spines with long, thin stems than in spines with short, stubby stems.

However, it should be noted that the lag in spines for spreading Ca$^{2+}$ was not due to a physical diffusion barrier as there was no lag in the change in $[\text{Co}^{2+}]$ between spine and parent dendrite when Co$^{2+}$ was substituted for Ca$^{2+}$ in the extracellular fluid (ECF). This suggests the possible importance of calcium pumps in keeping $[\text{Ca}^{2+}]_{sp}$ somewhat isolated from $[\text{Ca}^{2+}]_d$. However, notice that the degree of difference is considerably smaller than that suggested theoretically. This may in large part be due to the fact that calcium fluorescence techniques do not actually measure the Ca$^{2+}$ directly but rather the concentration of calcium bound to certain exogenous CaBPs which fluoresce when irradiated with light of certain wavelengths.

The idea that calcium dynamics are not always as intuitive as one might think is an
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important one when dealing with such a qualitative endeavor. Nowycky and Pinter [78] performed a rigorous numerical analysis of the motion of Ca\(^{2+}\) and CaBPs in model cells (for a less rigorous treatment of simulations of the movement of CaBPs, but one that is specific to spines see [45]). One of their chief results was the spatio–temporal distribution of Ca\(^{2+}\)–bound buffers and Ca\(^{2+}\) can be quite different. This is especially true for small, mobile calcium chelators such as fura-2 which are typically used in calcium fluorescence experiments. This may partially explain the paradoxical results of the above experiment.

### 2.7 The temporal nature of Ca\(^{2+}\) transients in LTP

The experimental data shows reasonable qualitative agreement with the theoretical models in terms of limiting the spread of Ca\(^{2+}\). Unfortunately, the same cannot be said of the temporal nature of the Ca\(^{2+}\) transients.

In a recent study [64], a photolabile Ca\(^{2+}\) chelator, diazo-4, which increases its affinity for calcium 1600-fold upon exposure to intense light, was used to determine some of the temporal features of the Ca\(^{2+}\) signal required to induce LTP. A stimulus which normally induces LTP (100 Hz stimulation for 1 s) was found to induce PTP if the light was applied 1 second after the beginning of the stimulus (1s of 100 Hz stimulation), STP or weak LTP if light was applied 1.5–2.0 seconds following the beginning of the stimulus, and full LTP if the light was shone 2.5 seconds or later after the beginning of the stimulus. While these results may possibly allow for the release of Ca\(^{2+}\) from intracellular stores such as the SER, they do rule out any possible influences due to long-lasting Ca\(^{2+}\) waves or oscillations. Furthermore, since the peak [Ca\(^{2+}\)]\(_i\) is reached within 1.5 seconds when only STP was induced, this indicates that incomplete activation of some quickly activated [Ca\(^{2+}\)]\(_i\) dependent process does not distinguish whether STP or LTP is induced. This is where the models discussed above fail. The Ca\(^{2+}\)–dependent processes that were assumed to underlie LTP were quick, and the peak level of [Ca\(^{2+}\)]\(_i\) in these simulations occurred
well before that noticed during experiments (this can be seen in the small value of $\tau_C$). Thus, the nature of the nonlinearity that is produced during LTP could not be of the type that would have been noted in the previous modeling efforts. We will need to consider some more results from the biology of LTP before attempting to proceed.

While a fairly robust result in LTP has been the requirement of increase in $[Ca^{2+}]_i$ and the activation of NMDARs, there are other possible sources of $Ca^{2+}$ such as the SER.

CA1 hippocampal pyramidal neuron dendritic spines contain a high concentration of mGluR5 [94], a metabotropic glutamate receptor, which catalyzes the hydrolysis of phosphoinositide bis-phosphate (PIP$_2$) to inositol trisphosphate (IP$_3$) and diacylglycerol (DAG). IP$_3$ acts on IP$_3$ receptors located in the SER to cause release of $Ca^{2+}$ (see [9] for a review), while DAG is a potent activator of protein kinase C (PKC). This is of some interest since it is known that IP$_3$ receptors are found in CA1 hippocampal pyramidal neurons but ryanodine receptors (RyRs) which are involved in calcium–induced calcium release (CICR) processes are not, even though they are typically found in high concentrations in dendritic spines [103]. Furthermore, IP$_3$ is produced during the induction of LTP [37], and topical application of IP$_3$ to their dendrites can result in a $Ca^{2+}$ wave which propagates throughout the dendrite [44]. Moreover, thapsigargin [7, 37] and dantrium [79], which act to inhibit the release of $Ca^{2+}$ from intracellular stores, can prevent the induction of LTP. A direct link between these results and a role for mGlur5 comes from agonist/antagonist studies. mGluR5 antagonists have been shown to prevent the induction of LTP [6], while agonists have been shown to be capable of potentiating LTP [20, 13, 14]. The effects of mGluR5 agonists could be prevented by thapsigargin which strengthen the link between intracellular $Ca^{2+}$ stores and the induction of LTP. In light of this evidence, I will now review a model of LTP which explicitly included the presence of intracellular calcium stores.
2.7.1 A Model of Ca$^{2+}$ Dynamics in Dendritic Spines

