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Anatomical specializations of neurons and their contribution to coincidence detection

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Within single neurons, there are biophysical mechanisms that can be used for coincidence detection. In some neurons, anatomical specializations have been identified that preserve timing information ultimately used for coincidence detection. For example, the calyces of Held are giant presynaptic structures in the medial nucleus of the trapezoid body that are used in sound localization. They relay timing signals carried by the cochlear nerve to brain regions where the relative timing of auditory stimuli between the two ears is compared.

Here we examine biophysical mechanisms in the neocortex and hippocampus that could be used for encoding and decoding timing signals in the millisecond range. Pyramidal neurons, the chief neurons in these areas, have a dramatic and complex morphology (*Fig. 70a*): dendrites that can extend for several millimeters with a variety of specialized regions, such as dendritic spines; an initial segment in the axon; axon collaterals, and nodes of Ranvier in their myelinated axons. What can these striking geometrical features contribute to coincidence detection on time scales ranging from less than one millisecond to 100 milliseconds? Pyramidal neurons encode integrated synaptic input into trains of action potentials (spikes) for transferring information over long distances. Converging experimental and neuronal modelling studies reveal that these neurons are capable of much higher precision in their spike timing and dendritic processing than hitherto imagined and

that spike timing may be an important variable for coding information in the brain. Just what contribution such mechanisms make to information processing remains to be investigated.

Spike generating mechanism

The timing of spikes in vivo is highly irregular, which has led some investigators to conclude that information about synpatic inputs can be conveyed reliably only by the average spike rate. However, evidence is growing that pyramidal neurons can fire in synchrony with millisecond precision. This indicates that the spike generating

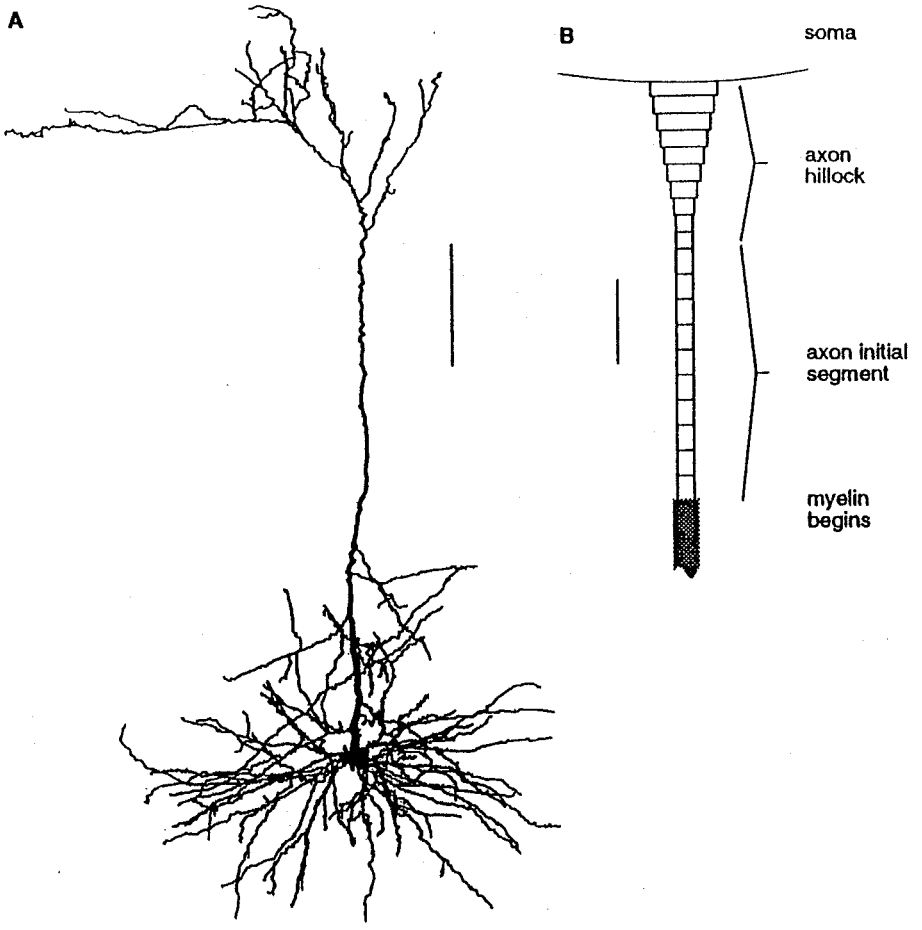

Figure 70. *Anatomy of the dendritic arborization, axon hillock and initial segment of a rat layer 5 cortical pyramidal neuron used to model initiation of the action potential (spike).* ***a****, drawing of a neuron filled with horseradish peroxidase.* ***b****, geometry of the axon hillock and initial segment. Modified from Mainen et al., 1995.*

mechanism must at least be capable of supporting such high temporal resolution.

