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Dendritic Discrimination of Temporal Input Sequences in Cortical Neurons

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Abstract

The detection and discrimination of temporal sequences is fundamental to brain function and underlies perception, cognition, and motor output. Applying patterned two-photon glutamate uncaging we found that single dendrites of cortical pyramidal neurons exhibit sensitivity to the sequence of synaptic activation. This sensitivity is encoded both by local dendritic calcium signals and by somatic depolarization, leading to sequence-selective spike output. The mechanism involves dendritic impedance gradients and non-linear synaptic NMDA receptor activation, and is generalizable to dendrites in different neuronal types. This enables discrimination of patterns delivered to a single dendrite, as well as patterns distributed randomly across the dendritic tree. Pyramidal cell dendrites can thus act as processing compartments for detection of synaptic sequences, implementing a fundamental cortical computation.

In sensory pathways, the relative timing of spikes from different neuronal populations can represent features of stimuli (1–4). A problem in cortical sensory processing is thus the discrimination of different spatiotemporal sequences of inputs (5). While networks composed of simple neurons can, in principle, decode temporal sequences, the size and complexity of such networks can be greatly reduced if individual neurons can perform temporal decoding (6, 7). Dendritic trees might contribute to this because they are highly non-linear devices that can locally process and integrate synaptic signals (8–10). For example, spatiotemporally clustered inputs trigger dendritic spikes (11–15), which can generate independent functional subunits, enhancing the computational potential of the neuron (16–18) and encoding spatial and temporal input synchrony. Whether these non-linear dendritic properties can be exploited to perform higher-order computations such as temporal sequence detection is unknown. In 1964 Wilfrid Rall predicted that, because dendrites act as a delay line, activation of synapses along a dendrite in different directions should produce different responses at the soma (19). While the dendrites of retinal neurons exhibit such direction selectivity (20–22), experimental investigation of the sensitivity to spatiotemporal sequences of synaptic activation in cortical pyramidal cell dendrites has been a challenge, due to the difficulty in delivering the spatiotemporal input patterns with the necessary sub-millisecond and sub-micron precision.

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To test the sensitivity of single dendrites to the order of activation of a defined set of synapses, we controlled spatiotemporal input patterns using multi-site two-photon glutamate uncaging at identified dendritic spines (15, 23) in layer 2/3 pyramidal neurons of somatosensory and visual cortex. We first studied the sensitivity of single dendrites to an ordered sequence of synaptic activation in opposite directions, selecting 8-10 spines on single basal and apical oblique dendritic branches (Fig. 1, A and B). Activating each site in isolation (Fig. 1C) produced synaptic responses (glutamate Excitatory Post-Synaptic Potentials - gluEPSPs) within physiological parameters (see fig. S1). Sequential activation of spines from the dendritic branch to the soma (IN) or from the soma to the tip (OUT) produced strongly directionally sensitive responses. The IN direction always produced a larger somatic response than the OUT direction (Fig. 1D and E; $31 \pm 4\%$ increase, $P < 0.0001$, $n = 20$, corresponding to a mean peak voltage difference of 2.8 ± 0.4 mV), even when stimulating only three inputs (fig. S2). EPSP peak was dependent on input velocity (Fig. 1, F and G), with optimal direction-sensitivity at 2.6 ± 0.5 $\mu\text{m}/\text{ms}$ (Fig. 1H). Axonal action potential output was highly directionally sensitive, with a higher spike probability in the ON direction than the OUT direction ($38 \pm 9\%$ increase, $P = 0.0013$, $n = 7$; Fig. 1, I-K).

The readout of sequence sensitivity was not just electrical, but also chemical, as local dendritic Ca^{++} signals also showed strong direction and velocity sensitivity (Fig. 2A-C). Peak local dendritic Ca^{++} signals were always larger in the IN vs OUT direction ($48 \pm 13\%$ increase, $P = 0.0047$; $n = 6$), and varied significantly with input velocity ($P = 0.0431$ and 0.0194 for IN and OUT respectively, ANOVA test), with the optimal velocity for direction sensitivity being similar to that of the somatic EPSP (2.0 ± 0.4 $\mu\text{m}/\mu\text{s}$; Fig. 2, D and E). The spatial Ca^{++} profiles along the dendrite were also different for IN and OUT directions (Fig. 2, C and F).

