Generation of directional selectivity by individual thin dendrites in neocortical pyramidal neurons.

NN Brief Communication.

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Patterned 2-photon glutamate uncaging and local GABA iontophoresis were used to test, in brain slices, whether basal and oblique dendrites possess the biophysical machinery to contribute to the directional selectivity exhibited by many sensory neocortical neurons. On average, Distal-to-Proximal (DP) sequences of glutamate stimuli along individual dendrites produced ~1.5-fold larger responses than the same stimuli in reverse order (PD). Proximal inhibition consistent with spatially-offset receptive subfields, preceding PD but following DP sequences, enhanced directionality to ~2.1-fold.

There is compelling evidence that many neurons in the brain are tuned to detect stimuli moving in a particular direction. Prominent directionality is seen in both visual[1] and somatosensory cortices[2]. In auditory cortex, many neurons respond differentially to increasing- versus decreasing-frequency tone sweeps[3] or to moving sound sources.

A striking feature of neocortical directional selectivity (and other responses) is the relatively long apparent duration of influence of some sensory inputs. In rat barrel cortex, for example, most neurons show directionally-selective responses to stimuli sweeping across the whisker field over 40-280 milliseconds[2]. In directional visual cortical neurons, multiple excitatory and inhibitory receptive subfields or 'subunits' influence action potential (AP) firing, with position-dependent delays ranging from ~40-300 milliseconds[4-6].The causes of these delays, and the directionality, remain unproven.

Dendrites have the machinery to function as non-linear subunits[7, 8] and can be important sources of lagged input influences[8] – and indeed directionality, for example, in retinal starburst amacrine cells[9] and in fly visual motion sensing neurons[10]. In neocortex, spiny sub-micron diameter basal and apical oblique dendrites, which receive the majority of inputs into pyramidal neurons, have high densities of voltage-dependent NMDA receptors (NMDARs). Brief synaptic inputs can elicit local dendritic NMDA spikes[11]. More intense brief stimuli can produce NMDA plateau potentials lasting hundreds of milliseconds[8]. NMDA spike/plateaus exhibit two other features highly relevant to directionality: (i) a strong increase in both amplitude and glutamate threshold from distal to proximal along a dendrite, paralleling the gradient in local input conductance, and (ii) *co-operativity*: depolarisation reduces their glutamate threshold[8]. For example, during a distal NMDA plateau potential, it is easier to trigger a more proximal NMDA spike in the same dendrite (Suppl. Fig. 1a). Thus a *brief* distal input can have strong effects on output, *delayed by several hundred milliseconds*. In models, a graded form of co-operativity can lead to directional selectivity[8] matching the time-courses commonly seen *in vivo*. By contrast, the spread of different passive cable delays or EPSP rise-times available to generate directionality[12] is only ~20 ms − an order of magnitude too brief.

To test experimentally whether basal and oblique dendrites have the biophysical capability of generating directional selectivity over the time scales seen *in vivo*, I used a combination of whole-cell recording from layer 5 pyramidal neurons in rat sensory neocortical brain slices, 2-photon microscopy, patterned 2-photon glutamate uncaging and local GABA iontophoresis (Suppl. A)[8].

Distal-to-proximal ('DP') sequences of 0.5-1 ms focal glutamate pulses, uncaged along a single dendrite at 90 spots near spines, generally produced bigger responses than exactly the same stimuli in reverse order ('PD'; Fig. 1; sequence durations mostly 135-180 ms). If the DP response was already 'saturating', close to the expected size of a proximal NMDA spike from that dendritic location[8], increasing the strength of some or all stimuli could reduce directionality, by increasing the PD more than the DP response (Figs. 1a3, b3). Similarly, in several cases where the PD response was close to the expected size of a proximal NMDA spike, there was little or no directionality (e.g. Fig. 2a2, b2). Each dendritic segment tested was capable of generating a range of directionalities, varying with the stimulus pattern, strength, sequence duration, and focal plane (Fig. 1d). The mean DP/PD directionality ratio found was 1.54 ± 0.50 (s.d.; >1 with $p<10^{-10}$; 129 different patterns, 23 dendrites, responses below AP threshold, Fig. 1c). When DP stimuli produced somatic APs, the underlying responses showed greater directionality, on average, with a mean DP/PD of 2.33 ± 1.10 (Fig.1c, 15 patterns, p=0.002; Suppl. A; PD responses all below AP threshold). For responses below AP threshold in both directions, the *maximum* directionality ratio obtained from each dendrite stimulated over >100 µm had a mean value of 2.11 ± 0.51 (range 1.28 to 3.40; 21 dendrites). Individual basal and oblique dendrites can therefore generate substantially directional responses to sequences of purely excitatory inputs lasting ~100 ms or more.

