

MATHEMATICAL MODELING

Over the last several lectures you have been exposed to a large number of signal transduction pathways that start with neurotransmitters acting on ionotropic or metabotropic receptors to activate voltage gated calcium channels or G-proteins that control various effectors and second messengers which can activate kinases and phosphatases.

OH 1 Some of the substances involved are calcium, adenylyl cyclase, cAMP, phosphodiesterase (PDE), PKA, PLC, PIP₂, DAG, PKC, IP₃, calmodulin, calcineurin, CaMKII, PP1, PP2A, Inhibitor-1, DARPP-32, tyrosine kinases, Raf/MAPK, MAPKK, arachidonic acid, tyrosine kinases, Ras/Raf, and CREB.

In addition you have been exposed to a number to pathways involving a number of substances and pathways involved in gene transcription, translation, regulation and expression, which I won't mention.

Because of biochemical experiments we have some idea of how individual substances react with specific other substances. But what does it all mean? How do all of these reactions interact? When you have enzyme cascades, feedback loops, and time delays, what happens in the cell? How can we understand what is actually going on?

This is where mathematical models come in. Mathematical models provide a conceptual framework to assemble complex data into concise pictures of structure and operation of signaling pathways.

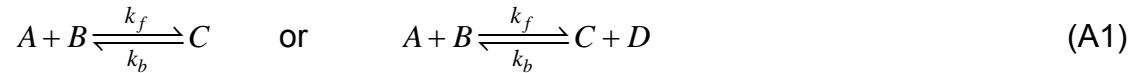
Experimentally we can knock out a substance and see what the effect is on the system. However manipulating one substance at a time does not let us see clearly what the metabolic or signaling network is doing. Our intuition may generally be good in making predictions of linear systems, but generally it fails miserably when systems are nonlinear and/or attain the complexity of biochemical networks present in cells.

The many systems you have seen over the last several lectures are not independent. The signaling pathways interact and their interaction allows emergent network properties to be observed such as i) integration of signals across time scales, ii) generation of distinct outputs depending on strength and duration of signals and iii) self-sustaining feedback loops. Feedback can result in bistable behavior and well defined thresholds.

Mathematical models provide the framework through which emergent behaviors of biochemical networks can be explored and studied.

So where do we begin? (Much of what I will discuss is either in Chapter 14 of your book or else in U.S. Bhalla and R. Iyenger, Emergent properties of networks of biological signaling pathways" *Science* 283:381-387, 1999).

OH 2 Signal transmission occurs through biochemical reactions among proteins or enzymatic reactions such as phosphorylation and dephosphorylation, or protein degradation or production of messengers. Consequently the place to start is representing these reactions in their standard forms:



We represent these equations mathematically with differential equations. In high school, what kinds of mathematical problems were generally considered the most difficult? Typically students had difficult times with “word problems” or “story problems”. A train leaves Baltimore at 2 PM traveling 60 MPH and another train leaves New York at 3PM traveling 45 MPH. At what time do they crash? Etc.

Well, biology is a bunch of word problems just waiting to be expressed mathematically. Fortunately biochemical reactions are perhaps the easiest type of problems to translate into mathematical equations.

To form a differential equation for this first reaction, we need to think about how the concentrations of A, B, and C change in time. We do this first for reactant A.

The concentration of A will decrease as A and B combine to form C. The rate of this decrease will be proportional to the current concentrations of both A and B. The proportionality constant is characteristic for a particular reaction and here we call it k_f . The constant k_f is usually called the forward rate constant and since there are two reactants (second order reaction), it has units of concentration⁻¹ time⁻¹ or, M⁻¹s⁻¹. Therefore the change in the concentration of A over time due to its reaction with B will be $-k_fAB$, where the minus sign indicates that the change is negative, and the k_fAB indicates that the change is proportional to the concentrations of both A and B.