What are the mechanisms underlying direction and velocity sensitivity in single dendrites? First, the somatic voltage responses to input sequences were markedly supralinear (Fig. 3A; peak voltage $223 \pm 9\%$ of the arithmetic sum, $P < 0.0001$) and voltage-dependent (fig. S3B-D). Unlike conventional all-or-none dendritic spikes (13, 15), this supralinearity did not exhibit a clear somatic threshold or a step increase in dV/dt (fig. S3A), but rather developed gradually with increasing numbers of activated synapses (Fig. 3B). Next, we investigated the role of NMDA receptors (NMDAR), given their important contribution to dendritic nonlinearities in pyramidal cells (13, 16–18, 24). The NMDAR blocker D-AP5 abolished EPSP supralinearity ($103 \pm 3\%$ of linear sum, $P = 0.336$, $n = 8$; Fig. 3C), direction sensitivity (Fig. 3D-F; IN response $8 \pm 3\%$ larger than the OUT response; peak voltage difference of 0.4 ± 0.4 mV, significantly smaller than control, $P = 0.0011$) and velocity sensitivity (Fig. 3G), as well as any detectable dendritic calcium signals (fig. S4).

To probe the biophysical basis of direction sensitivity we used a detailed compartmental model of layer 2/3 pyramidal cells. Direction sensitivity could be reproduced with a relatively simple model, with passive dendrites and synapses containing AMPA and NMDA conductances (fig. S5). The simulations showed that the mechanism of direction sensitivity in single dendrites results from the interaction of two factors: the gradient of input impedance along the dendrite combined with the highly non-linear voltage-dependence of the NMDAR conductance. This leads to asymmetric recruitment of NMDA receptors when

activating synapses in different sequences, such that sequences starting from regions of high input impedance (the tip of the dendrite) generate more initial local depolarization and hence more cumulative NMDAR activation. The differential recruitment of non-linearities between proximal and distal synapses was verified with calcium imaging and glutamate uncaging in different regions of the same dendrite (fig. S6). The relative simplicity of this mechanism suggests that it should be general; indeed, the vast majority of dendrites across a wide range of morphologies exhibit sufficient impedance gradients to permit substantial direction sensitivity (fig. S7). We confirmed this experimentally by showing that neurons with very different morphologies – layer 5 pyramidal cells and hippocampal dentate gyrus granule cells – also exhibit robust direction sensitivity (fig. S8). This is especially relevant in the latter, as they receive layered input from the entorhinal cortex, with the lateral cortex projecting distally and the medial cortex proximally.

Can this mechanism be used to discriminate more complex patterns of input? We first assessed discrimination of random patterns by a single dendrite (Fig. 4, A and B). In both the model (Fig. 4, A and C) and experiments (Fig. 4, B and D), different sequences of the same inputs produced a wide range of somatic EPSP amplitudes ($P < 0.05$, ANOVA on each cell; mean difference between experimentally tested patterns = $2.3 \text{ mV} \pm 0.22 \text{ mV}$; $39 \pm 8 \%$ of all possible sequence comparisons were significant, $P < 0.05$, t-test with Bonferroni correction; $n = 7$), which was dependent on NMDA receptor activation (mean difference between patterns = $0.69 \pm 0.13 \text{ mV}$, $P < 0.0001$; Fig. 4, C and D). This is because synapses at different locations along the dendrite differentially influence each other depending on their relative timing. The broad EPSP amplitude distribution reflects the discriminability of different sequences, with the likelihood of discriminating any two sequences ($> 1 \text{ mV}$ peak difference) being 40%. This distribution was insensitive to feedback inhibition, but shifted to a smaller mean with the same variance with feedforward inhibition, suggesting that the same NMDA-dependent mechanism can represent the combined spatiotemporal sequence of excitation and inhibition (fig. S9). While synaptic interactions are maximal if synapses are on the same dendrite, voltage can spread to other parts of the dendritic tree, the extent depending on the tree geometry and biophysical properties. We therefore examined whether neurons could also discriminate patterns of input delivered to multiple dendrites. By activating multiple spines across several dendrites, it was possible to discriminate the response to activation of different sequences of distributed input patterns (Fig. 4, F and H; mean EPSP peak difference = $4.0 \pm 1.3 \text{ mV}$, $P = 0.0036$, $n = 5$; tested sequences had no net directionality, see fig. S10C, D and supporting online material text). This discriminability was fully reproduced by our model (Fig. 4, E and G), and was severely reduced upon hyperpolarization ($0.5 \pm 0.4 \text{ mV}$, $P = 0.0075$; Fig. 4H) and by block of NMDA receptors (Fig. 4G). We further extended these results using the model, where input sequences could be reliably discriminated over a wide range of spatial input distributions (fig. S10A, B), and with electrical synaptic stimulation (fig. S11)

