

DENDRITIC SPINES

14-1. Dendritic spines have fascinated researchers for over a century. They are little appendages seen on many cell types where axons meet to form synapses. Why does the cell have these dendritic spines?

I've listed some review articles here about spines and I've given you two of my book chapters on spines for your reading pleasure.

Spines were first seen by Cajal and Berkeley over a century ago, but it was not until 1959 that Gray showed using the electron microscope that spines were sites of synapses.

There have been a number of observations about spines made through the years. First, lower numbers and densities were seen in post-mortem brains of people suffering from dementia. Second a decrease was observed with those suffering from acute alcoholic poisoning. Animals subject to sensory deprivation have fewer spines and those raised in enriched environments have increased spine numbers and densities. So it seems that having lots of spines is a good thing!

We will return to the debate about what spines do later.

One concern for modelers is how to include spines in models. Cells can have thousands of spines. A cerebellar Purkinje cell can have 100,000 spines. A CA1 hippocampal pyramidal cell can have 10-30,000 spines. A hippocampal dentate granule cell can have 4-10,000 spines.

Spine membrane can make up as much as 50% of the total membrane area of a cell, so clearly this must be included in a model some how. If we code separate compartments for each spine, the computation time will slow significantly, but clearly we cannot just ignore spines. So what do we do?

14-2. Two methods have been developed and tested for including spines in models implicitly.

The first method is one I came up with, which is to increase C_m and reduce R_m according to the fraction of membrane area due to spines, while keeping the product $R_m C_m$ constant. If including spines increases total membrane area by 33%, then multiply C_m by 1.33 and divide R_m by 1.33. If spines make up half of the total membrane area (or including spines doubles the total membrane area), then multiply C_m by 2 and divide R_m by 2.

The second method, due to Ken Stratford, is to keep R_m and C_m the same, but increase the length and diameter of the dendrite in such a way as to keep axial resistance constant. That is, keep $\text{length}/\text{diameter}^2$ constant. Given the increase in area due to spines and this ℓ/d^2 constraint, it can be shown that if F is the factor multiplying dendritic membrane area to include spines (i.e., if spines increase total area 33% then $F=1.33$), then length is increased by $F^{2/3}$ and diameter is increased by $F^{1/3}$.

GENESIS has the second method built in, but NEURON does not. NEURON has crude commands to deal with spines, but these are really not that helpful, or at least they are not

described in a way that seems helpful. `setSpineArea(area)` sets the area per spine and this area is added to the segment area, but it is not clear how this is done. `getSpineArea()` returns the area per spine and `spine3d()` returns 1 if a spine exists at a point and 0 otherwise. You may see these commands in some of the hoc files or morphology files we have looked at.

14-3. What are some implications for these spine compensation methods? First, both methods work just fine for passive models. Results are identical.

But what if there are voltage-dependent conductances?

The Rm-Cm method assumes spines are passive and will add to the passive conductance of the parent dendrite.

The len-diam method assumes that the spines have the same voltage dependent conductances as the dendrite. The length and diameter and thus area of the segments are changed, but the conductance densities are per area, so the number of voltage-dependent channels is increased, unlike with the Rm-Cm method.

What if spines have different voltage-dependent conductances than the parent dendrites? In this case it is relatively straight forward to figure out how to incorporate this into a hoc file, but it requires some thought. It can be done with either method.

What about calcium-dependent potassium conductances? Well this is a problem. It is unlikely that spine calcium concentrations will be similar to dendritic calcium concentrations (as we shall see later), so dealing with this is an open problem (or at least I have not come up with an easy solution myself).

14-4. So why not just ignore spines? Here is a simulation I did some years ago to show why this is a bad idea. I constructed a model where I could replicate branches as many times as I wanted. In this way I could explicitly code spines on one dendrite and then have the model replicate the dendrite. In this way I got a cell with thousands of spines explicitly represented. What happens if there is input to a few spines (not replicated)? The voltage response is shown with the open circles here on the plots. What happens if all other spines are ignored and only the dendrites are replicated? Then the voltage response is bigger, as you might expect—less membrane area should increase input resistance. However if Rm and Cm were changed to compensate for spines, then the voltage responses were identical.

