The Time Window for Generation of Dendritic Spikes by Coincidence of Action Potentials and EPSPs is Layer Specific in Somatosensory Cortex

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Abstract

The precise timing of events in the brain has consequences for intracellular processes, synaptic plasticity, integration and network behaviour. Pyramidal neurons, the most widespread excitatory neuron of the neocortex have multiple spike initiation zones, which interact via dendritic and somatic spikes actively propagating in all directions within the dendritic tree. For these neurons, therefore, both the location and timing of synaptic inputs are critical. The time window for which the backpropagating action potential can influence dendritic spike generation has been extensively studied in layer 5 neocortical pyramidal neurons of rat somatosensory cortex. Here, we re-examine this coincidence detection window for pyramidal cell types across the rat somatosensory cortex in layers 2/3, 5 and 6. We find that the time-window for optimal interaction is widest and shifted in layer 5 pyramidal neurons relative to cells in layers 6 and 2/3. Inputs arriving at the same time and locations will therefore differentially affect spike-timing dependent processes in the different classes of pyramidal neurons.

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Introduction

Timing is a central concept in cortical function. At the network level, information is encoded in the spiking of neurons and there is much debate about the level of precision that is important [1,2]. At the cellular level important processes have been hypothesized to be dependent on the timing of input and output such as spike-timing dependent plasticity "STDP" [3]. The notion of timing is particularly important in pyramidal neurons, the principle excitatory neurons of the neocortex. With their elongated dendritic trees spanning several cortical layers they can independently process different classes of synaptic input within the same neuron [4]. The synaptic inputs that can contribute to the input/output function for each pyramidal neuronal type is determined by the specific layers spanned by their dendritic trees and the laminar profile of activity throughout the cortex which is specific to each pyramidal cell class.

Recently it has become clear that the input/output function of pyramidal neurons is also profoundly influenced by the computational properties of the dendritic tree itself [5,6,7]. The dendrites of all cortical pyramidal neurons have been shown to have Na^+, K^+ and Ca^{2+} channels [8,9,10] that contribute to the active propagation of signals and to the generation of local spikes [11,12,13,14,15]. The final output from the pyramidal neurons is the generation of action potentials in the axon initial segment [16,17], but the computational power of the pyramidal neuron is greatly enhanced by the interaction of these APs with the sub-regions of the dendritic tree that generate local spikes [5,18].

Neocortical pyramidal neurons have a spike initiation zone in the apical dendrite [10,11,12,14,19]. The dendritic spike generated in this location is composed of an initial fast component that has been shown to be mediated by voltage-sensitive Na^+ channels followed by a slower Ca^{2+}-dependent component [10,20]. In L5 pyramidal neurons the 2nd component is particularly pronounced and typically drives the soma to fire a burst of APs [21,22,23]. In L2/3 and L6 neurons, the 2nd component contributes to further somatic depolarization but does not necessarily trigger axonal firing. The dendritic and axonal spike initiation zones are coupled by the influence of the backpropagating action potential (bAP) that lowers the threshold for the initiation of the dendritic spike. This phenomenon, known as "backpropagation activated calcium spike firing" (BAC firing) [18] is strongly dependent on the relative timing of input to the proximal and distal initiation zones. The generation of a dendritic spike under these circumstances represents a mechanism for pyramidal neurons to detect the coincidence of proximal and distal input to the dendritic tree.

In this paper, we investigated the time window of coincidence detection in L2/3, L5 and L6 pyramidal neurons of the somatosensory cortex in rats using simultaneous dual patch-clamp recordings from the cell body and apical dendrite and we show that all three types of pyramidal neurons have a specific time window for somato-dendritic spike interaction.

Materials and Methods

The study was approved by the Veterinary office of the Canton Bern, Switzerland, permission number 90/08.

