uniform to clumpy. While this particular family of stimuli is well suited to the generation of large numbers of randomized stimulus patterns, the magnitude of the cluster sensitivity effect, as represented in fig. 7, could be considerably increased if the stimulus family were specifically tailored to the electrotonic structure of the dendritic tree under study (unpublished observations).

Different Biophysics, Similar Behavior

While the four cases summarized in figs. 7BCDE are all highly cluster sensitive, it is essential to note that the biophysical conditions that hold within the dendritic trees in these 4 cases are very different from each other. The difference is particularly salient in comparing the NMDA-only case in B with the NMDA/fast/slow-spiking combination shown in E. Subsynaptic and somatic voltage traces for these two runs may be compared in fig. 9 for 100 active synapses in clusters of 5. In the NMDA-only case, the subsynaptic voltage traces achieve smooth steady state values, while in the combination case the subsynaptic voltage traces reveal a chaotic jumble of fast and slow spikes throughout the dendritic arbor. Direct intradendritic current injections in this latter cell configuration, as shown in fig. 10, confirm the complex nature of the voltage activity in the dendritic tree under these distributions of active membrane and synaptic conductances; the inset shows an intradendritic spiking trace recorded from a real neocortical pyramid for purposes of qualitative comparision (Amitai et al. 1993).

Dendritic Spike Propagation

If most or all of a dendritic tree contains excitatory conductances in sufficient quantity for the generation of action potentials (e.g., the "distal" condition described in section), then it would appear that any accidental concentration of synaptic input at a single dendritic locus could "light up" the entire tree, and thereby effectively destroy the cell's selectivity for the global spatial pattern of synaptic activation. In the apical tree of the cell used in this study, a single concentrated synaptic action can in fact "ignite" the entire apical tree, since the apical tree structure is qualitatively similar to a branched axon. An action potential initiated in a distal dendritic tip can propagate first proximally, and then distally to every other apical tip. However, even under these conditions, the apical tree remains strongly cluster-sensitive. As synaptic input becomes more clustered, action potentials are initiated at an increasing *number* of dendritic sites, leading to a higher frequency of activation of the apical tree as a whole. An experiment was run in which the entire apical tree (sparing the main apical trunk) was covered with the fast-spiking conductance complex, and was synaptically activated by 100 voltage-independent AMPA-type synapses. The soma was passive. As cluster size was increased from 1 to 15 in the apical tree, the average aggregate spike frequency in the apical tree doubled from 65 to 130 Hz, while the charge injected at the soma over the 100 ms stimulus period increased by 60%.

Discussion

Dendritic Cluster Sensitivity

The foregoing results support the conclusion that the responses of neocortical pyramidal cells, if endowed with a significant complement of excitatory membrane mechanisms, will be strongly dependent on the spatial pattern of synaptic activation across the dendritic arbor. The dependence is simple and is essentially statistical in nature: when synaptic input is relatively clumpy, cell responses tend to be large. When synaptic input is relatively diffuse, cell responses tend to be small.

How much greater is the actual current injection per synapse in clustered vs. unclustered stimulus conditions? The answer is complicated by the fact that the net current flowing into the cell is not a steady state quantity, since it depends on the time-changing channel activity across the entire dendritic tree. The *average* current flowing into the cell may be measured over an extended interval, but this quantity is not simply related to the cell's output firing rate during that interval. Other quantities are crucial, such as time spent above firing "threshold", but are more poorly defined.

Two results nonetheless shed light on this question. First, in a previously reported simulation experiment (Mel 1992a), total injected charge at the passive cell body was measured in a passive-dendrite/high-NMDA condition for varying cluster sizes. The maximum increase in average somatic current injection relative to an unclustered stimulus condition was 40% for basal dendrites and 18% for apical dendrites. The larger percentage increases typically seen in the number of somatic *spikes* produced in clustered vs. unclustered conditions—e.g., see range in fig. 7B to E—is due to the thresholding nonlinearity of the somatic spiking mechanism. In another run with 1) high-NMDA synapses, 2) fast-spiking in the distal basal dendrites, and 3) slow-spiking in the distal apical dendrites, average current injection at the passive soma showed a maximum increase of 70% for clustered vs. unclustered stimuli (using parameters as in Tables 1 and 2).