14-5. OK, so now we know how to include spines in models. So why do spines exist?

Here is a Table Gordon Shepherd put together some years ago about Theories of spine function. He quotes Cajal as saying that “In the absence of experimental evidence, theories proliferate” and this has certainly been true regarding the function of dendritic spines. Kristen Harris, who has done marvelous 3D serial EM reconstructions of spines and synapses was quoted as saying that “Most of what we suspect about spine function is based on computer simulations”.

Spines are the site of synaptic connections. This is well established, but is the function of the spine only to connect?

Are spines needed to increase dendritic surface area to make connections better during development?

Do spines serve as local input-output units (as in the olfactory bulb)?

Do spines modify synaptic potentials? Well yes they do. They attenuate synaptic potentials, but local potentials in the spine head could be large.

Are spines units for synaptic plasticity? Can changes in shape mediate learning and memory?

If spines have active properties, can they boost signaling?

Do spines behave as an information processing unit?

How about spines as coincidence detectors?

Are spines biochemical compartments where local calcium mediated reactions can take place safely?

Do spines increase the distance between synapses and is this important?

These are just some of the theories of spine function that have been proposed.

14-6. I will discuss a few of these theories. First, are spines involved in plasticity because of their electrical properties? Rall and Rinzel proposed that changes in spine shape, particularly the spine neck or stem, should underlie plasticity.

They considered the spine as an electric circuit where a voltage in the spine head V_{SH} is attenuated by the spine stem resistance R_{SS} to reach the dendrite V_{BI} and from there voltage goes to ground via the input resistance at the dendrite R_{BI} .

So in steady-state, the current through the spine stem resistance is $(V_{SH} - V_{BI})/R_{SS}$ by Ohm's law. The same current can be expressed going to ground as V_{BI}/R_{BI} or as $V_{SH}/(R_{SS} + R_{BI})$.

Equating the 2nd and 3rd of these expressions we see that voltage attenuation expressed as V_{BI}/V_{SH} (the inverse of what we considered previously) = $R_{BI}/(R_{BI}+R_{SS}) = 1/(1+R_{SS}/R_{BI})$.

So if $R_{SS} \ll R_{BI}$ then how much attenuation is there? Little or none. $V_{BI} = V_{SH}$.

If $R_{SS} \gg R_{BI}$ then there is nearly complete attenuation. $V_{BI}/V_{SH} \sim 0$

If $R_{SS} \approx R_{BI}$ then there is 50% attenuation, or the voltage drop is equal across R_{SS} and R_{BI}

14-7. So they plotted V_{BI}/V_{SH} as a function of R_{SS}/R_{BI} to obtain this curve. This curve shows a region of sensitivity where attenuation can change a lot with a small change in R_{SS}/R_{BI} . A change in R_{SS} will have the largest effect on V_{BI}/V_{SH} when R_{SS}/R_{BI} is between 0.1 and 10.

Therefore if spine stem shape changes underlie plasticity, then R_{SS}/R_{BI} must be in this region of sensitivity.

At this time, Peters and Kaiserman-Abramof were studying spine shape and they categorized spines into stubby, mushroom-shaped and long-thin spines. We still use these categories today and they are not just a continuum but seem to be real distinctions. Rall calculated R_{SS} for these shapes and came up with 10^5 - $10^6 \Omega$ for stubby spines, 10^6 - $10^8 \Omega$ for mushroom-shaped spines and 10^8 - $10^9 \Omega$ for long-thin spines.

14-8. Peters and Kaiserman-Abramof reported that stubby spines tended to be found proximally where $R_{BI} \approx R_N$. If $R_N \approx 20 \text{ M}\Omega$, then $R_{SS}/R_{BI} \approx 10^6/20e6 \approx 0.05$. Where does this stand on the previous plot? This is not a good value for plasticity by changing R_{SS} .

Mushroom-shaped spines found in mid dendritic regions where $R_{BI} \approx 50 \text{ M}\Omega$. Then $R_{SS}/R_{BI} \approx 10 \times 10^6 / 50 \times 10^6 \approx 0.2$. This is at the lower edge of the sensitivity range.