Na^+ and Ca^{2+} currents have recently been reported in the dendrites of neocortical pyramidal neurons, which has raised the possibility that spike generation is not exclusive to specialized regions of the cell body but may also occur in dendrites. The concentration of Na^+ channels in neocortical pyramidal neurons is comparable in the dendrites and cell body. Despite this, Stuart and Sakmann (1994) found that when current was injected directly into the dendrites, the spike was initiated first in the cell body and then propagated back into the dendrites. Add to this an observation of John Huguenard (personal communication) that when acutely dissociated neocortical pyramidal cells lose their initial segment, they produce only a feeble spike when current is injected, rather than a burst of action potentials. Could a differential distribution of Na^+ channels account for backward propagation of spikes in dendrites and if so, what implications does this have for the reliability of spike timing?

We have used a computational model of a rat neocortical neuron to demonstrate that the observations of Huguenard and Stuart and Sakmann are consistent with spike initiation in the axon initial segment (Mainen *et al.*, 1995). A neuron was filled with horseradish peroxidase and its detailed geometry divided into several hundred compartments, including the entire apical and basal dendrites, the axon hillock, initial segment and the myelinated axon (*Fig. 70b*). The electrophysiological recordings could be reproduced by the model only when the density of channels in the axon hillock and initial segment was about a thousand time greater than that in the cell body and the dendrites. Under these conditions, when current is injected into the dendrites, the membrane potential rises slowly until the spike initiates in the initial segment or sometimes in the first node of Ranvier, where the sodium channel density is highest (*Fig. 71*). A small structure with a high density of sodium channels requires less current to depolarize it to threshold. Injection of current into the dendrites causes a gradual depolariza-

tion that inactivates the dendritic sodium current; injecting current into the cell body induces a more rapid depolarization and hence less inactivation of sodium channels than in the dendrites.