Schiegg et al. developed a model [97] in 1995 which incorporated a mechanism for the release of Ca$^{2+}$ from intracellular stores as a result of the experimental results noted above. In particular, they were concerned with meeting the criterion for elevated levels of $[\text{Ca}^{2+}]_\text{sp}$ for $2 - 2.5 \text{ s}$ as noted in the study using the photoactivable calcium chelator, diazo-4 [64].

The modeling approach was to use a 10 compartment electrical model of a CA1 hippocampal pyramidal neuron split into a soma compartment, a basilar bush, a lower apical branch, and an upper apical branch which is subdivided into 6 dendritic compartments. The upper apical branch was subdivided to allow better spatial resolution in the neighbourhood of the synaptic input. A set of $N_{\text{spine}}$ identical spines were attached to the fourth dendritic compartment away from the soma on the upper apical branch. The spine morphology was as before, a cylindrical spine head attached via a spine neck, which was reduced to a lumped potential in the electrical model, to the parent dendrite. It further was assumed that there was synchronous activity in each spine, and thus the voltage and current loss to the dendrite was the same for each spine. As a result, only one spine needed to be explicitly included in the electrical model. The voltage channels used in this model were based on the NMDA and non-NMDAR currents used by Zador and Koch [122].

The calcium dynamics were essentially the same as in the ZK model. The spine head was split into three compartments in the discretization, with all synaptic channels located in the subsynaptic compartment. The spine neck also was subdivided into three compartments. There are a couple of differences worth noting. First, it was assumed that 10% of the current flowing through the NMDAR is due to Ca$^{2+}$, rather than the 2% used in the ZK model. Rather than model the four separate sites on CaM, which was assumed
to have a total concentration of \( \approx 30 \mu M \), it was assumed that there was a buffer with a single calcium binding site with \([Bt] = 120 \mu M\) in the spine. The forward and backward binding constants of the buffers were the same as in the ZK model. The form of the pumps were as in the ZK model, but the pump efficiencies were larger than the values used in the ZK model by a factor of 10. Rather than explicitly model the \([Ca^{2+}]_d\), it was assumed that \([Ca^{2+}]_d\) was clamped at 50 nM. However, by far the biggest difference between the two models is the inclusion of a CICR mechanism in this model.

Both the IP\(_3\) receptor and the RyR show a bell-shaped calcium response with little calcium release at low \([Ca^{2+}]_i\) and high \([Ca^{2+}]_i\). Rather than using a detailed model of both IP\(_3\) receptors and RYRs, Schiegg et al. combined the two in the form of one phenomenological equation with a bell-shaped calcium dependence. It was assumed that there was one common intracellular Ca\(^{2+}\) pool which occupied \(\frac{1}{10}\) of the spine head volume. In the discretization scheme used, this intracellular pool was placed in the second compartment, directly below the subsynaptic membrane. The release of Ca\(^{2+}\) from this store and into the second spine compartment is modeled by

\[
\frac{\partial [Ca]_{store}}{\partial t} = \rho X ([Ca]_{store} - [Ca]_2) \tag{2.23}
\]

where \([Ca]_{store}\) is the concentration of Ca\(^{2+}\) in the store, \([Ca]_2\) is the concentration of calcium in the second compartment, and \(X\) is the number fraction of open channels. \(X\) is calculated from

\[
\frac{dX}{dt} = -\frac{1}{\tau_{store}} [X - (RA)Re([Ca]_2)] \tag{2.24}
\]

where \(RA\) is the probability of receptor–agonist binding and \(Re([Ca])\) models the bell-shaped calcium response of the receptors. In these simulations, when the effects of intracellular Ca\(^{2+}\) release was thought to occur, \(RA\) was set equal to 1. This is equivalent to assuming the receptors are saturated by agonist molecules. The following functional
form was used for $Re$:

$$Re([Ca]) = \begin{cases} 
0, & [Ca] < [Ca]_\theta, \\
v([Ca]) \exp [1 - v([Ca])], & [Ca] > [Ca]_\theta, 
\end{cases} \quad (2.25)$$

$$v([Ca]) = \frac{[Ca] - [Ca]_\theta}{[Ca]_{max} - [Ca]_\theta}. \quad (2.26)$$

The form of the above equation is an $\alpha$–function (i.e. it has the form $cea^{-\frac{t}{\tau}}$ for $t > 0$, and 0 otherwise) which attains a maximum of 1 at $[Ca] = [Ca]_{max}$. In the simulations, $[Ca]_\theta$ was chosen to be 150 nM as it is thought that there is little CICR release when $[Ca^{2+}]_i$ is less than 150 nm. Thus the intracellular stores only will act as a source so long as $[Ca]_2$ is above 150 nM.

If $RA$ was set equal to zero, it was found that previous models could not sustain high calcium concentrations for longer than 100 ms after the end of the stimulus. If we refer back to the analysis done on linearizing calcium dynamics, we can see this is a result of the extremely small value of the chemical time constant. This is where intracellular Ca$^{2+}$ stores come into the picture. If four pulses are applied at 100 Hz with $N_{spine} = 30$ and $RA = 1$, it was found that $[Ca^{2+}]_{sp}$ could be kept above 400 nM for longer than 1 s. Because of the steep drop–off in $Re([Ca])$ for $[Ca] > 250$ nM, it was found that there was minimal involvement of intracellular stores until $[Ca]_2 \approx 400$ nM. The intracellular stores are able to maintain this concentration by compensating for the loss of calcium through pumps and diffusion if there is enough calcium present in the store, and if $\rho X$ (see (2.23)) is large enough. If $\rho$ or $X$ is reduced by a factor of 10, then it is not possible to sustain elevated $[Ca^{2+}]_{sp}$ under any stimulation protocols. If, however, $\rho$ and $X$ are large enough, the drop–off in $[Ca]_{store}$ is nearly linear in time from the time it begins until the time it ends, resulting in a quasi–steady-state in which $[Ca^{2+}]_{sp}$ is elevated for over 1 s after the end of the stimulation at a near constant value. The key factor that controls whether a spine is in the high X range is receptor–agonist binding which can be thought of as a function of IP$_3$ concentration. Thus, if a stimulus is large enough to result
in significant IP$_3$ release, it is expected that [Ca$^{2+}]_{sp}$ may be elevated for a significant amount of time. Otherwise, [Ca$^{2+}]_{sp}$ will decay quickly as the value of $\rho X$ will be too small to compensate for the loss of calcium through diffusion and pumps.

This model nicely builds a mechanism showing how intracellular calcium stores can explain the results of the diazo-4 experiment where the previous models fail, and it also helps explain the importance of IP$_3$ in LTP. Unfortunately, there is evidence suggesting that there are other calcium stores of importance in LTP, and that the biochemical cascade involved may be a bit more complicated than initially thought. It turns out that protein kinases (PKs), and protein phosphatases (PPs) may be quite important in LTP induction.

### 2.8 Protein Kinases and Phosphatases in LTP

As mentioned previously, there is ample evidence for the existence of L-type VGCCs in dendritic spines. However, typically it has been found that depolarization pulses alone could not induce LTP. Some suggested that this may be due to an inability to create the appropriate increase in [Ca$^{2+}]_i$, although it has been found that most of the increase in [Ca$^{2+}]_i$ during synaptic activity is due to VGCCs [72]. However, depolarization pulses coupled to LFS (2 Hz stimulation) which did not act through an increase in Ca$^{2+}$ influx could result in the induction of LTP [54]. This suggests that some aspect of glutamate release may be important in inducing LTP. Three immediate candidates for this effect are NMDARs, mGluR5, and a possible priming effect that action potentials have on the presynaptic cell. The problem with NMDARs as the candidate is that bath application of NMDA is capable of creating large Ca$^{2+}$ transients, but they only can induce STP and not LTP [12] (although, see [108]). The problem with the priming hypothesis is that postsynaptic glutamate iontophoresis is capable of inducing LTP [22]. Thus the most likely stimuli required for the induction of LTP is some combination of a sufficiently
large increase in \([\text{Ca}^{2+}]_i\) and the activation of mGluR5.