In addition the concentration of A will increase as C breaks down to form A and B. The rate of this increase is proportional to the concentration of C with proportionality constant k_b . This constant is called the backward rate constant, and since C does not combine with anything else for this reaction, k_b has units of time⁻¹ or, s⁻¹. Therefore the change in the concentration of A over time due to the breakdown of C will be $+k_bC$, where the plus sign indicates that the change is positive, and the k_bC indicates that the change is proportional to the concentration of C.

We indicate the change in the concentration of A over time with the differential dA/dt or the change in A with respect to a change in time. Putting all of this together we have:

$$\frac{d[A]}{dt} = -k_f [A][B] + k_b [C] \quad (\text{A2})$$

If we analyze the change in the concentrations of B and C in the same manner, we find that the differential equation for B is the same as for A, and the differential equation for C is the negative of that for A or B. This makes sense since A and B must decrease

at the same rate as they combine to form C and they must increase at the same rate as C breaks down. Similarly the change in C must be exactly opposite that of A and B. Consequently the differential equations for B and C are:

$$\begin{aligned}\frac{d[B]}{dt} &= -k_f [A][B] + k_b [C] \\ \frac{d[C]}{dt} &= +k_f [A][B] - k_b [C]\end{aligned}\tag{A3}$$

Often the individual forward and backward rate constants are unavailable. What may be available is the ratio of these rate constants, given, for example, as a dissociation constant K_D . The dissociation constant comes from a steady-state measurement. To go from the differential equations to the steady-state is quite simple. In the steady-state all derivatives with respect to time are zero. Consequently, from eq. A2 above we have

$$0 = -k_f [A][B] + k_b [C]\tag{A4}$$

which implies

$$\frac{k_b}{k_f} = \frac{[A][B]}{[C]} = K_D\tag{A5}$$

where the units of K_D are $\text{time}^{-1}/(\text{concentration}^{-1} \text{time}^{-1})$ or concentration. When only the K_D value is provided in an experiment, the modeler must choose what seem to be reasonable k_f and k_b values that are consistent with the K_D . Often the k_f may be diffusion limited and k_b is the major rate constant determining K_D .

Similarly, for the second reaction we have

$$\frac{d[A]}{dt} = -k_f [A][B] + k_b [C][D]\tag{A6}$$

OH 3 For enzyme kinetics we have what well known equation? The Michaelis-Menten equation. For an enzyme reaction



we often talk about the velocity of the reaction. For those who have had biochemistry, what is the Michaelis-Menten expression for the velocity of an enzyme reaction?

The velocity of the reaction will reflect the formation of product. So $v = dP/dt$ and

$$\frac{d[P]}{dt} = \frac{V_{\max} [S]}{[S] + K_m}\tag{A8}$$

so when you have reactions where a phosphatase is dephosphorylating a protein, the phosphatase can only work so fast. It becomes saturated. So in this case you would use the M-M form in your differential equation. Where does the enzyme concentration come into play here? It is in the V_{\max} because $V_{\max} = k_3[E_t]$ and $[E_t]$ is the amount of enzyme present.

Most (all but a few exceptions) of the biochemical signaling pathways we have encountered can be expressed in equations using the forms given above. The text goes into detail about incorporating allosteric modifications. This is a variation of the Michaelis-Menten kinetics and the exact form of the equation will depend on the number of modification sites in what is called the Hill equation. The modification is to include a

$\frac{[L]^n}{[L]^n + K_H^n}$ term where n is the number of effector sites (binding sites on enzyme).

Once we have all of the equations that describe our network, we then have to solve them. Fortunately, it is no longer necessary to write computer programs to do this with a particular numerical technique. Software packages such as Matlab or Stella or XPP or kinetikit within the GENESIS simulator allow you to specify differential equations much as I have written them (or even simpler) and the software will solve them for you. With Copasi (newer version of gepasi) you can just write the chemical equations and the software will generate and solve the differential equations. Copasi has a number of built in reaction forms that can be useful.

So to this point we have discussed converting the reaction equations to mathematical equations and have mentioned methods of solving them. But now, what reactions should we consider?

The way to approach this is to develop libraries of known reactions and reaction kinetics to the extent that they have been determined by experiments. This has been done for many reactions and a good source for this is Upi Bhalla's web-site as given in the Science article I mentioned earlier.