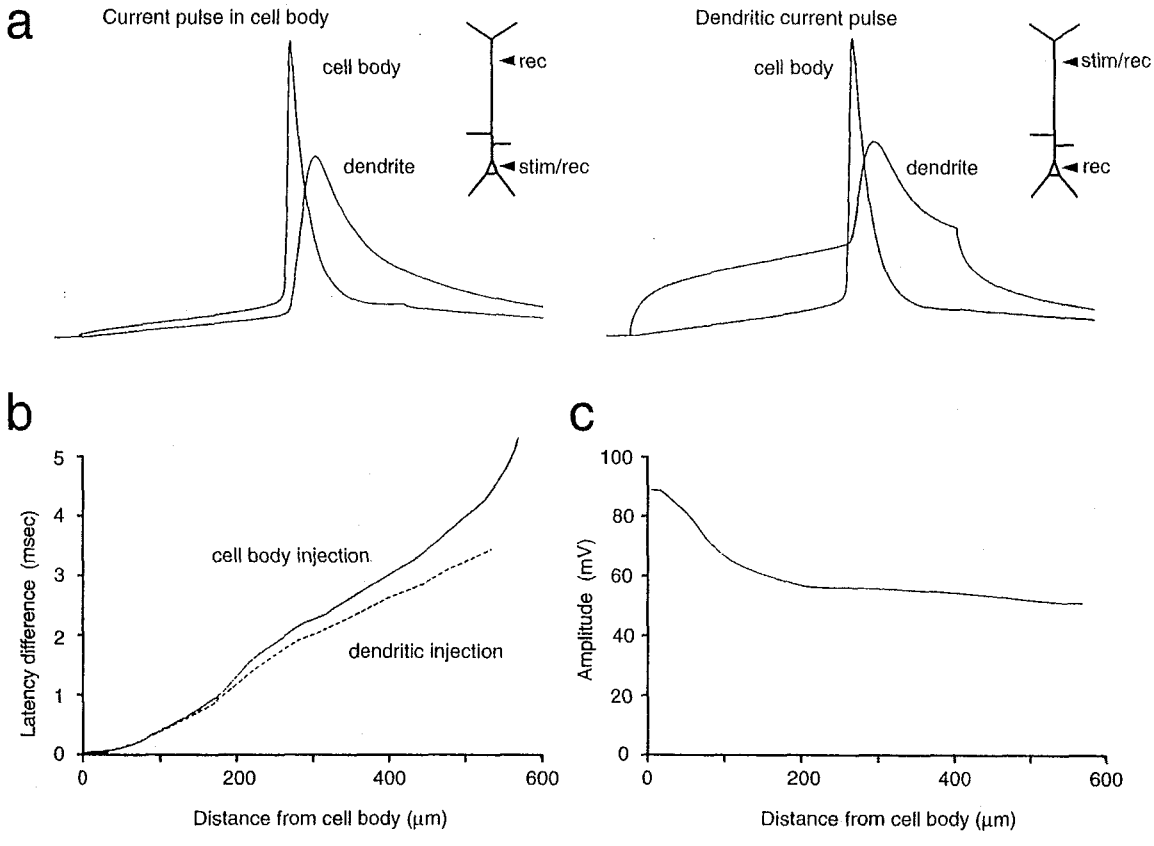

Figure 71. *Determining the site of spike initiation.* **a**, *voltage trace from the cell body and apical dendrite in response to current steps injected into either the cell body (left) or dendrite (right).* **b**, *plots of the latency differences between peak somatic and peak dendritic potential at different distances from the cell body. Spikes were elicited by current injection into the cell body (solid line) or dendrites (dashed line).* **c**, *spike amplitude as a function of distance of the current injection site from the cell body. Modified from Mainen et al., 1995.*

A high density of sodium channels in the axon hillock was indicated by the earliest physiological studies of spike initiation but there has never been any direct proof of this. A precedent can be found in the nodes of Ranvier, where the density of sodium channels is comparable to that required in the axonal hillock in our model. Indeed, the ultrastructure of the axon hillock and initial segment show the

same submembranal cisterns and thickening of the membrane as in the nodes of Ranvier, further support for a high density of sodium channels.

Reliability of spike initiation

The specialization of the axon hillock raises the question of how precise is the timing of spike initiation in neocortical pyramidal neurons. When a cell fires, either spontaneously or driven by stimulation, the intervals between spikes are irregular, with a coefficient of variation of one or more. If information is conveyed in the mean firing rate, then repeating exactly the same stimulus should produce the same number of spikes in every train but the timing of spikes should carry no information. On the other hand, if information is carried by spike timing, repeated stimuli should produce spike trains that line up very precisely.

We have studied the initiation of action potentials directly by recording from pyramidal neurons in neocortical brain slices using whole-cell patch clamp electrodes (Mainen and Sejnowski, 1995). Current was injected into the cell body of a pyramidal cell while glutamate synapses were blocked with CNQX and APV. During steady current injection, the average number of spikes per second was highly reliable. The first action potential always occurred at a precise interval after the onset of current injection but the time of initiation of subsequent spikes drifted (*Fig. 72a*). This is consistent with the idea that cortical neurons are relatively sloppy integrators and only the total number of spikes over a long period provides a reliable signal.

In its natural physiological state, however, a cortical neuron is continuously bombarded by EPSPs and IPSPs, so its normal input is a fluctuating rather than a constant current. We simulated this input by injecting a fluctuating current into the cell body. When the same noisy input was given repeatedly, the timing of the spikes produced by the neuron was highly reliable (*Fig. 72b*). In all the neurons tested, spike timing was precise to less than one milli-