Since a local, transient increase in \([\text{Ca}^{2+}]_i\) itself was never seriously expected to be the ultimate end of the biochemical cascade which results in LTP, the next question becomes what is? That is, there may be a number of biochemical cascades involved which all have one common endpoint — the activation of some critical molecule or biochemical process that once initiated results in a persistent increase in synaptic efficacy. It turns out there is such a candidate molecule, \(\text{Ca}^{2+} - \text{CaM}\) dependent protein kinase II (CaMKII) (for a review of this remarkable enzyme see [16]).

**A Brief Introduction to CaMKII**

CaMKII is a holoenzyme composed of 8-12 basically identical monomers. Each monomer possesses a catalytic site, a regulatory domain, and a site which allows it to bind to other monomers. Monomers are typically inhibited by their regulatory domain, but this inhibition is relieved when \(\text{Ca}^{2+}_i - \text{CaM}\) binds to the regulatory domain. In its \(\text{Ca}^{2+}_i - \text{CaM}\) bound state, each monomer has a very broad substrate specificity allowing it to regulate such diverse processes as carbohydrate metabolism, neurotransmitter release and resynthesis, cytoskeletal function, and ion flux [98]. Interestingly, CaMKII is the most common \(\text{Ca}^{2+}\)-dependent PK, comprising \(\approx 1\%\) of total protein in the brain, and \(\approx 2\%\) of total protein in the hippocampus, and 30-40\% of the total protein in the PSD. Given its high concentration in the PSD and hippocampus, it is not surprising that it is suspected of playing a major role in governing synaptic plasticity.

CaMKII has a unique set of attributes that allow it to respond to the distinctive properties of calcium signals which usually occur over a narrow range of concentrations, and are transient and pulsatile in nature rather than smooth and continuous. CaMKII handles such signals elegantly due to its multiple activity levels which are dependent not only on its monomers \(\text{Ca}^{2+}_i - \text{CaM}\) bound, but also their phosphorylation state. The
CaMKII subunits are involved in autophosphorylation reactions [71] – one subunit in its Ca$^{4+}$ – CaM bound state can phosphorylate a neighbor in the same state [34]. This phosphorylation has the effect of trapping Ca$^{2+}$ – CaM to a monomer through a dramatic decrease in the off-rate of Ca$^{2+}$ – CaM binding. Since there is very little effect on the on-rate of binding, this results in a dramatic increase in the monomer’s affinity for CaM – the $K_d$ goes from $\approx 45$ nM to $\approx 60$ pM, one of the highest known affinities for CaM [16]. While Ca$^{2+}$ – CaM typically dissociates from a monomer within a half-second, in its phosphorylated state, the typical dissociation time is on the order of hundreds of seconds. In this Ca$^{4+}$ – CaM bound, phosphorylated state, the monomer maintains 100% of its catalytic activity. Even after the Ca$^{2+}$ – CaM dissociates from the monomer, so long as the monomer is phosphorylated, it maintains 20–80% of its catalytic activity. Now it can be seen how the CaMKII molecule can decode calcium signals. If a Ca$^{2+}$ signal is arriving with a certain frequency, a certain number of subunits per holoenzyme are going to bind Ca$^{2+}$ – CaM. If two proximal subunits become activated, they may become involved in an autophosphorylation reaction which traps the Ca$^{4+}$ – CaM and leads to a potentiation of the calcium signal. If the frequency is low, the Ca$^{4+}$ – CaM would escape before the next spike (this can happen because there are phosphatases which also are activated by Ca$^{2+}$–CaM which dephosphorylate the subunits), but if the frequency is high, then there is the possibility of even more subunits becoming activated with each new spike. “This cooperativity of calmodulin trapping may allow the kinase to be a frequency detector with a threshold frequency beyond which it becomes highly active” [16].

A Model of CaMKII Dynamics

Using some estimates of experimental parameters [68], Michelson and Schulman [69] developed a model for the activation and transition between different activity states of a ten subunit CaMKII molecule.

The $j^{th}$ subunit, $S_j$, had five activation states: inhibited, $S_j^I$, Ca$^{4+}$ – CaM bound, $S_j^R$,
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Ca$_2^{2+}$ – CaM trapped, $S_j^T$, autonomous, $S_j^A$, and capped, $S_j^C$. In the capped state, it is phosphorylated at an additional site compared to the autonomous and trapped states. Once a subunit is bound to Ca$_2^{2+}$ – CaM, it can traverse the trapped, autonomous, and capped states. The transitions between these states were a function of the Ca$_2^{2+}$ – CaM concentration and the activation state of its right-hand neighbor.