Models are developed in stages. Note that second messengers produced by one pathway may be used as inputs to other pathways and enzymes regulated by one path are coupled to substrates in another path.

OH 4 The next two overheads give examples of known pathways. For example:

pathway C involves the Gs G-protein and its interaction with two forms of adenylyl cyclase. AC1/8 binds calcium-calmodulin, becomes activated and produces cAMP. Or AC 1/8 binds Gs, becomes active and produces cAMP (AC1 and 8 have a CaM binding site, 2 does not). Or GsAC2 synthesizes cAMP from ATP. cAMP is broken down by PDE (phosphodiesterase).

Pathway D shows the activation of G-proteins. Glutamate(1) binds to a receptor(2) forming a complex (3), which then changes conformation leading to binding of a G-protein $G_{\alpha\beta\gamma}$ (4) forming a complex (5). Normally GDP is tightly bound to the α -subunit but when the receptor complex binds, GDP dissociates, and GTP binds (6), and the complex breaks into the active forms $G_{\beta\gamma}$ (7) and G_{α} -GTP (8) and the complex dissociates from the glutamate receptor (to 3). Eventually the G_{α} -GTP gets dephosphorylated (9), allowing the $G_{\beta\gamma}$ to rebind (10 or 4).

The conversion of PIP₂ to DAG and IP₃ by PLC is shown in pathway F.

OH 5 Activation of PKA by cAMP is described by the scheme shown in pathway J. Recall that the inactive PKA has 2 regulatory subunits (R) and 2 catalytic (C) subunits. cAMP binds to the regulatory subunits (two per R subunit) and this releases the two catalytic subunits (C) which are now active.

Pathway M shows calcium binding to calmodulin and the interaction with Neurogranin which is thought to act as a calmodulin sink. Calmodulin is a dumbbell shaped molecule and two calcium ions bind to each "bell". Neurogranin binds calmodulin when calmodulin is free of calcium (usually), but phosphorylated neurogranin cannot bind calmodulin. Ng is phosphorylated by PKC and dephosphorylated by calcineurin.

Pathway I shows the activation of CaMKII by calcium-calmodulin its subsequent autophosphorylation and then dephosphorylation by PP1. We will return to this pathway in more detail later.

Pathway N shows the activation of calcineurin (PP2B) by calcium-calmodulin.

Pathway O shows the control of PP2A activation by Inhibitor-1, calcineurin, and PKA. DARPP-32 has functions similar to Inhibitor-1. PKA phosphorylates I1 and PP2A and calcineurin dephosphorylates I1*. Phosphorylated I1* binds to PP1 and inhibits it from doing its job as a phosphatase.

These libraries of reactions express what we think happens. When the mathematical models are done, it often becomes necessary to re-think how things bind.

For example, pathway I indicates that calmodulin must bind four calcium ions before calcium-calmodulin will bind to CaMKII. Mathematical models show a requirement for a large change in calcium to get calcium-calmodulin to bind to CaMKII. But experimental work has found CaMKII with bound calmodulin at much lower concentrations of calcium. Upon further investigation it has been found that CaMKII can have calmodulin with just two calcium ions bound to it. Such interaction between models and experiment helps improve our understanding of how things work.

So what can the models tell us? Let's look at an example on this next overhead

OH 6 Here the pathways are expressed in a block diagram. Calcium enters through NMDA receptors, binds to calmodulin and calmodulin activates CaMKII, calcineurin and phosphodiesterase, and a form of adenylyl cyclase. CaMKII can autophosphorylate.

These boxes on the left exert control over production of PP1 which dephosphorylates CaMKII. Note, the block diagram leaves out some of the reaction details expressed in modules C, I, J, M, N and O from the previous overhead.

The questions asked here are: Can the cAMP pathway gate CaMKII signaling through the regulation of PP1? Can interactions between CaMKII, the cAMP pathway, and calcineurin produce persistent activation of CaMKII, even after the calcium signal is terminated? Such activation is thought to be important for learning and memory.