Long-thin spines were found in distal regions where R_{BI} is $200 \text{ M}\Omega$. Then $R_{SS}/R_{BI} \approx 500 \times 10^6 / 200 \times 10^6 \approx 2.5$ and this is in the middle of the plasticity range.

Similar predictions can be made with peak transient V_{SH} instead of steady-state. Note that these results depend on R_a and R_{BI} .

So the hypothesis was that with learning, the neck diameter increases and this results in less voltage attenuation from the spine head to the dendrite and this could be the mechanism for plasticity. This set off a flurry of activity where people looked for spine shape changes after experimental manipulations.

Are there changes with long-term potentiation? Results were mixed.

Experiments with jewel fish showed that those with extensive social experiences tended to have wider spine stems.

Honey bees after their first flight show head swelling and stem shortening

14-9. So the general conclusion was that spine heads may swell and part of the spine stem may become spine head (stem shortening), but in general it was hard to claim that the stem diameter changed in a systematic way with these manipulations.

How might spines change shape? Spines have microtubule-associated proteins (MAP) and actin in high concentration. During depolarization, these fibers could contract and “twitch”, suggested Francis Crick. Perhaps stems would increase in diameter and calcium might be involved.

However, the general shape and not exclusively the stems is probably involved if such a mechanism exists.

So although Rall suggested that plasticity would occur for changes in R_{SS} , what about spine head swelling and stem shortening?

How can we calculate R_{SS} ? $R_{SS} = R_a \ell / (\pi r^2)$. So if the stem shortens, then R_{SS} will go down, but no where near as much as when the diameter increases, because of the squared term. If we do an equivalent analysis of the spine head, head lengthening and diameter swelling will partially compensate for each other (len in numerator and diam in denominator and both going up) so again this will partially work, but not as well as just stem diameter increasing.

14-10. Another theory of spine function is that spines exist to isolate inputs from each other.

Suppose we compare inputs on spines and dendrites, $I_{syn} = g_{syn} (V - V_{syn})$

For the same synaptic conductance, how would the peak voltage change compare?

$V_{\text{spine}} > V_{\text{dendrite}}$ because the input resistance to the spine is larger
What about the peak soma voltage?

$V_{\text{soma}} (\text{Spine input}) < V_{\text{soma}} (\text{Dendritic input})$ because of the attenuation from the head to the dendrite.

What about the synaptic current?

$I_{\text{syn}} (\text{Spine input}) < I_{\text{syn}} (\text{Dendritic input})$ because the driving force is reduced in spines.

So the conclusion is that spine inputs are less effective than dendritic inputs (although for small g_{syn} the differences are small)

But what about the voltage differences between adjacent spines?

$V_{\text{BI}}/V_{\text{SH}} = 1/(1 + R_{\text{SS}}/R_{\text{BI}})$ gives the magnitude of attenuation from the spine head to the dendrite, but what about from the dendrite to the spine head? Before answering this question, let's look at the conclusion above about spine inputs being less effective than dendritic inputs.

14-11. Let's look at the electric circuits for a spine input and a dendritic input as shown here. Let g_{syn} be the synaptic conductance and let V_{EQ} be the synaptic reversal potential.

Then at node V_{SH} the sum of the currents is $V_{\text{SH}}/R_{\text{SH}} + g_{\text{syn}}(V_{\text{SH}} - V_{\text{EQ}}) + V_{\text{SH}}/(R_{\text{SS}} + R_{\text{BI}}) = 0$ by Kirchoff's law. Because R_{SH} is very large we can neglect the first term.

At node V_{BI} the sum of the currents is $V_{\text{BI}}/R_{\text{BI}} + (V_{\text{BI}} - V_{\text{SH}})/R_{\text{SS}} = 0$.

We rearrange the former equation (assuming $V_{\text{SH}}/R_{\text{SH}}$ is negligible) to $V_{\text{SH}} = V_{\text{EQ}}/(1 + 1/[g_{\text{syn}}(R_{\text{SS}} + R_{\text{BI}})])$.