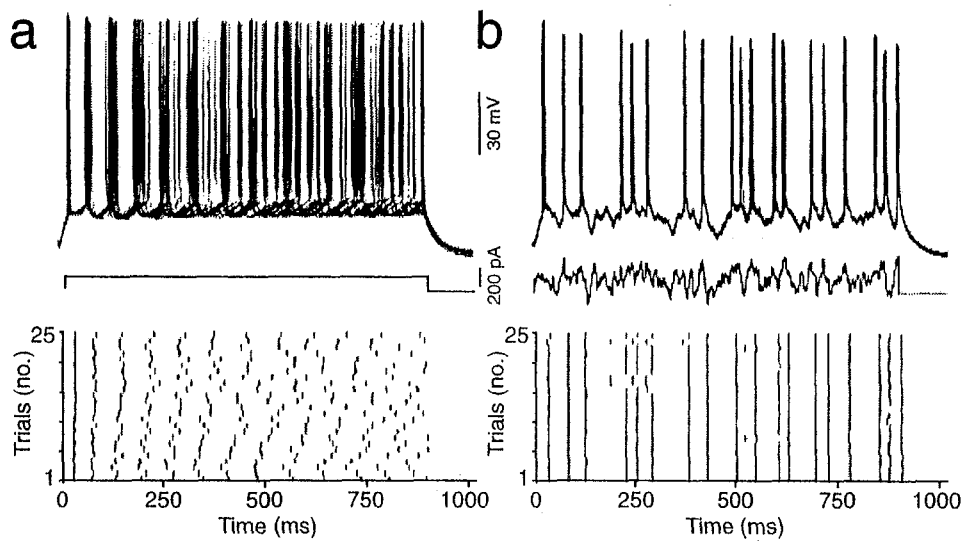

Figure 72. *Reliability of firing patterns of cortical neurons evoked by constant and fluctuating current.* ***a****, a constant current step elicits a train of spikes; 10 trials are superimposed (top) and shown as a raster (bottom).* ***b****, the same cell was stimulated 10 times with the same fluctuating stimulus. The spikes in the raster diagram are much more precisely timed than with the constant current step. Reproduced with permission from Mainen and Sejnowski, 1995.*

second (Mainen and Sejnowski, 1995). The spike generating mechanism is thus much more precise than it needs to be for a sloppy integrator.

It is not clear how the timing of spikes could be used by networks of neurons to code or process information. Evidence that it is used comes from the middle temporal (MT) area of the monkey visual cortex, which has a high proportion of directionally selective cells that detect motion. The stimulus was a field of randomly moving dots presented many times in precisely the same pattern. The cells in MT, which are at least 6 synapses away from the retina, preserve the information in the pattern of dots (Shadlen and Newsome, 1994). It may be that both the average firing rate and timing of spikes is used in the cortex to encode different types of information, depending on how rapidly the inputs are changing.

Excitation - spike potentiation

If information in a neuron flows antidromically from the cell body to the dendrites as well as orthodromically from dendrites to cell