A strong calcium signal was presented to the model as one might find in experimental conditions that induce LTP. Calcium bound to calmodulin and activated CaMKII, AC1, and CaN and also activated PKA through an increase in cAMP produced by activation of AC1/8.

The simulations predict that activation of AC1/8 overcomes the competing degradation of cAMP because of activation of CaM and PKA sensitive PDEs.

PKA activation leads to phosphorylation of Inhibitor-1 which inhibits PP1. When calcium returns to normal, CaN is rapidly deactivated (plot E), PKA is rapidly and then more slowly deactivated (plot C) and PPI is restored to basal activity in 20 minutes (plot D). With PP1 active, CaMKII gets dephosphorylated and deactivated.

Simulations were done with and without the cAMP gate. With the cAMP gate, there is an increase in CaMKII activation and a prolongation of its activity, but no persistent activation of CaMKII in this model. Nevertheless the prolongation of CaMKII activity can be significant. The model makes a prediction of how these pathways may interact, and this prediction can be tested experimentally.

But now, what if we add interactions with other pathways?

OH 7 The block diagram in A is the basis for Fig. 14.5 in your text. We know these pathways exist and interact, but again, what does it all mean?

At the synapse, we know that glutamate can act on 3 types of receptors—the NMDA receptor, the AMPA receptor and the mGlu receptor. The responses to mGlu activation involve G-proteins.

Calcium influx through NMDA receptor channels or voltage-gated calcium channels can activate CaMKII, PKC, PKA and MAPK. The PKC and MAPK pathways are linked and the CaMKII and cAMP pathways are also linked. The PKC pathway is also connected to the cAMP pathway via AC2 which can be phosphorylated and activated by PKC. So these kinases interact in a network.

In this network, can we get CaMKII activity that is persistently activated after the calcium signal has ended?

The network was analyzed in the model both with and without the feedback loop in the connection between PKC and MAPK pathways mediated by PLA2.

OH 8 Without the feedback loop, CaMKII, CaN, PKA and PP1 concentrations behave similarly to the previous case.

Initially you also see PKC activation (PKC spikes) (plot B) but PKC concentration goes back to rest quickly and there is little MAPK formation (plot C) (can only get to MAPK via PKC).

- 1) With the feedback loop in place after the first PKC spike there was a second rise in PKC activity from stimulation by DAG and AA (the first rise was due primarily to calcium and activation of PLC stimulating DAG production).
- 2) Now we get a steady rise in MAPK activity that persists (ultrasensitive MAPK switch).
- 3) Also, there is a smaller increase in PKA due to activation of AC2 by PKC. With PKC activation becoming sustained, we see sustained activation of PKA above basal levels (plot D).
- 4) This sustained increase in PKA regulates PP1 by phosphorylating Inhibitor-1 which allows Inhibitor-1 to inhibit PP1 (plot F).
- 5) With PP1 persistently inhibited, CaMKII activation reaches a steady-state at a level where the active enzyme concentration is doubled (plot E).
- 6) PP2B (calcineurin) cannot dephosphorylate inhibitor-1 and restore PP1 activity because its activation depends on the calcium signal (plot G).

So in this case, we can get a sustained increase in CaMKII activation and this sustained activity does not require changes in protein expression. It occurs only through the biochemical properties of the system.

Of course, MKP (MAP kinase phosphatase) induction may turn off this feedback loop and transcriptional events initiated in parallel with MKP induction may produce gene products which can then reach the synapse. So the extra feedback loop may gate incorporation of the gene products into the cytoskeleton to produce a long term change.

OH 9 So these are just some **examples of how mathematical models can be used to make predictions** about how the myriad of pathways may interact, and without the conceptual framework provided by the models, it would be very difficult to make much sense of the biochemical data.

By considering the networks instead of individual pathways, network properties emerge.

1. We may see extended signal duration. by coupling fast and slow processes, output can be regulated for an extended period of time after the initial signal transient.