The second result relevant to the magnitude of the cluster-sensitivity effect was discussed earlier: In contrasting the plots in fig. 8 A and B, it was seen that the maximum increase in cell response due to synaptic clustering (from 0 to 4 spikes over 100 ms) was comparable to that associated with a 25-50% increase in total synaptic conductance input to the dendritic tree. For an additional point of reference, in the case of passive dendrites, a 25% increase in direct somatic current injection (0.6 to 0.8 nA) can account for an increase in somatic spiking rate from 0 spikes to 4 spikes in a 100 ms stimulus period for the somatic fast-spiking parameters as in Table 2. We conclude that substantial percentage increases in somatic current injection can be due to synaptic clustering alone, in the range of 20 to 70% depending on assumptions.

It is important to note that the family of stimuli used in these experiments, parameterized by cluster size, is only a crude abstraction of the continuum of distributions of synaptic activity in a dendritic tree that ranges from

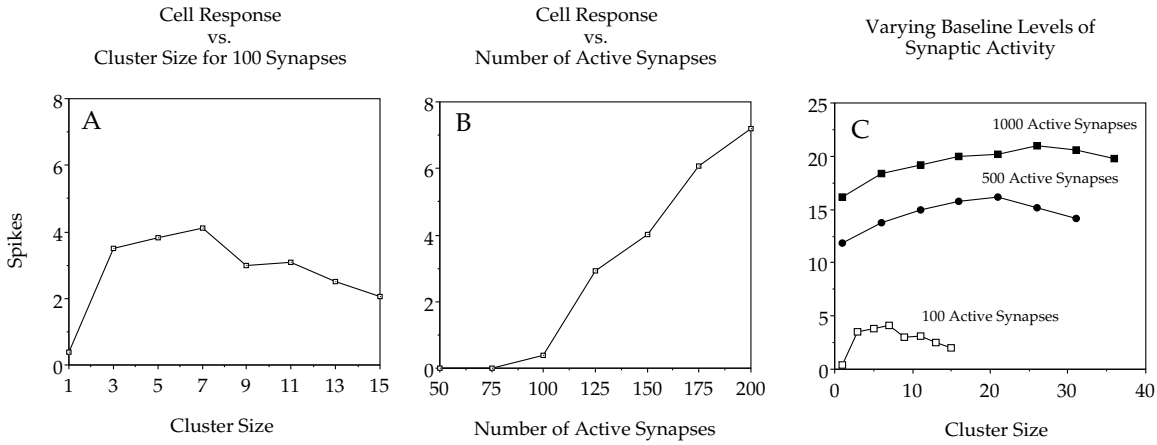

Figure 8: Plot of cell responses (spikes/100ms) as a function of cluster size (A) and number of active synapses (B). In C, number of active synapses is increased from 100 (as in A) to 500 and 1000 to see effect of response saturation on cluster sensitivity. Same synaptic strengths were used in all three cases; values were those optimal for case of 100 active synapses. A modest initial regime of cluster sensitivity is thus conserved even when baseline level synaptic drive is increased by an order of magnitude. Parameters were as in Tables 1 and 2.

configured as in fig. 7E (reproduced in B). The number of active synapses was varied from 50 to 200; the runs at 100 active synapses were equivalent to those of plot B at cluster size 1. Beginning with 100 active synapses, increases in synapse number of approximately $25-50\%$ cause increases in cell responses comparable to the maximum response increase due to clustering alone. This result indicates that optimal sensitivity to changes in the degree of synaptic clustering can only be accomplished under conditions of relatively tight control on the number of active afferents impinging on a cell's dendrites from moment to moment. It is also a crucial that the average number of active synapses is not so large as to produce very strong or maximal cell responses for unclustered layouts, as this would preclude further significant response increases due to variations in the spatial distribution of activated synapses. As shown in fig. 8C, the initial regime of cluster sensitivity is mostly, but not entirely, eliminated when the number of active synapses is increased by an order of magnitude (from 100 to 1000) leaving all other parameters unchanged. Note that the response levels for 1000 activated synapses are in excess of 160 Hz, suggesting that these levels of synaptic activation may be unrealistically high for a neocortical pyramids. Definitive data are not yet available, however, as to i) the average number of activated synapses impinging on a pyramidal cell during the normal functioning of cortex, or ii) the variance in this number from moment to moment.