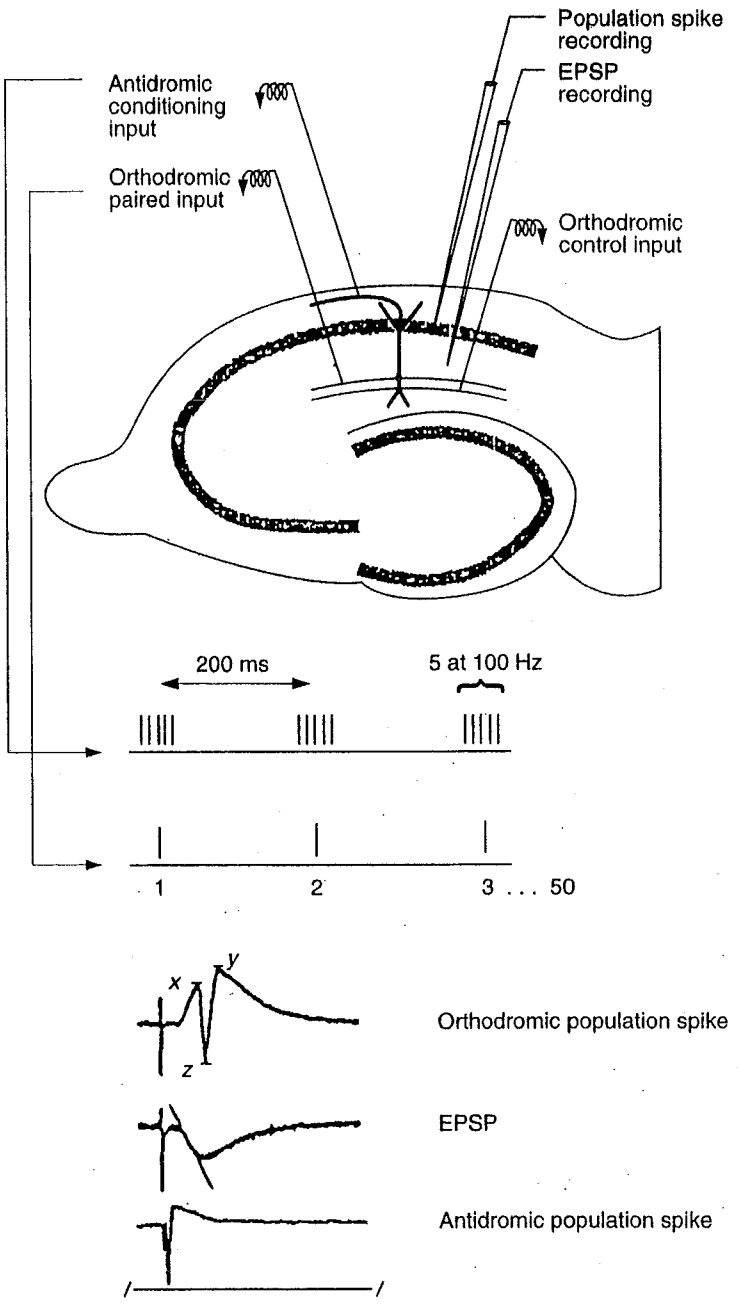

Figure 73. *Demonstration of potentiation of the EPSP - spike coupling. Schematic drawing of the hippocampal slice preparation (top) shows electrode placements; the paired orthodromic and antidromic stimulation protocols are depicted in the middle, and typical population recordings are displayed at the bottom. Modified from Jester et al., 1995.*

body, there is potential for coincidence detection between the two signals. Because the spike generation mechanism is precise, coincidence detection to within few milliseconds is possible. We have investigated what happens when antidromic and orthodromic stimuli are paired, firing the initial segment and the dendrites simultaneously. It has previously been observed that when depolarizing current is injected into the cell body and paired with weak orthodromic stimulation, even a single pairing is enough to induce long-lasting potentiation (see Bliss and Collingridge, 1993). In a cell with passive dendrites, naturally occurring impulses in the cell body cannot inject enough current into the dendrites to open the NMDA receptors at distal locations, so potentiation does not occur. However, if sodium and calcium currents in dendrites could be activated in coincidence with stimulation of the cell body, the spike would be boosted.

To test this in the hippocampus, cell bodies of CA1 pyramidal neurons (*Fig. 22*) were stimulated antidromically while activating many axon terminals and synapses on the apical dendrites with a single orthodromic stimulus (Jester *et al.*, 1995). The slope of the extracellular EPSP was measured to test for potentiation of synaptic potentials (*Fig. 73*). Surprisingly, paired stimuli produced potentiation of the population spike but slope of the EPSPs was unchanged. Thus, spike generation can be potentiated without involving classical synaptic LTP; also unlike LTP, potentiation took 10-20 minutes to reach full expression.

The origin of this increase in excitability following orthodromic and antidromic pairing remains uncertain. Its induction was blocked by APV, so an NMDA component is essential. $GABA_A$ blockers, such as picrotoxin or bicuculline, had no effect, indicating that the potentiation occurs in pyramidal cells. $GABA_B$ blockers, which block a slow inhibition known to be mediated by second messengers that can last for hundreds of milliseconds, prevent the expression of this potentiation but not its induction.

This type of excitation - spike potentiation provides a mechanism for neurons to change their excitability when they spike at

high frequency and simultaneously receive low-frequency synaptic input. Conditions such as this, which probably exist in the cortex, would have an important influence on the overall output of neurons by varying the coupling between the dendrites and the spike generating mechanism. It may be that some aspects of memory are stored not at synapses but at voltage-dependent mechanisms that couple synaptic inputs with spike initiation.

The significance of timing

Although these results are incomplete and fragmentary, they do indicate that spike encoding and coincidence detection in the millisecond range may be important for cortical function. The classical form of synaptic LTP is also sensitive to coincidences between presynaptic transmitter release and postsynaptic depolarization in the range of a few milliseconds. The regulation of the coupling between distal synaptic events and spike initiation in the axon hillock by active currents in dendrites may also have a timing function. The greater the excitability, the faster a spike will be initiated for the same synaptic input. The presence of precise spike-timing mechanisms and their significance for information coding and processing are important problems that deserve further investigation.