2. Activation of feedback loops. Feedback loops are common and in the example just presented, we saw that the multiple modes of stimulating PKC were essential for the expression of the feedback loop. In the model MKP and PKC levels could be determinants of CaMKII activity levels and this is not intuitively obvious without considering the whole network.
3. Definition of threshold stimulation for biological effects. It is the system that defines which external stimuli are capable of evoking a response.
4. Multiple signal outputs. Needing multiple kinases to be activated together in the proper amounts provides a safety factor to insure that only the desired outcomes occur.

Caveats: Finally, let me say that the results I have shown are dependent on numerous assumptions and rate constants and the actual results from the models are predictions and the quantitative values should not be taken as truth. There are a number of competing models out there arguing for the persistent activation of CaMKII. The model of CaMKII activation used in the simulations I've shown you has some significant omissions. The scheme pictured in your book in Fig. 14.3 is misleading, (but Fig. 12.15 gets it right as far as it goes) and I may give you the most recent version next time.

Nevertheless, the simulations have provided insights and they will be revised continuously as new biochemical data become known. However, without the models, there would be little hope of understanding how all of these pathways might come together.

The biochemical reactions discussed previously and the corresponding equations were developed for a well-mixed homogeneous medium. The models developed with these biochemical networks provided insights and predictions that proved to be useful and in some cases have been verified by experiment (the cAMP gate for CaMKII activation).

Unfortunately the neuron is not a well-mixed homogeneous environment. To better understand how the biochemical signaling mechanisms work, it will be necessary to put the biochemical reactions in their proper context, which means putting them in the volume of a dendritic spine.

OH 10 As you know, many cells in the brain, such as cortical pyramidal cells, hippocampal pyramidal cells, Purkinje cells of the cerebellum, etc. possess dendritic spines where input is received (in contrast motoneurons do not have spines). The spines are small mushroom-shaped or lollipop shaped or bump shaped appendages protruding from the dendritic shaft. They may occur in high densities (1-5 per um of dendritic length) and typically are 1 um long. The synapses are formed on the spine head and the synapses are almost exclusively excitatory.

The typical thing to do is to model the spine as a sequence of cylindrical compartments. The biochemical machinery that we have discussed is thought to exist primarily in the part of the spine nearest the synapse called the post-synaptic density or PSD.

The complication this adds is that calcium that enters the spine head through NMDA channels or voltage-gated calcium channels can do many things. Besides binding to calmodulin, calcium can diffuse through the spine head and down the spine neck, calcium can be pumped out of the spine by Ca-ATPase or Na-Ca exchangers, and calcium may activate additional calcium release from intracellular stores (IP₃ mediated perhaps). Then again, Ca may be pumped into internal stores as well.

This requires more specialized modeling which I will discuss in the Computational Neuroscience course next quarter, so I will be brief here. We have to consider what happens to calcium in each portion of the dendritic spine. To be very general about this, we can say that

$$d[Ca]_i/dt = + \text{influx} - \text{buffer} - \text{pumped out} - \text{diffuse to next compartment} + \text{entry from adjacent compartments} + \text{release from stores} + \text{release from buffer} - \text{pump into stores}$$

where the subscript *i* refers to compartment *i*. Equations will be needed for each substance in each compartment that the substance may occupy, so you can see that the number of equations can increase quickly.

The take home point is that the magnitude and time course of the calcium signal that triggers the networks of biochemical reactions can be affected by many factors and these must be appreciated to understand how the network reactions proceed.

OH 11 Let's consider two of these factors that are important for understanding the magnitude and time course of the calcium signal: 1) the buffer capacity of spines and 2) how fast calcium extrusion (diffusion and pumps) is.

Estimates of buffer capacity ((Bound + free)/Free) in spines is about 20 (meaning 95% of calcium is bound to buffers) and this capacity is much lower than that of dendrites (> 100).

Estimates of extrusion of calcium are that it is a fast process, taking < 50 ms.

However these are difficult to measure. Why? The probes that are used (Dr. Colvin described how the probes are used earlier) themselves bind calcium. 1) They are themselves a calcium buffer. 2), the probes tend to be more mobile than actual calcium buffers. For example, calmodulin is thought to diffuse slowly if at all because of the large number of calmodulin binding proteins near the synapse.