If we let $S_{j+1}^X$ denote the activation state of the right-hand neighbor, the following transition matrix describes the probability of transfer between the various activation states:

$$
\begin{bmatrix}
  p_{II}([Ca_4CaM]) & p_{IB}([Ca_4CaM]) & 0 & 0 & 0 \\
p_{BI}([Ca_4CaM], S_{j+1}^X) & p_{BB}([Ca_4CaM], S_{j+1}^X) & p_{BT}([Ca_4CaM], S_{j+1}^X) & 0 & 0 \\
0 & p_{TB}([Ca_4CaM], S_{j+1}^X) & p_{TT}([Ca_4CaM], S_{j+1}^X) & p_{TA}([Ca_4CaM], S_{j+1}^X) & 0 \\
p_{AI}([Ca_4CaM], S_{j+1}^X) & 0 & p_{AT}([Ca_4CaM], S_{j+1}^X) & p_{AA}([Ca_4CaM], S_{j+1}^X) & p_{AC}([Ca_4CaM], S_{j+1}^X) \\
0 & 0 & 0 & p_{CA}([Ca_4CaM], S_{j+1}^X) & p_{CC}([Ca_4CaM], S_{j+1}^X)
\end{bmatrix}
$$

where the nonzero entries in each row are non-negative and sum to 1.

The method used to solve the problem was Monte Carlo simulation. The transition probabilities were based on half-life estimates of the various activity states from experimental data [68]. It was assumed that the transition state probabilities could be fitted to an exponential distribution.

The results of simulations suggest that the trapped state of the subunits can act as a "capacitance" that allows for significant temporal summation even at low frequencies. In fact, a single 1µM Ca$_4^{2+}$ – CaM spike was capable of increasing the activity of CaMKII for minutes following the stimulation. Another observation from the application of sinusoidal Ca$_4^{2+}$ – CaM transients is that while the proportion of subunits in inhibited, bound and trapped state is sinusoidal, the proportion in the autonomous and capped state do not and thus appear to be acting capacitively.
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CaMKII and LTP

The idea of CaMKII acting as a frequency detector of high frequency calcium signals, coupled to its high concentration in the hippocampus and PSD immediately suggests that it may be very important in LTP induction. This has been recognized and a very prominent theory in the field has been put forward suggesting that the activation of CaMKII is not only necessary and sufficient for the induction of LTP but may actually be the genesis of LTP and LTD expression [57, 60, 58, 59]. The basic idea is that low levels of \([Ca^{2+}]_i\) which are thought to be involved in LTD induction may preferentially activate protein phosphatases which will dephosphorylate CaMKII subunits and result in a decrease in synaptic efficacy. However, a large increase in \([Ca^{2+}]_i\) will directly lead to the phosphorylation of CaMKII and the resulting increase in CaMKII activity will lead to an increase in synaptic efficacy. In between synaptic weight change episodes, the level of phosphorylation of CaMKII will be set by the competing reactions of dephosphorylation by protein phosphatases and autophosphorylation within CaMKII holoenzymes. In support of this theory, it has been found that postsynaptic injection of \(Ca^{3+} - CaM\) can lead to the induction of LTP [112], while postsynaptic injection of CaM inhibitors [62] or CaMKII inhibitors [62, 65] can block LTP induction. Furthermore, evidence for the long-term activation of CaMKII following LTP induction has been found [26]. An interesting question now becomes how might CaMKII increase synaptic efficacy. One possibility that has been suggested is that CaMKII directly phosphorylates synaptic glutamate receptors and results in an increase in conductance. In this light, it has been shown that several glutamate receptor subunits have consensus phosphorylation sites for CaMKII [67]. Furthermore, CaMKII can phosphorylate and increase the current through kainate receptors (this is a type of non-NMDAR which is quite similar to the AMPAR) three- to four-fold in vitro. A corollary to this CaMKII hypothesis for LTP should be that if CaMKII already is maximally activated or cannot be activated at all, then it should be
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impossible to induce LTP. This is, in fact, observed. Transgenic mice without genes for CaMKII are incapable of undergoing LTP [30], and when a constitutively active CaMKII was introduced into hippocampal slices, it resulted in an increase in synaptic efficacy but prevented further induction of LTP [85].