Slice preparation

Experiments were performed in somatosensory neocortical slices from postnatal day 28–49 Wistar rats (n = 26) using procedures described previously [9]. Briefly, rats were decapitated and the brain was quickly removed into cold (0–4°C), oxygenated physiological solution containing the following (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 1 $MgCl_2$, 2 $CaCl_2$, and 25 glucose; pH 7.4. Parasagittal slices, 300 µm thick, were cut from the tissue block with a vibratome (Microm) and kept at 37°C for 30 min and then at room temperature until use.

Electrophysiology

All experiments were performed at 32.0±0.5°C. Single pyramidal neurons were identified using infrared Dodt gradient contrast or oblique illumination and a CCD camera (CoolSnap ES, Roper Scientific). Slices were perfused with the same extracellular solution mentioned above. Recording pipettes were filled with intracellular solution containing the following: 130 mM K-gluconate, 5 mM KCl, 30 mM HEPES, 10 mM Phospho-kreatine, 4 mM MgATP, and 0.3 mM GTP; pH 7.3. The somatic pipette contained in addition 10–50 µM Alexa 594 (Invitrogen), 100 µM Oregon Green BAPTA-1 (OGB-1, Invitrogen), and 0.2% Biocytin (Sigma). Dual whole-cell voltage recordings were performed from the soma and dendrites (6–10 and 20–40 MΩ pipette resistances respectively) using Axoclamp 2A (Axon Instruments) and Dagan BVC-700A amplifiers (Dagan Corporation). Data were acquired with an ITC-18 board (Instrutech) and custom software written for the Igor environment (Wavemetrics). After recordings, slices were fixed and stained as described previously [14] for later reconstruction of the investigated neurons. Data analysis was performed using Igor software (Wavemetrics) and Excel (Microsoft).
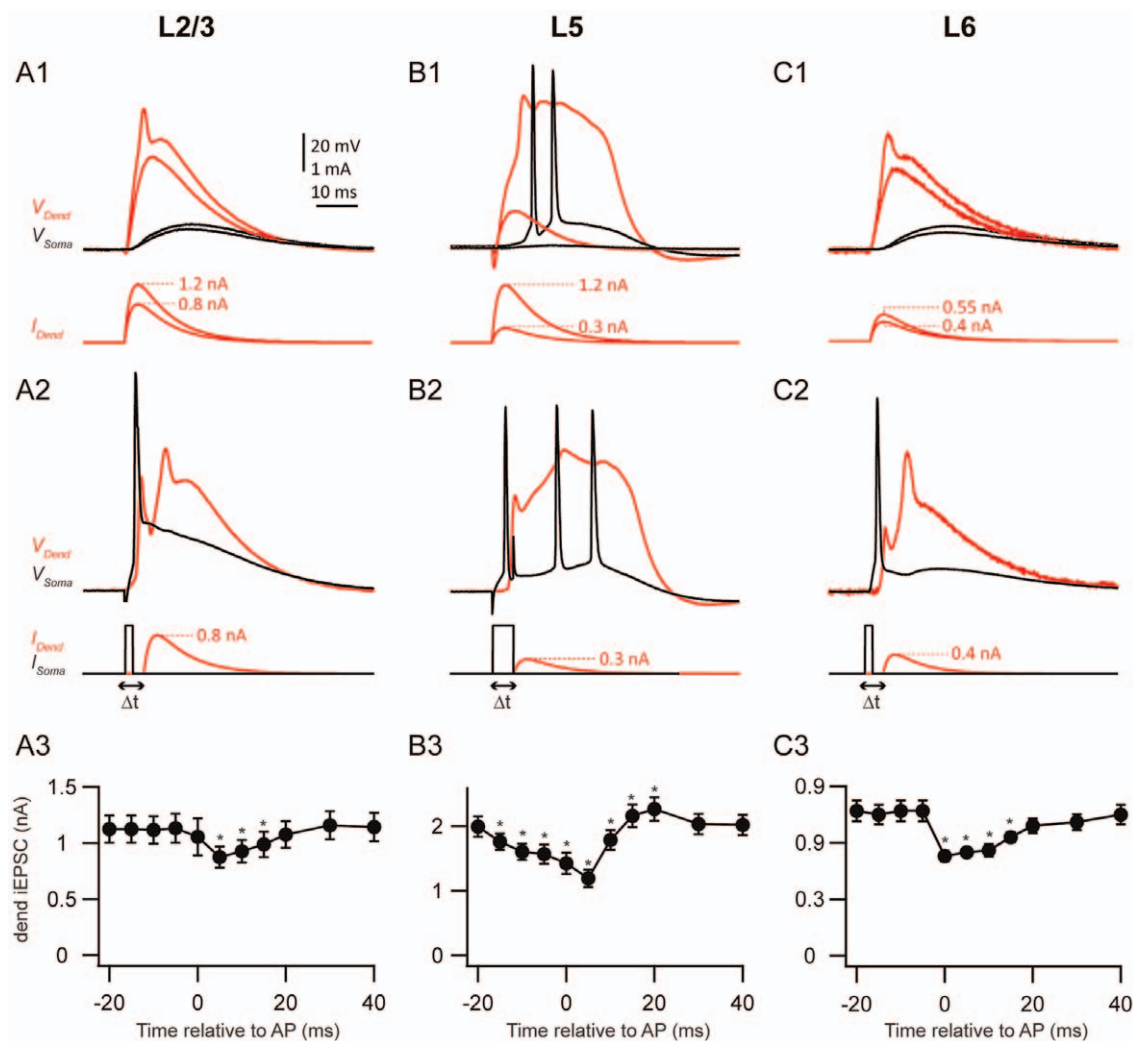