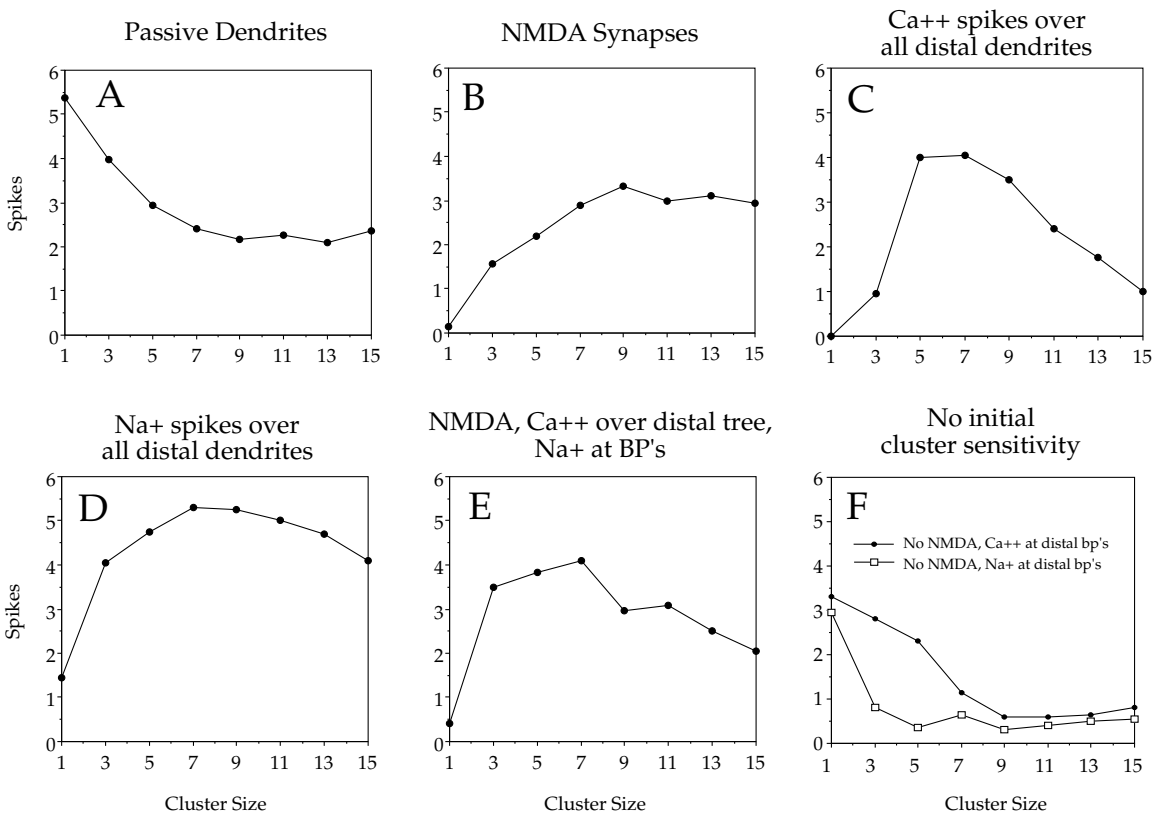

Figure 7: A. Response of passive dendrites and AMPA synapses as cluster size is increased (reproduced from fig. 5). B. In high-NMDA condition, direction of effect is reversed: cell displays an initial *increasing* responsiveness to clustered synaptic stimuli, with an subsequent falloff due to saturation as in A. C. With slow-spiking complex in the "distal" configuration but no NMDA, initial "cluster senstivity" is pronouced. D. With fast-spiking complex in distal configuration (i.e., dendrite is made to look like an axon), strong cluster-sensitivity remains. E. Combination of high-NMDA, slow-spiking distributed distally, and fast-spiking distributed at branch points. Cluster-sensitivity is among strongest of all studied cases. F. In two cases when no NMDA was present, and dendritic spiking conductances were sparse as in branch-point configuration, cluster-sensitivity was abolished.

