Site of Impulse Initiation: How geometry affects where the impulse initiates

Goals:

1. Where is the action potential initiated?
2. What parameters determine the site of AP initiation?
3. How does the site of initiation affect spiking response?

Motivation:

The experimenter's problem: For most slice preparations, physiologists are limited to somatic recordings. The soma is orders of magnitude larger (in diameter) than dendrites and particularly axons, as shown in a biocytin-stained cortical pyramidal cell (Figure 1A). Moreover, under IR-DIC visualization, the dendrites are visible only under ideal conditions and axons are rarely visible due to their small diameter (Figure 1B). Due to these limitations, most experimentalists are not aware of the timing or amplitude of voltage fluctuations that occur in the axons.

Site of action potential initiation is the axon:
The first proposal that the site of action potential initiation is the axon was derived from early studies in the motoneurons (Coombs, Curtis and Eccles, 1957). This hypothesis was based on recordings in the motoneuron showing a "two stage invasion" or two component action potential. In experiments in which antidromic spikes were evoked, it had been previously proposed that these two components represent the spikes in the axon (small spike, attenuated because of the somatic recording site) and soma (larger spike). In the case of synaptic input to the motoneuron, it was observed that the same two components were observed, but the time interval between the small spike and large spike was smaller (Eccles, 1957). Coombs, Curtis and Eccles (1957) showed that the axonal spike was initiated at a lower voltage threshold than the somatic spike and proposed that the spike was therefore initiated in the axon.

More recent studies using experimental tools that were not previously available have confirmed the hypothesis that the action potential is initiated in the axon. In a difficult set of experiments, Colbert and Johnston (1996) demonstrated that orthodromic action potentials are first initiated in the axon and then invade the soma by recording simultaneously from the axon initial segment and the soma of hippocampal pyramidal cells. Figure 2A shows the IR-DIC visualization of the hippocampal soma and axon with the two recording pipettes. Figure 2B shows the morphological reconstruction of this biocytin-filled neuron, showing the approximate locations of the recording electrodes. The soma was recorded in whole-cell configuration, while the axon was recorded using a
cell-attached configuration. Figure 3 shows that when spikes are initiated in the soma by a current injection (50ms, 200pA; Figure 3A), the voltage deflection in the soma occurs after the deflection in the axon (Figure 3B). The first and second peaks observed in the axon patch recording represent the axonal spike and somatic spike, respectively. A second recent study also provides direct evidence to support that the action potential initiates in the axon. Palmer and Stuart (2006) used voltage-sensitive dyes to record voltage deflections in the soma and along the length of the axon of cortical layer 5 pyramidal cells. The experiments revealed that onset latencies to a spike evoked by a somatic current injection were shortest in the axon segment. Figure 4A shows the voltage deflections, based on the illumination of voltage-sensitive dyes, in the soma and across the axon. Figure 4B is a graph illustrating that the shortest onset latency to spike, as determined by the voltage-sensitive dyes, occurs in the axon, about 40 microns from the soma.

Importance of studying the AP initiation site: The action potential initiation site is perhaps the most important part of the neuron, because this is where the decision is made about whether or not to convert the synaptic inputs to an output signal. Since the discovery that the axon initial segment is the AP initiation site, many studies have focused on the ionic channels and receptors at this specific location that may contribute to this vital decision (ie: Sarmiere et al., 2008; Cruz et al., 2004). Moreover, it has been shown that specific types of inhibitory interneurons may target this region to control spiking of cortical pyramidal cells (Somogyi et al., 1982). Therefore, knowing the precise location of action potential initiation and knowing what factors determine this site are essential to understand the input-output function of specific neurons. This Neurons in Action (Moore and Stuart)
tutorial allows us to determine the site of action potential initiation and which parameters can change this site. Finally, this report will examine an issue not addressed by the tutorial: whether a morphological change that occurs to pyramidal cells during development may affect the site of spike initiation and impact spiking responses.

**Stylized neuron model:**

The neuron for this tutorial has been simplified considerably to represent simple morphological aspects. This model is shown in Figure 5 along with a biocytin-filled pyramidal neuron to illustrate how the morphology is represented. The neuronal dendritic arbors (Figure 5A - blue) are collapsed into a single passive cylinder with a diameter and length (Figure 5A). Single synapses (alpha function) can be positioned along the length of this dendritic trunk. The soma is a cylinder with a diameter and has voltage-gated HH channels. The unmyelinated axon is represented as a single cylinder with length and diameter and contains voltage-gated HH channels.