Figure 1. Somato-dendritic coupling for pyramidal neurons in different layers of the neocortex. Cell types are arranged in columns (A, L2/3; B, L5; C, L6). Row 1) Injection of EPSC-waveform current (lower panels) into the apical dendrite below and above threshold for the generation of a dendritic spike (red traces) which propagated to the soma (black traces). Row 2) Sub-threshold current injection from row 1 5 ms after an axonal AP elicited by somatic current injection (black traces in bottom panels). Row 3) Average threshold current at the dendritic electrode for the generation of a dendritic spike in the presence of a backpropagating AP for various time intervals (Δt). Values are presented as mean with standard error. Asterisks indicate significant deviation from baseline (threshold determined in row 1) tested with the Holm-Šidák.
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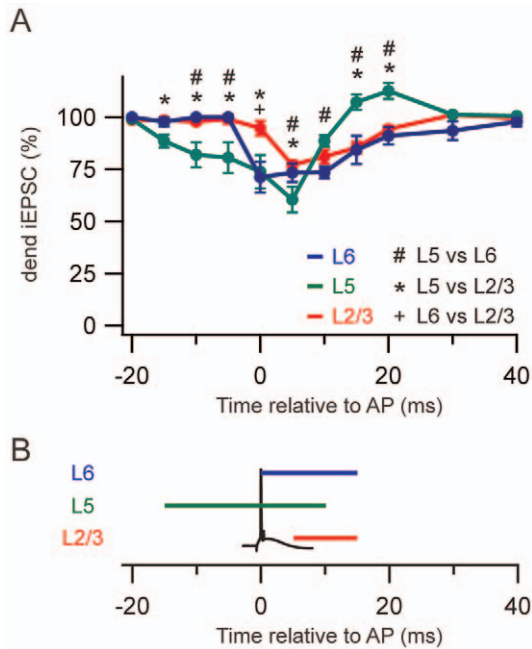
Figure 2. Time windows for AP/EPSP coincidence detection. A) Average normalized dendritic spike thresholds for L6 (blue), L5 (green) and L2/3 (red) pyramidal neurons for different dendritic versus somatic times. Statistical difference is indicated for comparisons between cell types (#, L5 vs. L6; *, L5 vs. L2/3; +, L6 vs. L2/3) using Holm-Šidák (p<0.05). B) Time windows for coincidence detection showing intervals where the threshold was significantly lower than baseline (Holm-Šidák).
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The dendritic recording was made at least 20 min after establishing the somatic recording to allow intracellular spread of the dyes from the soma. Dendrites were targeted with infrared-scanning gradient contrast (IR-SGC) [24] or an overlay of the separately acquired epifluorescence image with an obliquely illuminated IR image using custom software. We used a Leica TCS SP2 confocal scanner or an Olympus BX-51WI microscope with a 60X objective. Dendritic spikes were elicited with direct dendritic current injection by a pipette placed in the spike initiation zone [10,19,23]. The regenerative component of the dendritic AP was calculated by subtracting the predicted non-regenerative component (using the previous sub-threshold traces) from the suprathreshold dendritic recording [10,19]. We found no evidence that the precise location of current injection (inside the initiation zone) alters the timing of coincidence detection.