We have shown experimentally that single cortical pyramidal cell dendrites can encode the temporal sequence of synaptic input. The underlying mechanism relies on the interplay between non-linear activation of synaptic NMDA receptors and the impedance gradient along dendritic branches, two fundamental biophysical features common to most neurons in the brain. The active, regenerative nature of this mechanism contrasts with the classic

passive directional selectivity proposed by Wilfrid Rall (19), which requires electrotonically very long dendrites. Instead, the NMDA-dependent mechanism produced strong sensitivity to the direction of synaptic input even in short pyramidal cell dendrites, making it more general and sensitive to synaptic input, robust against timing jitter, and further enhanced and tunable by depolarization (such as in network UP states (25, 26)). Different input sequences also lead to differential dendritic Ca^{++} signals, raising the possibility that they will engage plasticity mechanisms to different extents (27, 28). The large dynamic range conferred by NMDA receptor activation allowed for high discriminability of multiple temporal sequences both when inputs were on the same dendrite or dispersed over the dendritic tree. This further extends the range of computational and plasticity mechanisms that have recently been described in dendrites (10, 13, 15–18, 29, 30). In particular, this sensitivity to temporal input sequences may be relevant for detecting features of sensory stimuli, and for encoding the speed and directionality of waves of activity propagating in the cortex (31–33). It is also especially relevant for circuits with layered input such as the hippocampus, where this mechanism could be used by dentate gyrus granule cells to directly detect the sequence of entorhinal cortex activation. These computations are conventionally thought to be implemented at the level of neural populations, and thus our results represent a demonstration of the power of dendrites for solving computational problems in the brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Single dendrites are sensitive to the direction and velocity of synaptic input patterns. (A) Layer 2/3 pyramidal cell filled with Alexa 594; yellow box indicates the selected dendrite. (B) Uncaging spots (yellow) along the selected dendrite. (C) Average individual uncaging responses at the soma. (D) Somatic responses to IN and OUT directions at 2.3 $\mu\text{m}/\text{ms}$ (average in bold). (E) Comparing peak amplitudes for IN and OUT sequences at the optimal velocity for direction selectivity (green circle: example shown in D). (F) Direction-selective responses at different velocities. (G) Relationship between peak voltage and input velocity (values normalized to the maximum response in the IN direction for each cell, $n =$

15). **(H)** Relationship between direction selectivity and input velocity ($n = 15$). **(I)** Direction selectivity of spike probability; population data shown in **J** ($P = 0.0013$, $n = 7$). **(K)** Relationship between spike probability and velocity ($n = 7$, average of both directions).

Fig. 2. Dendritic calcium influx is direction and velocity sensitive.

(A) Basal dendrite of a layer 2/3 pyramidal neuron; uncaging locations indicated in yellow, linescan profile used for Ca^{++} imaging in red. (B) Spatiotemporal profile of Ca^{++} signals triggered by IN and OUT input patterns at two different input velocities. (C) 3D plot of the data in B (2.3 $\mu\text{m}/\text{ms}$). (D) Relationship between Ca^{++} signals and input velocity ($\Delta F/F$ values normalized to the mean $\Delta F/F$ of all velocities in the IN direction of each cell). (E) Relationship between direction selectivity of Ca^{++} signals and input velocity. (F) Average

spatial profile of the integrated Ca^{++} transient across the dendrite ($n = 5$ cells; lines indicate s.e.m.; bar indicates region of statistical significance).

Fig. 3. NMDA receptor activation is required for robust velocity and direction coding.

(A) Somatic responses to IN and OUT sequences (coloured traces) and linear sum of the individual synaptic responses (gray traces). (B) Left, activating more synapses (1-7) produces EPSPs that are progressively larger than the linear sum of the individual responses. Right, summary plot of 8 cells. (C) As in A, but in the presence of AP5, which blocks the supralinearity. (D) Velocity and direction sensitivity is abolished in AP5. (E) Comparing peak amplitudes for IN and OUT sequences at the optimal velocity for direction selectivity (green circle: example shown in C; cf. Fig. 1E). (F) Summary plot of direction selectivity vs velocity (cf. Fig. 1G). (G) Reduced velocity sensitivity in AP5 (EPSPs normalized to the average IN maximum in control cells in the absence of APV; cf. Fig. 1H).

Fig. 4. Dendritic discrimination of complex input sequences.

(A) Random patterns of 9 inputs were generated and sorted by their directionality index (see Materials and Methods). Four sample patterns (numbers showing activation order) and corresponding directionality index are shown. Patterns were played into a single dendrite in a pyramidal cell model or B, experimentally in single pyramidal cell dendrites; peak response is different for different patterns, and responses become similar with hyperpolarization (or without NMDA receptors in model). (C) Pattern separability (measured by somatic peak EPSP distribution) was much greater with NMDA conductances

present. **(D)** Peak EPSP voltage measured experimentally depends on input pattern similarly to the model (panel **C**); stars indicate significant differences between EPSP peaks for the example shown; hyperpolarization reduces the ability to discriminate different patterns. **(E, F)** Layer 2/3 pyramidal cell with uncaging spots (yellow) randomly distributed across 8 dendrites. Somatic responses to sequential uncaging in forward (red traces) or reverse sequence (blue traces; average in bold) are different at resting potential both experimentally **(H)** and in a compartmental model of the same neuron **(G)**. Responses to the two sequences become similar upon hyperpolarization **(H)** or by removing synaptic NMDA receptors **(G)**.