Dendritic Spiking Mechanisms

It was necessary to establish whether the cluster sensitivity seen in the passive-dendrite/high-NMDA case was an inherent property of a dendritic arbor containing any form of excitatory voltage-dependent conductance in sufficient quantity, or whether the behavior was particular to some aspect of the kinetics, voltage-dependence, peak conductance, or sparse pattern of activation of NMDA synapses in particular. Simulation experiments were thus repeated using various combinations of dendritic spiking mechanisms in the dendritic arbor, both with and without NMDA synapses. Figure 7 C, D, and E show the results for three representative cases. In case C, the dendrites contained the slow-spiking calcium channel complex in the "distal" dendritic configuration (fig. 3B). Zero-NMDA synapses were used. In case D, the dendrites contained only the fast-spiking condutance complex in the distal configuration, again with zero-NMDA. In case E, a combination of all three voltage-dependent mechanisms was used: NMDA synapses, the slow-spiking mechanism in the distal configuration, and the fast-spiking mechanism in the branch-point configuration. In all three of these test cases, cell responses showed pronounced sensitivity to the degree of spatial clustering of the synaptic activation pattern.

A number of other cases were studied. In general, sufficient criteria for cluster sensitivity were 1) a high-proportion of synaptic current due to NMDA channels, or 2) a spiking conductance complex over most of the dendritic tree. Thus, a configuration with a spiking conductance complex only at branch points, and with no NMDA channels at activated synapses, led to cell responses with no initial cluster-sensitive regime (fig. 7F). A combination of NMDA channels *and* fast and slow spiking mechanisms lead to the most pronounced initial cluster sensitivity seen in these experiments (fig. 7E). No qualitative differences were seen in the cluster-sensitive behavior of the dendritic tree when the full calcium-mediated slow-spiking mechanism was replaced with the simpler voltage-mediated slow-spiking mechanism, suggesting that the signaling intermediary between inward and outward currents, whether voltage or calcium concentration, was not of primary functional importance for the effects studied here.

Variations in Number of Active Synapses

Cell responses also depend on the *number* of active synapses, or similarly, the total stimulated synaptic conductance. We contrast the range of cell responses seen under variations in cluster size with the range of cell responses seen under variations in the number of active synapes with cluster size fixed at 1. This comparison is important: if the total number of active synapses varies significantly from moment to moment, then changes in cell responses due to differential clustering of synaptic inputs could be washed out. If this were the case, then the cluster-sensitivity property could not be used to establish the selectivity of cell responses.