Statistics

All statistics were calculated using commercial software (SigmaStat, Systat Software Inc.; San Jose, CA). If not otherwise indicated values represent means ± s.e.m. All data were tested for normality and equal variance. Statistical comparisons of spike thresholds were performed using 2-way repeated measurement ANOVA to test for effects of time versus baseline (Fig. 1) or for time versus cell type (Fig. 2). A significance level of 5% was chosen.

Results

The aim of this study was to investigate the coupling of the tuft dendrite with the cell body across the pyramidal cell classes of the cortex. The coupling was assessed in terms of the coincidence time window during which a backpropagating AP influenced the threshold for the generation of a dendritic spike. We carried out dual whole-cell patch clamp recordings from the dendrites and somata in layers 6, 5 b (thick-tufted cells) & 2/3 in the somatosensory neocortex of rats (see Table 1 for detailed experimental parameters). A transient current resembling a compound EPSC (EPSC$_{inj}$) was injected into the dendrite (Fig. 1, upper panels; for further details see Methods). Axonal APs were evoked with 2-ms somatic current injection just above the AP threshold (Fig. 1B, middle panels).

We first determined the threshold for a dendritic spike using only dendritic current injection (Fig. 1, upper panels). The threshold for dendritic spikes was lowest in L6 pyramidal neurons (avg 770±192 pA, n = 5; Fig. 1C1; Table 1), highest in L5 neurons (avg 2011±553 pA, n = 9; Fig. 1B1) and intermediate in L2/3 neurons (avg 1142±419 pA, n = 12; Fig. 1A1). However, this threshold decreased when the cell fired an axonal AP 5 ms before the dendritic spike (Fig. 1, middle panels; Table 1). We assessed the reduction in threshold for time intervals (Δt) between −20 and 40 ms (Fig. 1, lower panels). One way repeated measures ANOVA for each group of pyramidal neurons revealed that there was a significant effect of time on the threshold for dendritic spike generation (L2/3: F17 = 43, L5: F8 = 11.91, L6: F6 = 10.08, p<0.001 for all layers)

To compare the time windows for somato-dendritic coupling between the different pyramidal cell classes we normalized the values at the different Δt's to the threshold for generating a dendritic spike without an axonal AP (Fig. 2A). 2-way repeated measurement ANOVA revealed that there was a significant effect of time (F17 = 27.17, p<0.001), no significant effect of layers (F2 = 1.13, p = 0.33) but a significant effect of the interaction between layers and time (F34 = 6.90, p<0.001). Post hoc test showed that the threshold reduction was significantly different for L5 pyramidal neurons compared to L6 and L2/3 for many time points, whereas L6 and L2/3 pyramidal neurons were only

Table 1. Experimental parameters and cell properties across pyramidal cell types.

	L2/3	L5	L6
Soma location, distance from pia (μm)	582±50	1093±111	1548±65
Dendritic patch location, distance from soma (μm)	238±45	699±102	399±52
Baseline threshold for dendritic spike (pA)	1142±419	2011±553	770±192
Threshold for dendritic spike combined with AP (pA)	858±350	1144±480	500±100
Average age of recorded rats (days post natal)	31±3	41±8	29±1
n	12	9	5

Values are given as means with standard deviations.
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different from each other at one time point. The presence of an AP had the greatest effect on L5 pyramidal neurons reducing the threshold by $41\pm7\%$. Furthermore, the coincidence detection time window for L5 was extended relative to L6 and L2/3 pyramidal neurons (Fig. 2B).