(1) **Where is the action potential initiated?**

To determine where the action potential is initiated, voltage traces are generated at 3 sites along the neuron: the dendrites (blue), the soma (red) and the axon (black) (Figure 6A). Currently, it is not possible to experimentally record whole-cell voltages simultaneously from these three locations. To initiate an action potential, a suprathreshold excitatory postsynaptic potential (EPSP) is delivered to the middle of the dendrite. Figure 6B shows the time course of the action potentials (voltage over time) at these 3 electrode recording locations. The stages of the AP generation are as follows: (1) Synaptic input to the dendrite leads to a uniform increase in voltage in the dendrite (blue trace) and soma (red trace). (2) The voltage spreads passively to the axon, which generates an AP in the axon. (3) The AP, first initiated in the axon, backpropagates into the soma and dendrites to generate a delayed and stunted spike. Because the dendrites have an infinite resistance at their tips (closed end), the dendrite is at an isotential and closely follows the soma. Figure 6C is a freeze-frame of the movie, showing the voltage deflection (Y axis) as a function of location across the neuron (X axis). The peak of the AP initiates in axon initial segment.
AP occurs at the initial segment of the axon, about 100 microns from the soma. It is apparent that for these parameters the AP is initiated in the axon. The next set of simulations will determine the parameters that control the relative timing and amplitude of the AP in the axon, soma and dendrites.

(2) What parameters determine the site of AP initiation?

**Strength of Synaptic Input**

To determine whether the site of AP initiation is affected by the strength of the synaptic input, the maximum conductance was varied (gmax). The synaptic input is modeled using the alpha function:

\[ I = G \cdot (V - E) \]
\[ G = G_{\text{max}} \cdot \frac{(t - \text{onset})}{\tau} \cdot e^{-(t - \text{onset})/\tau} \]

where \( I \) is the synaptic current, \( G \) is the conductance, \( V \) is the membrane potential, \( E \) is the reversal potential for the synapse, \( G_{\text{max}} \) is the maximum conductance, and \( \tau \) is the synaptic time constant. An alpha function (gmax = 1) is shown in Figure 7. Figure 8 shows the result of doubling and then redoubling gmax from 13.7µS to 28µS and then 56µS. Increasing the synaptic strength leads to a faster onset for APs in the axon (black trace), and faster and stronger APs in the dendrite and soma (blue and red traces) (Figure 8A, time course for APs). At 4x the default conductance (56µS), the APs occur almost simultaneously in the soma, dendrites and axon. Figure 8B shows the freeze-frame when the AP reaches the peak along the length of the neuron. The peak of the AP remains in the axon at all gmax tested. However, at the strongest synaptic input tested (gmax = 56µS), the voltage is elevated in the soma and dendrites during the axonal AP, representing a nearly simultaneous, but slightly stunted spike.
**Synaptic Location**

To determine whether the synaptic location can influence the position of the AP initiation, the location was varied from the center of the dendrite, to the soma, and then to the distal dendrite (Figure 9).

![Figure 9](image)

**Figure 9**: Biocytin-filled neuron showing the simulated varying locations of the synaptic input. Input 1 is to the middle of the dendritic arbor, input 2 is to the soma, and input 3 is to the distal dendrite.

The input to the soma (input 2) produced voltage traces and an AP initiation site similar to the input to the middle dendrite (Figure 10). However, the input to the distal dendrite led to delayed APs in the axon, soma, and dendrite. Note that the somatic and dendritic depolarizations are smaller due to the Na+ channel inactivation that occurs while these regions are slowly depolarizing (Figure 10A). However, similar to changes in synaptic strength, changing the synaptic location did not alter the location of the AP peak, which continued to occur in the initial segment of the axon (Figure 10B).

**Figure 10**: (A) Voltage versus time plot, showing the time course of the AP at t3 recording sites at 3 different synaptic locations. Note the delayed and smaller APs in response to the distal synaptic input. (B) Freeze-frame of the simulated movie showing the position (x axis) of the onset and highest point of the AP, (voltage y axis). Despite changes in synaptic location, the AP peak remains in the axon.