Figure 8A shows the averaged results for 30 runs in a cell biophysically

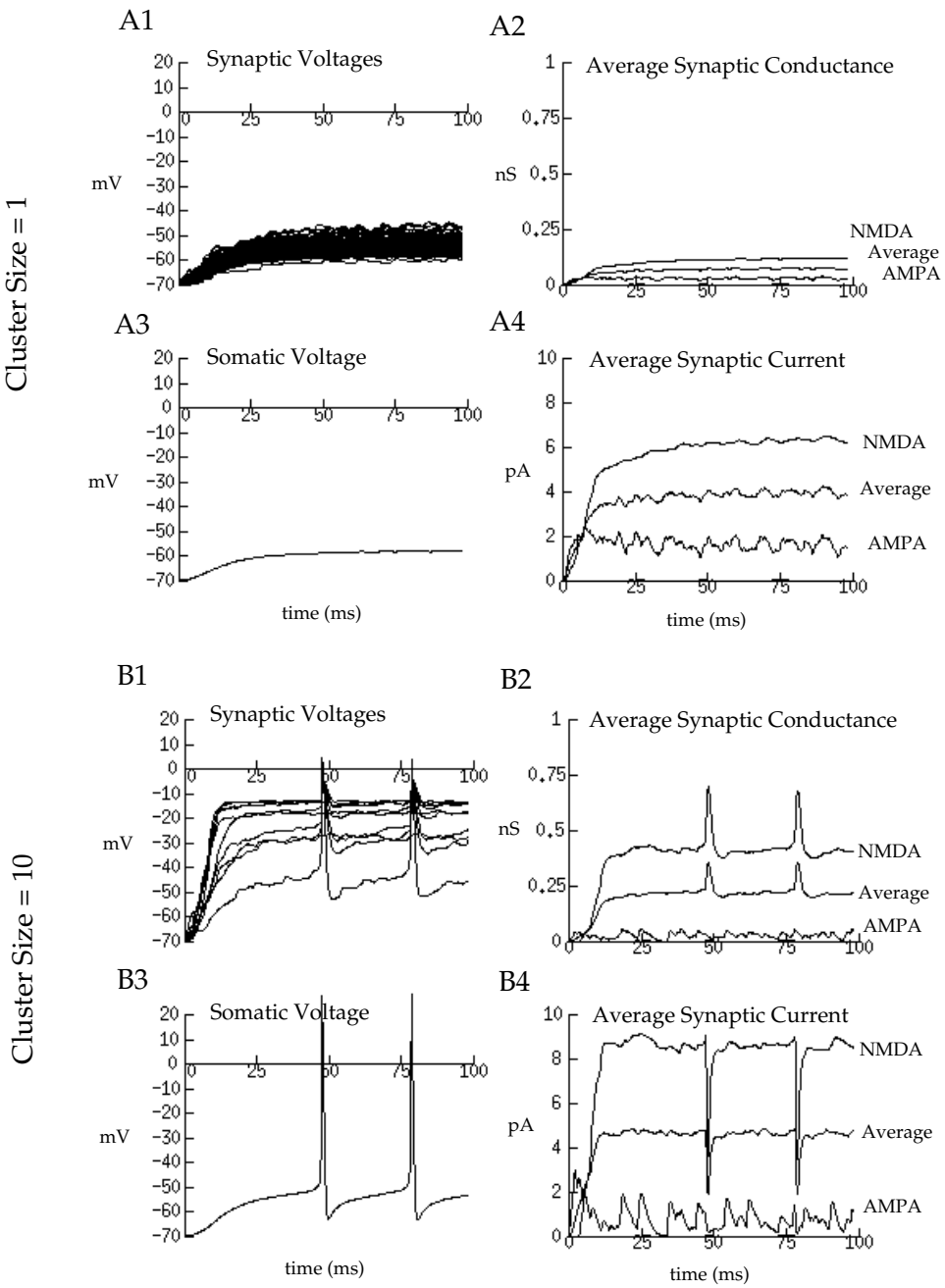

Figure 6: A. Response of cell to 100 active synapses in high-NMDA condition with cluster size 1. 100 sub-synaptic voltage traces are shown in A1; since each synapse acts "alone", synapses remain relatively polarized. As a result, average NMDA conductance (A2) and current (A4) remain modest. No somatic spike is generated in this case (A3). B. Same stimulus conditions, except synapses are grouped in clusters of 10. Subsynaptic voltage traces are much elevated (B1 vs. A1), as are NMDA average conductance (B2 vs. A2) and current (B4 vs. A4). Two somatic spikes are generated in this case (B3). Total peak synaptic conductance allocated to 100 active synapses was 8% less in case B than in case A.

unlike the baseline case, synapses were deployed in the high-NMDA condition. Peak conductances were as shown in Table 1. Fig. 6 exemplifies cell behavior for clusters of size 1 (A) and size 10 (B). As seen in fig. 6A1, subsynaptic voltage traces are relatively little depolarized from rest, since each of the 100 synapses acts "alone". The NMDA channels remain relatively polarized and hence relatively inefficient. Average NMDA conductance (A2) and current (A4) remain modest. The somatic voltage trace (A3) shows that no action potentials were generated during this run. By contrast, when synapses are clustered in groups of 10 (fig. 6B), subsynaptic voltage traces are boosted into a more favorable voltage range for the NMDA channels (B1), and average NMDA conductance (B2) and current (B4) are correspondingly increased. In this case, two action potentials are generated at the cell body (B3).