Discussion

In summary, we found that the coincidence timing curve for the initiation of dendritic spikes in L5 pyramidal neurons was wider than for L6 and for L2/3 pyramidal neurons. L6 and L2/3 pyramidal neurons exhibited similar coincidence detection windows to each other but were narrower than in L5 cells implying these cells require more precise synaptic inputs for this effect. The bAP had the greatest relative effect on dendritic spike generation in L5 neurons however the baseline threshold in L5 neurons was much larger than in L2/3 and L6 neurons (Table 1). Thus, the absolute dendritic spike threshold following a bAP was similar in all types of pyramidal neurons.

What are the implications of timing differences between pyramidal cell classes? We predict that processes in the dendritic tree which are influenced by the coupling of bAPs with local dendritic membrane potential such as STDP [25,26,27,28,29], local intrinsic excitability [30,31], and release of retrograde messengers [32] will follow similar timing rules to those shown here. This has already been shown in the case of STDP in L5 pyramidal neurons where the STDP timing corresponds to the time window for dendritic spike generation and is reversed [29,33] relative to the normal STDP time window in other neurons or for proximal inputs in pyramidal neurons [34,35,36,37,38,39].

The active and passive properties of L6, L2/3 and L5 pyramidal tuft dendrites are similar but not identical [9,10,11,12,14,40,41]. This presumably also explains why the timing of BAC firing is different from cell type to cell type. The fact that there is a negative component to the time window for L5 cells, for instance, might reflect the influence of EPSPs on back-propagating APs which has been observed in these neurons before [20,42]. Most importantly, when compared to L5 pyramidal neurons the amplitude and duration of the distal dendritic spike is reduced in L6 [10] and even more so in L2/3 neurons [19]. Under our conditions in vitro, L6 and L2/3 neurons therefore do not display bursts of axonal action potentials in response to an apical dendritic spike unlike the stereotypical bursting behaviour of L5 pyramidal neurons [21].

However, L2/3 pyramids have been shown to burst in vivo in the awake but not the anesthetized state [43,44]. Along the same lines, dendritic activity has been shown to be greatly elevated in L5 neurons in awake versus anesthetized rats [45,46]. This is consistent with the hypothesis that the awake state leads to an overall increase in dendritic excitability and shapes the output firing pattern in all pyramidal cell classes. The coupling of bAPs with dendritic input might therefore be even more crucial under physiologically relevant conditions.

The functional consequence of coincidence detection in pyramidal neurons depends also on the particular inputs that are associated. The cortical layer of the cell bodies and basal dendrites of pyramidal neurons determines the proximal input [47] and therefore determines the timing of APs propagating back into the tuft dendrite. The tuft dendrites of L2/3 and L5 both reach in to the uppermost layer of the cortex (L1) whereas L6 pyramidal neurons receive tuft input from upper L5 and L4. Since L1 receives long-range cortico-cortical feedback input, it has been suggested that L2/3 and L5 neurons can associate this input with the feed-forward and recurrent input in lower layers [18,48,49]. The cortex is also in constant dialogue with the thalamus via projections from L5 and L6 neurons and reciprocal connections from the thalamus to L4 and L1 [50,51,52]. Determining the functional implications of somato-dendritic coupling therefore awaits more precise data about the connectivity and timing of inputs to the different cortical layers under physiologically relevant conditions.

In conclusion, we have shown that all pyramidal neurons of the rat somatosensory cortex can associate inputs arriving at their distal and proximal dendritic trees in a limited time window that varies between cell classes. This suggests that pyramidal neurons operate in a similar way on the input which reaches the different cortical layers they are covering.

Author Contributions

Conceived and designed the experiments: DL MEL. Performed the experiments: DL MEL. Analyzed the data: DL MEL. Contributed reagents/materials/analysis tools: MEL. Wrote the paper: DL MEL.