Inhibitory receptive subfields are often 'sensori-topically' offset from excitatory subfields[1-6]. A dendrite's directionality could be enhanced by proximal inhibition from a receptive subfield on the preferred-direction side of its excitatory receptive subfield: pre-emptive inhibition would be produced by anti-preferred-direction stimuli moving through the inhibitory subfield before reaching the excitatory subfield. To test the extent to which single dendrite directionality could be improved by this mechanism, a double-barrelled GABA iontophoresis electrode was advanced to within ~1 µm of the proximal part of selected dendrites (Fig. 2a, b)[8]. A local iontophoretic IPSP preceding PD sequences (IPD) but following DP sequences (DPI) increased directionality by 1.5 ± 0.74-fold, on average, to a mean DP(I)/IPD ratio of 2.15 ± 1.03 (Fig. 2c; 37 patterns, 11 dendrites, $p=3\times10^{-5}$). Single basal and oblique dendrites therefore contain enough GABA conductance for their directionality to be enhanced substantially by inhibition timed to mimic a sensori-topically offset inhibitory subfield.

The *maximum* directionality ratio obtained (with or without inhibition) from each dendrite stimulated over >100 µm had a mean value of 2.49 ± 1.09 (range 1.28 to 6.54; 21 dendrites). Both the above mechanisms could therefore make important contributions to directionality *in vivo* if a sensory surface was mapped sensori-topically onto particular dendrites, so that stimuli moving in the preferred direction across the receptive field evoked first distal, then middle, then proximal excitatory inputs, and finally, proximal inhibitory inputs. Shorter-range local inhibitory-excitatory interactions could also contribute (Suppl. Fig. 1d). In support of these mechanisms, has been shown *in vivo* (in visual cortex) that NMDARs contribute prominently to responses in the preferred direction, that GABAergic inhibition reduces NMDAR responses in the non-preferred direction, and that GABA blockers can reduce directionality[13].

Single dendrite directionality occurred over a substantial range of response amplitudes in brain slices (Fig. 1c). Input conductances of neurons in behaving animals can be many times higher than in brain slices, so responses from individual dendrites can be scaled down several-fold *in vivo*, for example, due to shunting by perisomatic inhibition. In simulations (Suppl. Fig. 2), directionality was seen with DP responses as small as ~1 mV at the soma. Sparse coding during natural stimuli may

activate only a subset of dendrites at any given time. Axon arbors and receptive fields (mapped onto the cortical surface) are typically much wider than dendritic arbors, therefore dendrites on different sides of a neuron could be 'wired up' to have similar directionalities (Suppl. Fig. 3). Responses from multiple directional dendrites will sum at the soma, where their aggregate directionality can be enhanced by AP threshold[14].

Simulations showed that single dendrites were able to generate directional responses to random dot sensory stimuli (Suppl. Fig. 4) and, as a consequence, exhibited tilted spatio-temporal receptive fields (Fig. 2d). The latter are often associated with directionality *in vivo*[2-6]. This is a key result, linking directionality in brain slices and at the systems level. Another link is the recent discovery of orientation/direction-sensitive Ca^{2+} 'hotspots' in basal and oblique dendrites of hyperpolarised visual cortical layer 2/3 neurons in anaesthetised mice[15]. The amplitudes, time courses and spatial extents of the hotspot Ca^{2+} transients are consistent with those seen in dendritic shafts following clustered multi-synapse inputs sub-threshold for NMDA spikes[7], but strong enough to generate NMDA spikes at more depolarised potentials. Further *in vivo* experiments are needed to test for dendritic directional selectivity using a wider range of stimuli and conditions that do not suppress NMDAR conductances (no hyperpolarisation, minimal anaesthesia). Directionally-selective basal and oblique dendrites would be expected to exhibit directionally-selective Ca^{2+} transients in excess of any AP-related transients[7, 8, 11].

Many neocortical neurons exhibit directional selectivity, but the underlying mechanisms are still not rigorously understood (Suppl. H). The data presented here demonstrate that basal and apical oblique dendrites possess ample biophysical machinery to contribute to directionally-selective responses over the 50-200 ms time scale commonly seen *in vivo*.