Averaged responses from 50 randomized runs at several cluster sizes are shown in fig. 7B. In opposition to the passive case reproduced in fig. 7A, the NMDA-rich cell at first shows an average *increase* in cell response as cluster size is increased. At larger cluster sizes, the attenuating effects of synaptic saturation begin to offset the excitatory effects of the NMDA voltage-dependence. Note that the specific cluster size at which the cell response peaks depends on a number of variables including the elemental peak synaptic conductance, the afferent firing frequency, the number of active synapses, and the proportion of NMDA to non-NMDA conductance at each synapse. The average firing rate shown on the ordinate is likewise dependent on simulation parameters, including the excitability of the cell body, the peak synaptic conductance, and the presence or absence of background synaptic activity. In spite of this quantitative dependence on simulation parameters, the general pattern of dendritic "cluster sensitivity", characterized by an initially increasing, peaked cell response to increasingly clustered synaptic input, proved to be extremely robust under wide ranging parameter variations. As reported in (Mel 1992a), parameter variations tested in the high-NMDA/passive-dendrite case included membrane resistivity from 10,000 to 100,000 Ωcm^2, membrane capacitance from 0.9 to 2.0 μF/cm^2, cytoplasmic resistivity from 100 to 362 Ωcm (several configurations were taken from Stratford *et al.* 1989); synaptic conductance waveforms in either saturating or non-saturating conditions, spine membrane correction used or not, and count of activated synapses varied from 80 to 200. Most runs in this ealier study were carried out with peak synaptic conductances assigned uniformly across the dendritic tree, rather than scaled to local input resistance as was done here. Spine neck resistance was increased in one run from 173 MΩ 6-fold to 1038 MΩ, which led to a significant reduction, but did not abolish, the cluster sensitivity (i.e. there persisted a 50% increase in average cell response as clusters grew to from size 1 to size 3). Finally, in a pilot study on a smaller layer 3 pyramidal cell, cluster sensitivity was seen at stimulus frequencies of 20 Hz and 100 Hz, or when only a single spike was delivered to each synapse.