References

1. Usrey WM (2002) The role of spike timing for thalamocortical processing. Curr Opin Neurobiol 12: 411–417.
2. Kumar A, Rotter S, Aertsen A (2010) Spiking activity propagation in neuronal networks: reconciling different perspectives on neural coding. Nat Rev Neurosci 11: 615–627.
3. Dan Y, Poo MM (2006) Spike timing-dependent plasticity: from synapse to perception. Physiol Rev 86: 1033–1048.
4. Spruston N (2008) Pyramidal neurons: dendritic structure and synaptic integration. NatRevNeurosci 9: 206–221.
5. London M, Häusser M (2005) Dendritic computation. AnnuRevNeurosci 28: 503–532.
6. Branco T, Clark BA, Hausser M (2010) Dendritic discrimination of temporal input sequences in cortical neurons. Science 329: 1671–1675.
7. Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons in vivo. Nature 464: 1307–1312.
8. Migliore M, Shepherd GM (2002) Emerging rules for the distributions of active dendritic conductances. NatRev Neurosci 3: 362–370.
9. Waters J, Larkum M, Sakmann B, Helmchen F (2003) Supralinear Ca 2+ influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons in vitro and in vivo. JNeurosci 23: 8558–8567.
10. Ledergerber D, Larkum ME (2010) Properties of layer 6 pyramidal neuron apical dendrites. J Neurosci 30: 13031–13044.
11. Kim HG, Connors BW (1993) Apical dendrites of the neocortex: correlation between sodium- and calcium-dependent spiking and pyramidal cell morphology. JNeurosci 13: 5301–5311.
12. Amitai Y, Friedman A, Connors BW, Gutnick MJ (1993) Regenerative activity in apical dendrites of pyramidal cells in neocortex. CerebCortex 3: 26–38.
13. Stuart GJ, Sakmann B (1994) Active propagation of somatic action-potentials into neocortical pyramidal cell dendrites. Nature 367: 69–72.
14. Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. Journal of Physiology-London 505: 605–616.
15. Schiller J, Major G, Koester HJ, Schiller Y (2000) NMDA spikes in basal dendrites of cortical pyramidal neurons. Nature 404: 285–289.
16. Stuart G, Spruston N, Sakmann B, HÑusser M (1997) Action potential initiation and backpropagation in neurons of the mammalian CNS. Trends in Neurosciences 20: 125–131.
17. Kole MH, Ilschner SU, Kampa BM, Williams SR, Ruben PC, et al. (2008) Action potential generation requires a high sodium channel density in the axon initial segment. Nat Neurosci 11: 178–186.
18. Larkum ME, Zhu JJ, Sakmann B (1999) A new cellular mechanism for coupling inputs arriving at different cortical layers. Nature 398: 338–341.
19. Larkum ME, Waters J, Sakmann B, Helmchen F (2007) Dendritic spikes in apical dendrites of neocortical layer 2/3 pyramidal neurons. J Neurosci 27: 8999–9008.
20. Larkum ME, Zhu JJ, Sakmann B (2001) Dendritic mechanisms underlying the coupling of the dendritic with the axonal action potential initiation zone of adult rat layer 5 pyramidal neurons. Journal of Physiology-London 533: 447–466.