What are the consequences of these problems? First, the buffer capacity may be overestimated because of the additional capacity provided by the probe. Secondly, calcium removal may be estimated to be too fast. The probes will diffuse faster than the buffers and thus will carry calcium out of the spine faster than buffers would.

Comparisons of experiment to model must compensate appropriately and estimates of buffer capacity and calcium extrusion need to be done with care.

OH 12 Because of its importance in learning and memory, activation of CaMKII has received much attention. Dr. Colvin promised that both of us would say more about CaMKII when he first introduced it and I will do so now. I want to describe how CaMKII gets activated and why bistability may be important if it indeed occurs. John Lisman in particular has built a theory of how learning and memory work based on the persistent activation of CaMKII (paper topic for grad students?)

CaMKII is a holoenzyme composed of 12 subunits arranged in two 6 subunit rings. When we talk about CaMKII activation we refer to subunit activation, as each subunit is capable of kinase activity independently.

Dr. Colvin has already shown you how during a calcium signal, calcium binds to calmodulin and calcium-calmodulin binds to a CaMKII subunit causing the CaMKII subunit to change conformation. This conformation change allows the subunit to be phosphorylated at position T-286. However, how this phosphorylation takes place is a bit unusual.

When calcium-calmodulin binds to a subunit, the subunit is active and can perform kinase activity. If two neighboring subunits in a ring both have calcium-calmodulin bound to them, then one can phosphorylate the other as indicated here in this figure. At this point it is still not clear if the phosphorylation is directional or not (i.e., can a bound subunit phosphorylate both neighbors or only the one to the right or only the one to the left).

Nevertheless there are two requirements for a subunit to be phosphorylated at T286: 1) calcium-calmodulin must be bound to the subunit and 2) the neighboring subunit must be activated.

If a calcium signal is small and calcium-calmodulin binds to 2 non-neighboring subunits then no phosphorylation will occur. Once a subunit has been phosphorylated, the calmodulin is “trapped” on the subunit. It remains bound to the subunit for a considerable period (10s of seconds) after the calcium signal is over.

Also as Dr. Colvin showed you, once calmodulin unbinds from the subunit, the subunit can be phosphorylated in the region of the calmodulin binding site (T-305,306). This reaction seems to be intrasubunit (the subunit phosphorylates itself) although there is evidence it can be intersubunit too. CaMKII has to be dephosphorylated at these sites before the subunit can bind calmodulin again. These autonomous (no CaM, phos at T-286) and capped (no CaM, phos at T-286 and 305,306) states are also active states of the kinase, although activity is less as Dr. Colvin showed you.

So to get CaMKII activated there are a couple of nonlinearities. The first is that calcium from a calcium signal must bind to calmodulin. It is generally thought that 4 calciums must bind although recent work suggests fewer may also work. The second is in the autophosphorylation requirement of neighbors being activated. Once enough subunits bind calmodulin, the holoenzyme can quickly become autophosphorylated.

We have a phosphatase, PP1, as shown here that removes the phosphate groups once CaMKII is translocated to the PSD (PP2A dephosphorylates CaMKII in the cytoplasm). However once autophosphorylation really gets going, the phosphatase is saturated and cannot keep up with the autophosphorylation, at least as long as calmodulin is trapped on subunits. Once calmodulin comes off, the subunit can be dephosphorylated and will not be able to be phosphorylated again until another calcium signal allows calmodulin to bind again.

OH 13 So how might this process result in bistability? If we have a small calcium signal that pushes CaMKII activity up to point 1 on this figure (Fig. 14.3), then when the calcium signal is over, CaMKII activity returns to basal levels. If the calcium signal is strong enough (point 2 on this figure), then calcium-calmodulin binds most subunits and autophosphorylation occurs and we see a large increase in activated CaMKII and we go immediately to point 3 in the figure.

At point 3, PP1 removes phosphate groups, but it cannot keep up with autophosphorylation.