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Soma Diameter

To determine the effect of changing the somatic size on the AP initiation site, the soma diameter was varied from 200µM to 100µM to 800µM (Figure 11).

Figure 12 demonstrates the effect of changing the diameter of the soma. Changing the diameter increases the surface area and therefore increases the number of voltage-gated HH channels. This increase in HH channels allows the somatic membrane to depolarize faster, which drags the dendritic voltage along. When the diameter is 800µm, the onset of the AP in the soma and dendrites actually leads the AP in the axon. However, the amplitude of the AP is still highest in the axon. Decreasing the diameter to 100µm significantly decreases the amplitude of the AP in the soma and dendrites and increases the lag time.

**Figure 12**: (A) Voltage versus time plot, showing the time course of the AP at the 3 recording sites at different values of soma diameter. When the soma diameter is increased to 800µm, the dendritic/somatic AP (blue/red) actually leads to axonal AP (black). (B) Freeze-frame of the simulated movie showing the position (x axis) of the onset and highest point of the AP, (voltage y axis). Increasing the soma diameter leads to a larger voltage deflection in the soma and dendrites during the axonal AP.
**Soma Length**

**Figure 13** demonstrates the effect of changing the length of the soma on the site of AP initiation. Similar to increasing the diameter, increasing the length of the soma increases the somatic surface area and thus the number of H\(\text{H}\) channels. Therefore, when the length is doubled from 200\(\mu\)m to 400\(\mu\)m, the lag between the dendritic/somatic AP and axonal AP is reduced. Moreover, the somatic/dendritic AP is increased in amplitude. Conversely, when the length is halved from 200\(\mu\)m to 100\(\mu\)m, the somatic/dendritic AP is reduced in amplitude and becomes more delayed.

![Figure 13](image)

**Axon Diameter**

To determine how axon diameter affects the AP initiation site, the axon diameter was varied from the default value of 15\(\mu\)m to half this value (7.5\(\mu\)m) and 4x this value (60\(\mu\)m). Decreasing the diameter did not change the AP initiation site, but decreased the amplitude of the dendritic/somatic AP (**Figure 14**). Increasing the diameter of the axon 4x increases the spike threshold, such that the original value of gmax does not lead to an AP in the axon or dendrites/soma. However, if the gmax is increased such that the cell fires, the lag between the somatic/dendritic and axonal AP is reduced. These simulations suggest that the leading axonal AP is partly due to the axon morphology.
Channel Densities

In addition to the morphological properties discussed above, which may endow the axon initial segment with a lower spike threshold, it has been proposed that this region may contain a higher density of voltage-activated Na⁺ channels (Dodge and Cooley, 1973). Although this is still a controversial issue, some experimental evidence supports this theory. For example, human cortical pyramidal cells show a high density of Na⁺ channel immunoreactivity at the axon initial segment (Figure 15; Inda et al., 2006).

The next set of simulations is designed to assess the effects of changing the densities of voltage-gated Na⁺ and K⁺ HH channels at the axon initial segment. As shown in Figure 16, doubling both the Na⁺ and K⁺ channels leads to a faster and higher amplitude AP in both the axon and soma/dendrite compartments. Moreover, this increases the lead of the axonal AP. Conversely, if the densities are decreased by ½ the default values, the AP is delayed and stunted in the axon and the voltage deflection is barely visible in the soma/dendrite due to the slow inactivation of the Na⁺ channels before the axon reaches threshold. Doubling the Na⁺

Figure 14: (A) Voltage versus time plot, showing the time course of the AP at the 3 recording sites at different values of axon diameter. When the axon diameter is increased to 60µm, the spike threshold increases and the cell does not fire. If the gmax is increased such that the cell fires, the lag between the somatic/dendritic AP and axonal AP is reduced.

(B) Freeze-frame of the simulated movie showing the position (x axis) of the onset and highest point of the AP, (voltage y axis). Decreasing the axon diameter does not affect the AP initiation site. Increasing the axon diameter and gmax increases the amplitude of the somatic/dendritic AP during the axonal AP.