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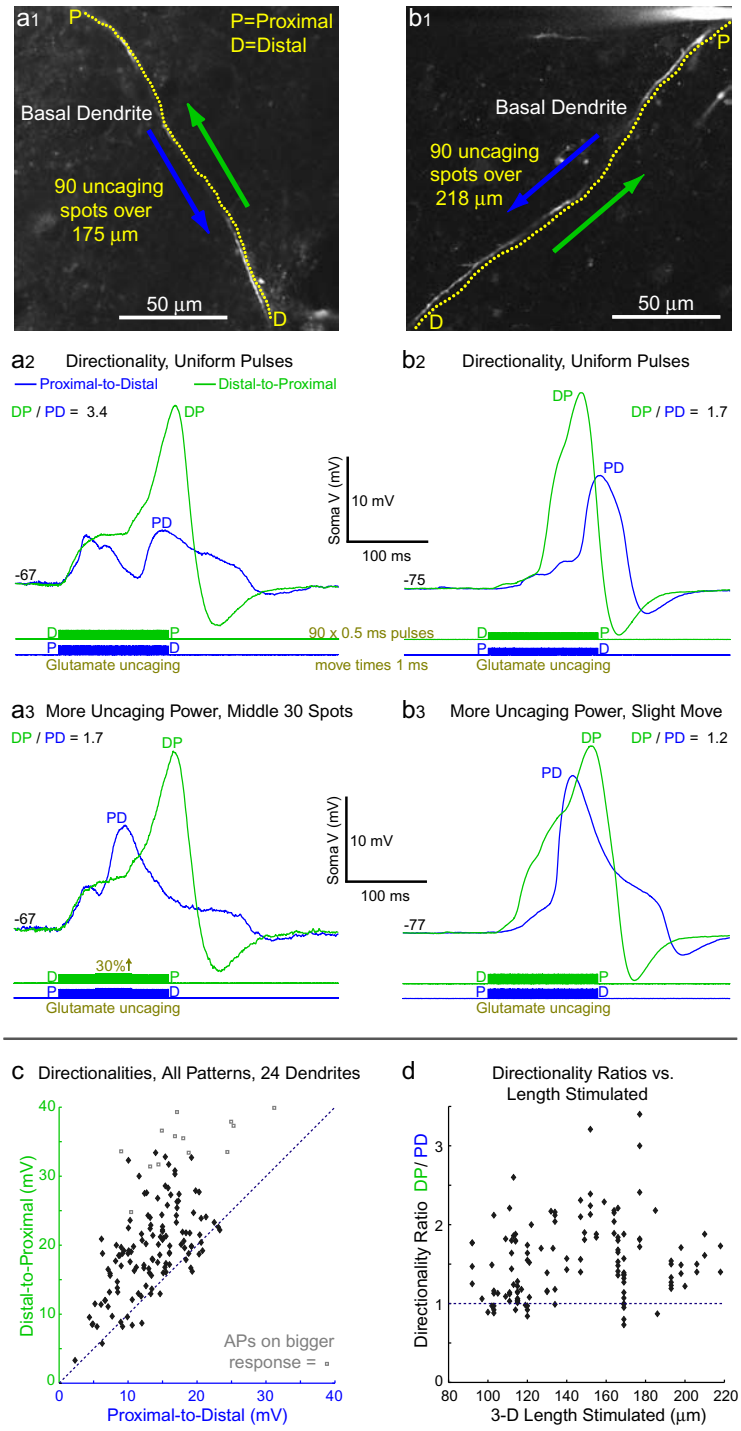

Fig. 1. Directionally selective responses to sequences of glutamate stimuli along individual basal and apical oblique dendrites.
a, **b**. Basal dendrites from different neurons, stimulated by sequential glutamate uncaging at yellow spots (0.5 ms per spot; 1 ms move time to next spot). Membrane potential responses at cell body.
a2, **b2**. Uniform uncaging pulses. Directionality ratio = peak DP/ peak PD response.
a3. ~1.3-fold increase in glutamate uncaged at spots 31-60;
b3. All pulses increased (uncaging x ~2.7, with spots moved <2 µm further from dendrite).
c. Peak DP vs. peak PD responses for 144 different stimulus patterns (19 basal, 4 oblique dendrites).
d. Spread of directionalities found for each dendritic segment tested.