If calcium goes down (point 4 in the figure), we do not suddenly drop back to the lower part of the curve because 1) we still have autophosphorylation out-dueling PP1. 2) Calcium levels may be sufficient for enough calcium binding to calmodulin and enough calcium-calmodulin binding to CaMKII so that subunits that are dephosphorylated and have lost calmodulin may still get phosphorylated, and this process occurs fast enough so that PP1 cannot return CaMKII activation to basal levels (point 4 in the figure). CaMKII activity remains high after the Ca signal is gone.

So how might we get persistent CaMKII activation? Lisman would argue that the region of bistability includes resting calcium. Resting calcium levels would be included in the left side of the shaded area in the figure. Whether this is true or not remains to be seen.

Does bistability exist in the real world? Although models predict this bistability, it is not known if bistability really exists. The one group that has tried to address this question experimentally has concluded that bistability does not exist and that CaMKII is merely an ultrasensitive switch.

However, this does not settle the question. First, the PP1 kinetics found in these experiments was much faster than anyone else had previously reported and even some models do not predict bistability with such fast PP1 dynamics. Secondly, the experiments were done without the cAMP gating system or other biochemical pathways as given in previous overheads intact. The experiments were done to consider only a

limited number of variables, and with a full system we may have PP1 modulation as shown by the modeling results I showed earlier. (Bradshaw PNAS 2003 grad student paper topic). Finally it has been argued that PP1 may not be able to reach the T286 site for dephosphorylation, but this is not widely accepted currently.

So whether CaMKII is a bistable switch or an ultrasensitive switch and whether or not it can be persistently activated is still an open question.

OH 14 There is another consideration that has not received very much attention that I want to discuss. Let's consider the dendritic spine. If resting free calcium concentration is 100 nM, how many free calcium ions are there in the spine? Suppose, to make the math easy, the spine head is a cube 0.5 μm on a side. Let's get some guesses. The volume of the spine head would be $1.25 \times 10^{-13} \text{ cm}^3$ or 1.25×10^{-16} liters. This multiplied by $100 \times 10^{-9} \text{ M}$ gives 1.25×10^{-23} moles. Multiplying this by 6.02×10^{23} gives us 7-8 calcium ions. This is not a big number and would create a problem for Lisman's persistent CaMKII activation theory.

How many CaMKII holoenzymes are there in a spine head? The latest estimates have brought the number down to about 40, at large synapses. The holoenzymes are large being 20 nm x 20 nm.

What do these small numbers mean? They mean that stochastic variations can be quite large. When you think of gene networks and how transcription works, then again the number of molecules involved is not very large. It really makes you wonder how the system works effectively at all. As I mentioned above, the networks of pathways and their interactions may provide a safety factor that confines outcomes to a narrow range, despite the small number of molecules involved.

Just as an example, let's consider the openings of NMDA receptor channels at a synapse. As I'll mention next time, the number of receptors is small and the number that reach the open state is even smaller. Here are results of two modeling studies in response to the same input. One modeling study looks at each NMDA channel individually while the other takes an average view. The deterministic average shows that on average less than one NMDA channel is open for this input (and of course this cannot happen). When channels are followed individually, the channel is either open or closed and transitions are stochastic. A second stochastic simulation would show the NMDA openings at different times. How much of an effect or how important this variability is remains to be determined.

The take home point is that when the number of molecules are small you must consider stochastic variations as important factors in how biochemical networks operate.

Lastly, these biochemical signaling reactions do not occur in a well-mixed heterogeneous environment or even in a locally well-mixed heterogeneous environment such as at the spine head. The post-synaptic density is aptly named. This postsynaptic region below the synaptic cleft is crowded with various proteins and other substances. Many of the kinases and phosphatases we have discussed are anchored to the membrane or to parts of the actin cytoskeleton. CaMKII for example seems often to be bound to NMDA receptors. Major components of the postsynaptic density have limited mobility. The fact that reactions can take place in such a crowded environment where diffusion can be limited makes it difficult to do experiments that truly mimic the biological situation and also makes the development of appropriate models difficult.