Figure 15: Human cortical pyramidal cells showing that Na⁺ immunoreactivity is most profound at the axon initial segments (Inda et al., 2006).
channel density alone leads to an even higher amplitude AP and a wider AP. This leads to a faster AP and gives the axonal AP a greater lead.

Figure 16: (A) Voltage versus time and freeze-frame of AP peak at default Na+ and K+ densities. B) Doubling the densities of both Na+ and K+ channels leads to a faster and higher amplitude AP in both the axon and soma/dendrite. Decreasing both densities to ½ default values leads to delayed, smaller spikes in both the axon and soma/dendrite. (C) Doubling the Na+ channel density only leads to a faster and higher amplitude AP in both the axon and soma/dendrites.

How does the site of initiation affect spiking response?

Finally, to address a question that was not included in the tutorial, I asked whether morphological changes that occur during development may affect spiking responses by changing the AP initiation site. Figure 17 shows preliminary anatomical data suggesting that the dendrites of cortical pyramidal cells increase in length considerably during postnatal development. Biocytin-labeled pyramidal cells are shown from the auditory cortex of gerbils aged postnatal day (P) 10 and postnatal day 26.

Figure 17: Biocytin-labeled pyramidal cells from the auditory cortex of gerbils aged postnatal day (P) 10 and P26. Note the extension of dendritic length that occurs during this developmental period.
It was estimated from several cells that there is about a 3-fold increase in dendritic length for these cells across this developmental period. Given that previous simulations suggest that morphological changes can have profound effects on the AP initiation site, it was hypothesized that these changes in dendritic length will move the initiation site. **Figure 18** shows that increasing the dendritic length by 3x to represent the older animals ('old cells') leads to a significantly greater lag between the axonal AP and the somatic/dendritic AP. As dendritic length increases, the capacitive load increases. Increasing the dendritic surface area does not increase the number of voltage-gated channels as we saw with increasing the somatic length, because these channels are not present in the dendrites in this model.

Based on the results from **Figure 18**, it was hypothesized that the increase in the lag time between the axonal and somatic/dendritic APs in the older cells due to the increase in dendritic length may limit firing frequency. In the young cells, the APs in the axon and soma/dendrite depolarize almost simultaneously. However, in the older cells, the total time of the combined APs in the axon and soma/dendrites is much greater due to the lag between them. Therefore, it was predicted that the 'young cells' may fire at higher frequencies.

**Figure 19**: Simulations showing the spiking of 'young cells' and 'old cells' in response to trains of 10 synaptic inputs at various frequencies. 'Old cells' are modeled using a dendritic length 3x to that of the 'young cells'. This change in dendritic length makes the 'old cells' fire less to trains of synaptic inputs.
This was tested using trains of 10 synaptic inputs at several frequencies (50, 67, 200Hz). As shown in Figure 19, the young cells were able to fire at higher frequencies in response to the synaptic trains. The maximum conductance to reach spike threshold was lower in the 'young cells' as well (gmax threshold young cells = 5µS; gmax threshold old cells = 10µS). Figure 20 summarizes the increase in spiking of the 'young cells' in response to synaptic trains of various frequencies. It is apparent from this figure that the most robust increase in spiking in the 'young cells' compared to the 'old cells' occurs at a frequency of about 200Hz. This simulation suggests that experimentally observed changes in dendritic morphology may contribute to other developmental changes to increase the excitability of the neurons in early development. Such increased excitability may be important for the heightened plasticity mechanisms that are essential during this time.

![Figure 20: Summary graph showing the number of spikes in response to trains of 10 synaptic inputs in 'young cells' and 'old cells'. 'Old cells' are modeled with 3x the dendritic length of the 'young cells'. This morphological change leads to less spiking, particularly at about 200Hz.](image)

**References:**


Cruz DA, Eggan SM, Azmitia EC, Lewis DA (2004) Serotonin 1A receptors at the axon initial segment of prefrontal pyramidal neurons in schizophrenia. *Am J Psychiatry* 161: 739-42.


Sarmiere PD, Weigle Cm, Tamkun MM (2008) The KV2.1 K+ channel targets to the axon initial segment of hippocampal and cortical neurons in culture and in situ. BMC Neurosci 13: 9-12.