Now the second equation yields $V_{\text{SH}} = V_{\text{BI}} ([R_{\text{SS}}+R_{\text{BI}}]/R_{\text{BI}})$ and substituting this in gives $V_{\text{BI}} = V_{\text{EQ}}/[1 + 1/(g_{\text{yn}}R_{\text{BI}}) + R_{\text{SS}}/R_{\text{BI}}]$.

If we do the analysis for the dendritic input we get the same expression except without the $R_{\text{SS}}/R_{\text{BI}}$ term. So the same input applied to the dendrite gives a larger V_{BI} with the difference depending on $R_{\text{SS}}/R_{\text{BI}}$.

14-12. So back to our question, what about attenuation from the dendrite to the spine head?

Suppose we have an input to spine #1. How different are

V_{SH1} and V_{BI1} ? Depends on R_{SS} , $V_{\text{SH1}} > V_{\text{BI1}}$ and the difference could be large depending on R_{SS} .

V_{BI1} and V_{BI2} ? These are essentially the same. There may be 1 μm between them in the same dendrite so the decay over this distance is negligible.

V_{BI2} and V_{SH2} ? It turns out that these are virtually the same. The current tends to run towards the soma. The sealed end boundary condition at the end of the spine is felt here.

Spines are a particular case of asymmetric attenuation of the type we have discussed earlier in this course.

So, spines do not isolate synapses electrically, but how about chemically?

Still there are questions about the attenuation. Attenuation depends on R_a and stem dimensions and it is not clear that R_a is the same in spines as in the dendrites. The Cytoplasm of spines stains more darkly and cross-filaments exist. Some spines have a spine apparatus.

14-13. Do spines exist to amplify charge transfer, if voltage dependent conductances exist on spines. In the passive case spines will never amplify charge transfer, because they reduce the driving force, but if voltage-dependent conductances exist on spines, this could change.

A high R_{SS} could lead to a higher V_{SH} in spines than would occur for the same input on the dendrite. This could activate voltage-dependent conductances.

Amplification can be very sensitive to R_{SS}

Spine head action potentials are potentially possible

Is there an optimal R_{SS} range at which you can get maximum amplification? It turns out that there is as shown in this plot. Maximum amplification occurs for an R_{SS} of about 600 M Ω . Why does a maximum occur? For low R_{SS} the voltage change is too small to activate voltage-dependent conductances. For large R_{SS} the voltage change is large enough to have an effect on the driving force. In between there is a maximum

If there is an action potential in a spine, what happens in neighboring spines?

It is possible to get a chain reaction of spine head action potentials spreading proximally and distally. If this is so, then fewer channels are needed to produce dendritic hot spots if such channels are on spines. Alternatively Na inactivation could prevent action potentials from forming on spines activated later.

14-14. Rall and Segev simulated dendrites with a few active spines and many passive spines. They found that it did not take many excitable spines to make a big difference.

14-15. However, there are problems with this analysis. They had to have densities of Na and K channels 5X the usual densities. The question arises as to whether spines have voltage-dependent Na and K channels. No experimental evidence has been presented for this. There is much evidence for Na and K channels on dendrites, but spines are quite small and any antibody staining would be hard to pick up. But regardless, there are not Na and K channels with the densities required by the Rall and Segev study.

What about Ca channels? Evidence, particularly from Svoboda's lab suggests that R-type calcium and possible other types of calcium channels exist on spines. Plus there is much evidence for Ca channels on dendrites.

If Ca channels exist on spines a decrease in R_{SS} (or stem shortening) could reduce peak Ca concentration (since less Ca enters) and this could affect Ca dependent reactions.

Could high [Ca] trigger shape changes? Maybe, but after shape changes, the lower [Ca] might have little effect.

There have been a few studies of multi-spine interactions. Rall and Segev proposed that excitable spine clusters may exist and Shepherd and Brayton suggested that depending on the conductances, spines can function as AND gates, OR gates or AND-NOT gates.

14-16. Here is a figure from the Rall and Segev study. Inputs on spines can activate other spines and thereby amplify the signal. Combinations of passive and active spines can have significant effects.