Now the hypothesis that increased receptor conductance leads to an increase in synaptic efficacy seems intuitively obvious, but one has to remember that efficacy here refers to the ability of presynaptic activity to result in action potential generation at the axon hillock. In fact, it has been show that under certain circumstances this may not be true [117]. This will be dealt with in more detail when I review results from electrical modeling of spines later.

Now there is one last question which I should delve into before finishing the LTP portion of this thesis. Is an increase in $[\text{Ca}^{2+}]_i$ under normal physiological conditions sufficient for the induction of LTP?

2.8.1 The role of PKC and sufficiency of Ca$^{2+}$ in LTP induction

The question of the sufficiency of increases in $[\text{Ca}^{2+}]_i$ in LTP is very contentious. The first of many positive results came from experiments with a calcium chelator, nitr−5, which releases Ca$^{2+}$ upon exposure to light [63]. However, many different experimental results have been found, and there is no clear consensus. One interesting experiment alluded to earlier [112] found that postsynaptic injection of Ca$^{2+}$ or CaM alone could not induce LTP while the injection of a calcium/CaM mixture could in a manner that was inhibited by CaMKII and PKC inhibitors. The role of CaMKII in LTP was discussed previously and will not be discussed again. PKC was mentioned previously as a PK which is activated by DAG which is produced during mGluR5 activation (along with IP$_3$). It has been a fairly consistent observation that PKC is activated during LTP induction [51], and its inhibition prevents LTP induction [3, 111, 91].

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The link between PKC activation and CaMKII activation is not immediate as neither has any direct effect on the other. However, it has been discovered that PKC phosphorylates a protein, RC3 (also called neurogranin), found in high concentration in dendritic spines [113]. RC3 is a calmodulin-binding protein which decreases its affinity for CaM when it is phosphorylated [28]. It has been shown that RC3 can modulate calmodulin availability and decrease the activity of Ca\textsuperscript{2+} - CaM-dependent enzymes [66]. For example, in the presence of 1\muM of RC3 (a more physiological concentration is 2\muM), 10\muM of Ca\textsuperscript{2+} was required to achieve half-maximal velocity of nitrous oxide synthase (NOS), a Ca\textsuperscript{4+} – CaM-dependent enzyme. Only .55\muM of Ca\textsuperscript{2+} was required in the absence of RC3. It was found that inclusion of activated PKC could prevent the RC3-mediated inhibition of NOS. This effect could be negated by inclusion of alkaline phosphatase which dephosphorylates RC3. This is of some interest since RC3 is dephosphorylated by PP1 and calcineurin, both of which are found in dendritic spines [102]. The specific link between RC3 and LTP comes from an experiment in which it was found that antibodies to RC3 which inhibit its PKC-mediated phosphorylation could prevent the induction of LTP [25]. One possible reason for this effect may have been due to some effect on CaM availability. Some have suggested that “RC3 is a biochemical ‘capacitor’ in that it transduces Ca\textsuperscript{2+} fluxes into kinetic parameters affecting the availability of Ca\textsuperscript{2+}/CaM and the rapidity with which it is made accessible to other enzymes” [28]. Thus, RC3 may be acting as a PKC-mediated CaM store, and this is the connection between PKC and CaMKII activation. However, there is some evidence suggesting a role for phosphorylated RC3 in mobilizing of intracellular Ca\textsuperscript{2+} stores in Xenopus oocytes [21].

2.9 Concluding remarks about LTP

What exactly LTP is at this moment is not at all clear, but there seems to be a very strong connection between it and dendritic spines. The connections between LTP and
learning/memory are less clear. The only solid connection between LTP and learning
appears to be that mechanisms which reduce the capacity for LTP induction seem to
impair spatial memory in rats. This is consistent with the hippocampus being very
important in spatial memory. Otherwise, there seems to be little effect for the most
part. One has to be a bit cautious because here LTP means LTP induced at the Schaffer
collaterals in the stratum radiatum of CA1 hippocampal pyramidal neurons. Thus, LTP
induction may be dependent on similar but slightly different mechanisms elsewhere in
the CNS. On the other hand, the local learning memory paradigm may not be the way
most memories are stored in the CNS. This should not denigrate, however, the work that
has been done in this field. LTP is a very robust memory phenomena which may provide
insights into the way in which memories are encoded in the CNS, and this is why it is
so extensively studied. Moreover, LTP offers an excellent example of theoreticians and
experimentalists working side-by-side in deciphering the riddle of biological complexity.