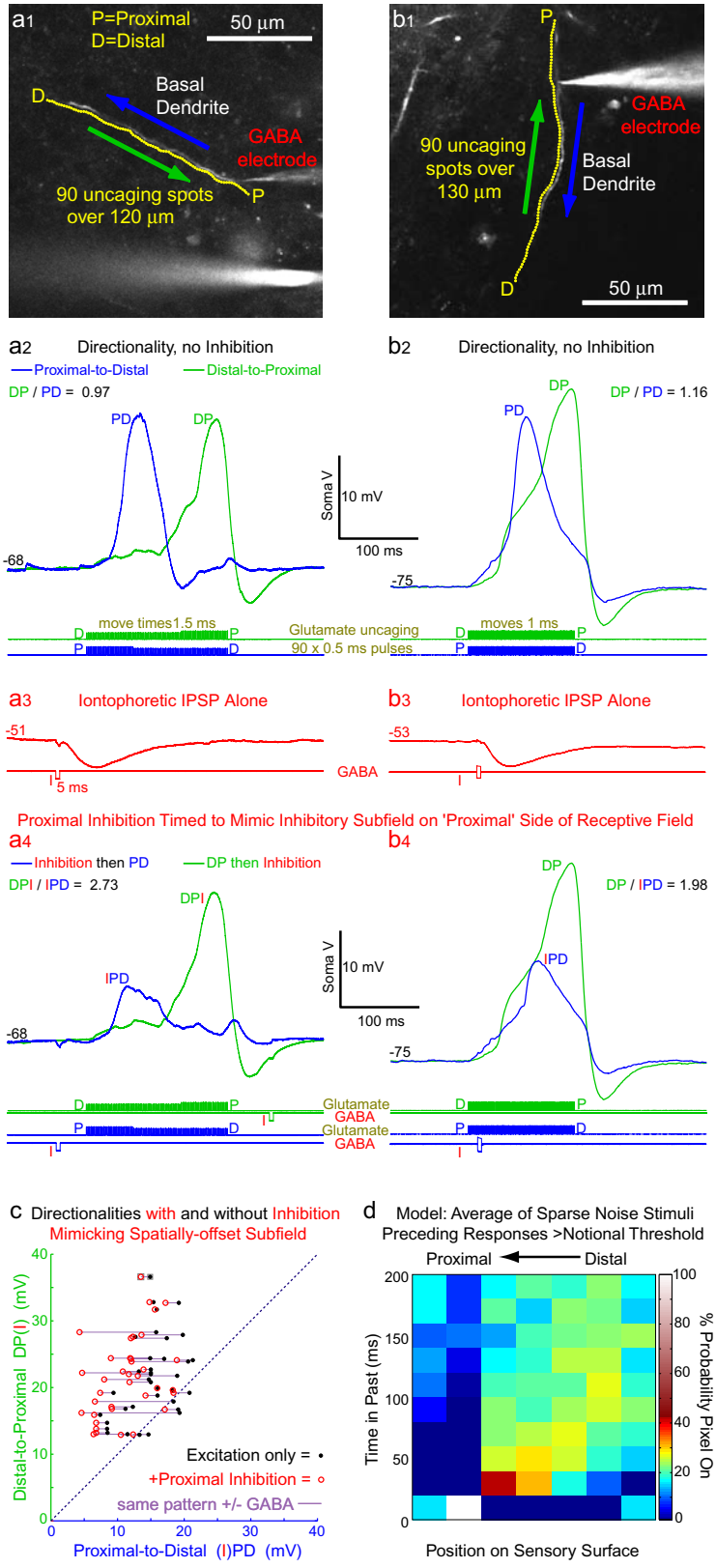

Fig. 2. Directionality enhanced by proximal inhibition, timed to mimic inhibitory receptive subfield on preferred-direction side of excitatory subfield (Suppl. Fig. 3c).

a, **b**. Basal dendrites from different neurons, with proximal GABA iontophoresis electrode (fluorescein in one barrel).
a2, **b2**. Responses to excitatory sequences alone.
a3, **b3**. IPSP alone (depolarised); 5 ms GABA pulse.
a4, **b4**. Proximal IPSP reduced a following PD response (IPD), but peak DP response unaffected by a following IPSP (DPI in **a4**). For all patterns tested, peak DP response was equivalent to peak DPI response (IPSP always occurred after peak).
b3, **b4**. Positive GABA iontophoresed from 1st barrel (pH 2.8), negative GABA from 2nd (pH 11.5).
Same scale bars a2-4, b2-4.

c. *Population data*; 37 glutamate uncaging patterns compared, with and without proximal inhibition timed to mimic sensori-topically offset inhibition (8 basal, 3 oblique dendrites). Solid black = excitation only DP vs. PD, hollow red = DP(I) vs. IPD; each lilac horizontal line connects the pair of points corresponding to a particular excitatory pattern.

d. *Simulated single dendrite can generate spatio-temporal receptive field tilted in time in its preferred direction*, in response to sparse 1-D noise (random dot) stimuli.
Model (Suppl. Fig. 4): 1-D sensory surface (7 binary pixels) projected sensori-topic maps of glutamate and spatially- or temporally-offset GABA inputs onto a single basal dendrite in a compartmental model of a reconstructed layer 5 pyramidal neuron.
Space-time plot:
average of stimuli preceding larger responses, which crossed a notional threshold 6.5 mV above rest; the majority of these involved chance right-to-left (DP) overall trends of 'on' pixels over time (forward direction of time is downwards); colours indicate average probability that a particular sensory pixel was 'on' at a given time before threshold crossings.