14-17. However there is a problem with these theories. What is R_{SS} ? These excitable spine clusters required R_{SS} to be about 600 M Ω . If one does the simple calculation for a spine with neck length of 1 μm and diameter of 0.1 μm and R_a of 100 Ωcm , then R_{SS} turns out to be 127 M Ω . However, it may be the case, as we suggested earlier, that R_a in spines is different from that in dendrites. So what is R_{SS} really?

Svoboda et al measured the diffusional exchange between the spine head and dendrite using fluorescence recovery after photoactivation. They estimated R_{SS} from the quantitative relation between diffusion and electrical conduction. Their conclusion was that R_{SS} is 4–50 M Ω , with an upper bound of about 150 M Ω . If this is the case then spine neck geometry does not have a significant role in plasticity. However, at the upper bound, amplification might occur if voltage-dependent calcium channels exist in spines (i.e., a depolarization of 10 mV or more) and Segev and Rall have argued that this might happen.

14-18. Do spines exist as a means to concentrate calcium? Calcium is very important as I'm sure you know. It is important for metabolic processes, it may be involved in spine shape changes and its involvement in long-term potentiation (synaptic modification) is well known.

There are two particular issues here:

1) Calcium entry might be different for different shaped spines? Why? Spines may differ in their R_{SS} value creating a different driving force for Ca channels and may also provide a different level of activation for voltage-dependent Ca channels. Also, calcium entry via NMDA receptor channels because of the voltage dependence of Mg block of NMDA channels.

2) Calcium concentration might be different for different shapes of spines receiving the same calcium entry. Why? Spine heads may have different volumes and different abilities for calcium to diffuse out of spines.

TO study this investigators have modeled spines as a series of compartments where calcium enters a spine through its outer surface and then diffuses to neighboring compartments, gets pumped out or gets buffered.

14-19. This is modeled with standard methods. Diffusion is modeled by considering diffusion from the center of one compartment to the center of the next compartment where any asymmetries in dimensions are taken care of by this delta term. The pump is modeled as a first

order equation as shown here or else as a Michaelis-Menten expression combined with a leak entry term. The buffer is modeled as simple binding.

So far we have seen various ways NEURON can handle buffer, pump and diffusion and these methods can be used here too.

This results in equations for calcium concentration for each compartment and equations for buffer concentration for each compartment and these are solved.

14-20. To address whether spines exist to concentrate calcium, consider spine models with 3 shapes, long thin, mushroom-shaped and stubby with dimensions as given here.

To do the model we will need other parameters for the buffer (types, concentrations, kinetics) and pump (rates and types) and knowledge of any subsequent reactions.

When the models are done, we find that for a hypothetical calcium current, a larger [Ca] peak occurs in long-thin spines than in mushroom-shaped or stubby spines. IN the early models this occurred because the calcium buffers got overwhelmed, but subsequent models have made different buffer assumptions and overwhelming buffers is not necessary to get the differences in [Ca] for different spine shapes. The duration of the calcium entry also matters.

Models have shown that spines may allow coincidence detection as backpropagating action potentials amplify Ca signals in spines. We discussed this last quarter as well.

14-21. So what does [Ca] look like in spines with different shapes? Here are results of an early model. Calcium gets highly concentrated in long-thin spines, here to 28 μM at the peak, but is not so highly concentrated in mushroom-shaped or stubby spines (peak between 1 and 2 μM). Also note that [Ca] in the stubby spine decays more rapidly than in the other spines. The neck provides a diffusional barrier and it is harder for Ca to get out of long-thin or mushroom-shaped spines.

14-22. The duration of the input matters as well, but the general pattern of higher concentrations in long-thin spines is preserved.

14-23. So what might [Ca] do in spines? It seems that Ca can be concentrated in spines at levels that might kill the cell if these concentrations occurred generally, but spines compartmentalize these large concentration changes. Calcium in spines can bind to calmodulin and activated calmodulin can activate a number of enzymes, particularly calcium-calmodulin dependent protein kinase II or CaMKII. This is thought to be very important for long-term potentiation.