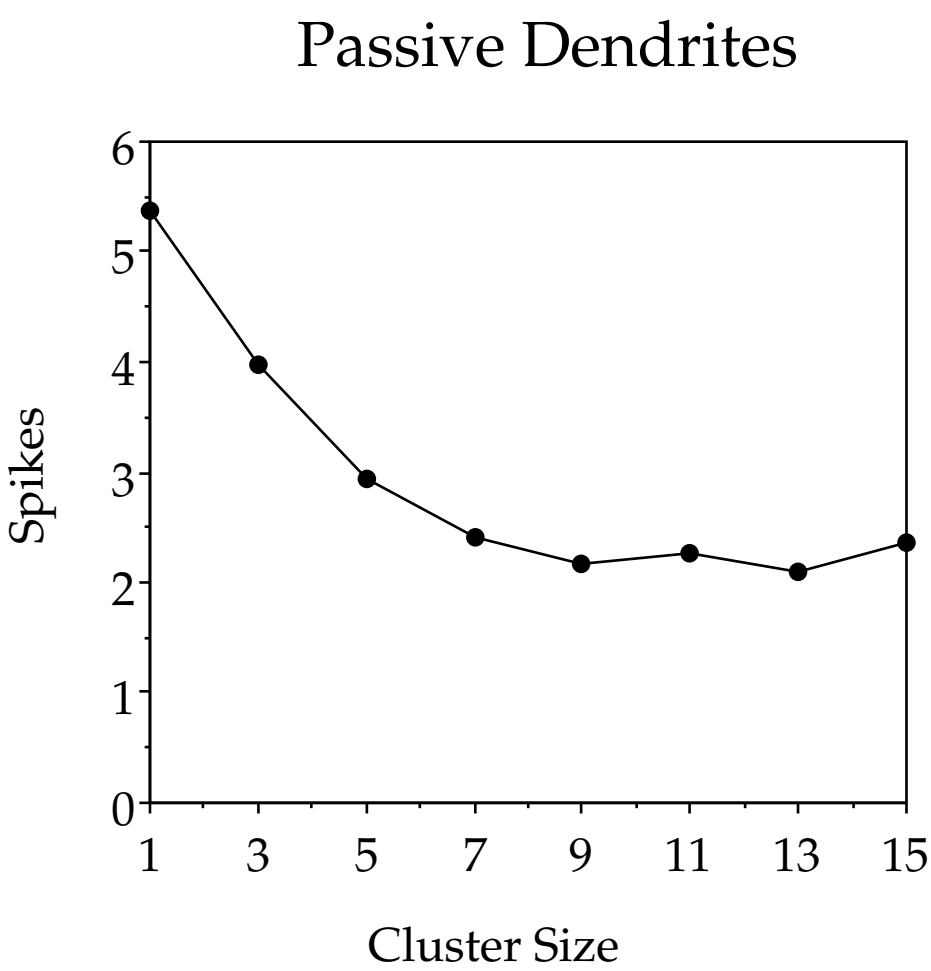

Figure 5: Response of cell with passive dendrites to 100 active synapses, as function of cluster size (1 to 15). Ordinate axis represents number of somatic spikes counted over a 100 ms stimulus period. Response falls off monotonically due to increased synaptic saturation with increasing cluster size. Results are averaged over 100 runs.

then placed on spines in $100/k$ randomly located clusters of k spines each[1]. The probability of each cluster site was uniform in dendritic length. Spines within a cluster were generally attached to the same isopotential dendritic compartment representing 40 μm of dendritic length. It is important to reiterate that the term "cluster" as used here (see also Rall & Segev 1987) does not refer to anatomical clumping of spines along a stretch of dendrite; spines were in fact assumed in these experiments to be uniformly distributed across the dendritic arbor (a simplification; see Larkman 1991). Instead, a cluster designates a dendritic neighborhood where the density of synaptic *activation* is increased relative to other parts of the dendritic arbor. A 100 Hz spike train was delivered asynchronously to each of the 100 preselected synapses. The train delivered to each synapse was generated by adding a uniform random jitter of ± 5 ms to the arrival time of each spike. The number of action potentials generated at the cell body was then counted over the first 100 ms of synaptic stimulation. Passive-soma response measures were also used with similar results, including peak somatic potential, and time integral of somatic potential. Cluster sizes were systematically varied, ranging from size 1 (unclustered) to size 15. The cell responses for each cluster size were averaged across a number of randomized runs, in order to wash out the variation from run to run in the locations of the activated synapses relative to the cell body.

Results

Passive Cell Baseline

The first block of simulation runs served as an experimental baseline, consisting of an electrically passive dendritic tree, driven by 100 voltage-independent AMPA-type synapses. The results averaged over 100 runs are shown in fig. 5. As cluster size increased from 1 to 15, average cell responses decreased monotonically. This result is due to the well known saturating nonlinearity associated with summation of conductance inputs: as clusters grow larger, the membrane under each cluster is progressively depolarized by the increased local synaptic activity, but the driving force at each synapse is correspondingly reduced as the local membrane potential approaches the synaptic reversal potential. Each synapse thus becomes a less effective current injector as clusters grow larger. In the passive case, the optimal stimulus configuration is one in which the activated synapses are distributed as widely as possible throughout the dendritic arbor (Rall, 1964, 1967; Koch et al. 1982; Bush & Sejnowski 1992).

NMDA Synapses

The next block of simulation trials was a replication of the main effect reported in (Mel 1992a). The dendritic tree was again electrically passive, but

[1] A single cluster of size 100 mod k was also included when k did not divide evenly into 100.

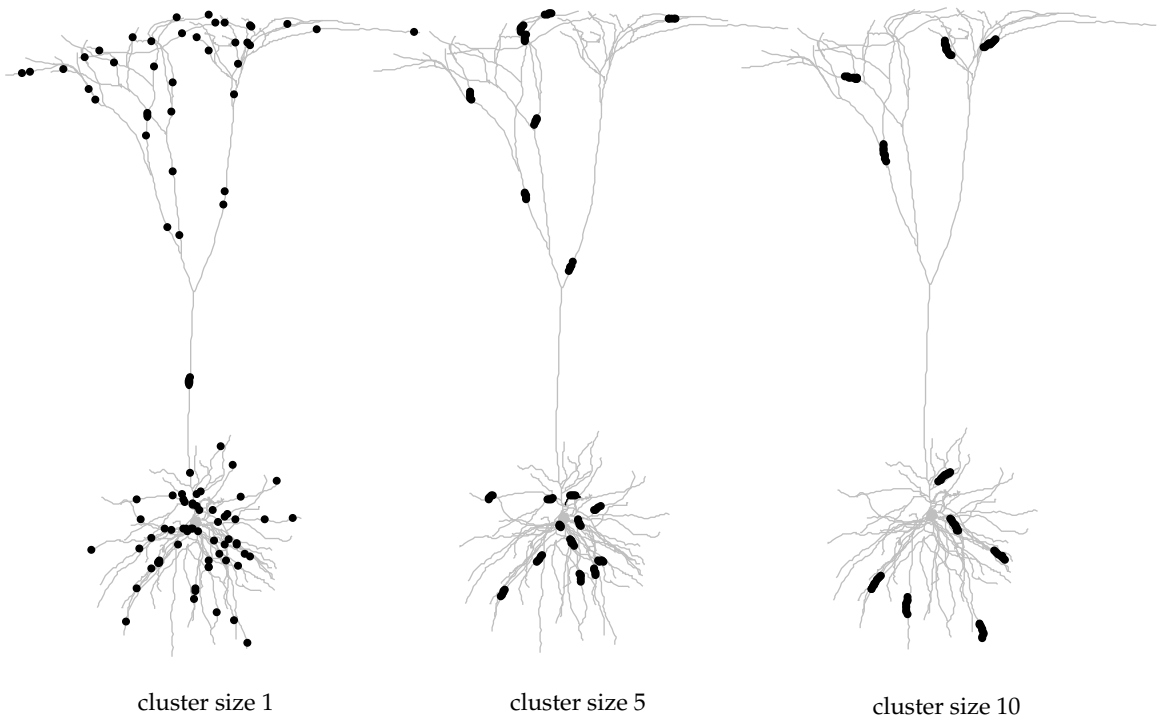

Figure 4: In each simulation run, 100 synapses were activated in randomly distributed clusters. Each active synapse is designated by a black dot. Shown are representative runs with cluster sizes 1, 5, and 10.

were studied. The conductance values for the dendritic spiking mechanisms were chosen heuristically, usually just large enough such that full-blown dendritic spikes were readily generated in a branch during concentrated synaptic stimulation. Peak synaptic conductance values were chosen such that diffuse synaptic stimulation of the dendritic tree gave relatively weak, relatively rare cell responses. This procedure was used in order to establish a low firing baseline against which increased responses due to spatial patterning of synaptic activation could be clearly resolved.

Stimulus/Recording Paradigm

As motivated by the sigma-pi hypothesis, the stimulus paradigm was designed to test for differences in the steady-state input-output behavior of the pyramidal cell as a fixed number of activated excitatory synapses was increasingly spatially clustered in the dendritic arbor. As intracortical network properties were not the object of this investigation, the neuron was as if dissociated from the brain and studied in isolation. Neither background synaptic activity, nor inhibitory input due to local circuit interneurons was modeled in these studies.

Each simulation run was parameterized by a single variable k, the cluster size (fig. 4). At the outset of each simulation run, the entire cell was set to a resting potential of -70 mV. One hundred synapses to be activated were