21. Williams SR, Stuart GJ (1999) Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. Journal of Physiology-London 521: 467–482.
22. Schwindt P, Crill W (1999) Mechanisms underlying burst and regular spiking evoked by dendritic depolarization in layer 5 cortical pyramidal neurons. JNeurophysiol 81: 1341–1354.
23. Larkum ME, Zhu JJ (2002) Signaling of layer 1 and whisker-evoked Ca^{2+} and Na$^+$ action potentials in distal and terminal dendrites of rat neocortical pyramidal neurons in vitro and in vivo. Journal of Neuroscience 22: 6991–7005.
24. Nevian T, Sakmann B (2004) Single spine Ca2+ signals evoked by coincident EPSPs and backpropagating action potentials in spiny stellate cells of layer 4 in the juvenile rat somatosensory barrel cortex. JNeurosci 24: 1689–1699.
25. Bi GQ, Rubin J (2005) Timing in synaptic plasticity: from detection to integration. Trends Neurosci 28: 222–228.
26. Kampa B, Letzkus JJ, Stuart G (2006) Requirement of dendritic calcium spikes for induction of spike-timing dependent synaptic plasticity. JPhysiol.
27. Nevian T, Sakmann B (2006) Spine Ca2+ signaling in spike-timing-dependent plasticity. JNeurosci 26: 11001–11013.
28. Holthoff K, Kovalchuk Y, Konnerth A (2006) Dendritic spikes and activity-dependent synaptic plasticity. Cell Tissue Res 326: 369–377.
29. Letzkus JJ, Kampa BM, Stuart GJ (2006) Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. J Neurosci 26: 10420–10429.
30. Frick A, Johnston D (2005) Plasticity of dendritic excitability. JNeurobiol 64: 100–115.
31. Losonczy A, Makara JK, Magee JC (2008) Compartmentalized dendritic plasticity and input feature storage in neurons. Nature 452: 436–441.
32. Zilberter Y, Harkany T, Holmgren CD (2005) Dendritic release of retrograde messengers controls synaptic transmission in local neocortical networks. Neuroscientist 11: 334–344.
33. Sjöström PJ, Häusser M (2006) A cooperative switch determines the sign of synaptic plasticity in distal dendrites of neocortical pyramidal neurons. Neuron 51: 227–238.
34. Markram H, Lubke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science 275: 213–215.
35. Bi GQ, Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: Dependence on spike timing, synaptic strength, and postsynaptic cell type. Journal of Neuroscience 18: 10464–10472.
36. Debanne D, Gahwiler BH, Thompson SM (1998) Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. JPhysiol 507(Pt 1): 237–247.
37. Feldman DE (2000) Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. Neuron 27: 45–56.
38. Sjöström PJ, Turrigiano GG, Nelson SB (2001) Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. Neuron 32: 1149–1164.
39. Froemke RC, Poo MM, Dan Y (2005) Spike-timing-dependent synaptic plasticity depends on dendritic location. Nature 434: 221–225.
40. Stuart G, Schiller J, Sakmann B (1997) Action potential initiation and propagation in rat neocortical pyramidal neurons. JPhysiol 505: 617–632.
41. Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J (2009) Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle. Science 325: 756–760.
42. Stuart GJ, Hausser M (2001) Dendritic coincidence detection of EPSPs and action potentials. NatNeurosci 4: 63–71.
43. de Kock CP, Sakmann B (2008) High frequency action potential bursts (>or = 100 Hz) in L2/3 and L5B thick tufted neurons in anaesthetized and awake rat primary somatosensory cortex. J Physiol 586: 3353–3364.
44. Greenberg DS, Houweling AR, Kerr JN (2008) Population imaging of ongoing neuronal activity in the visual cortex of awake rats. NatNeurosci 11: 749–751.
45. Potez S, Larkum ME (2008) Effect of common anesthetics on dendritic properties in layer 5 neocortical pyramidal neurons. J Neurophysiol 99: 1461–1477.
46. Murayama M, Larkum ME (2009) Enhanced dendritic activity in awake rats. Proc Natl Acad Sci U S A 106: 20482–20486.
47. Helmstaedter M, de Kock CP, Feldmeyer D, Bruno RM, Sakmann B (2007) Reconstruction of an average cortical column in silico. Brain ResRev 55: 193–203.
48. Spratling MW (2002) Cortical Region Interactions and the Functional Role of Apical Dendrites. Behavioral and Cognitive Neuroscience Reviews 1: 219–228.
49. Raizada RD, Grossberg S (2003) Towards a theory of the laminar architecture of cerebral cortex: computational clues from the visual system. CerebCortex 13: 100–113.
50. Sherman SM, Guillery RW (2002) The role of the thalamus in the flow of information to the cortex. PhilosTransRSocLond B BiolSci 357: 1695–1708.
51. Rubio-Garrido P, Pérez-de-Manzo F, Porrero C, Galazo MJ, Clascá F (2009) Thalamic input to distal apical dendrites in neocortical layer 1 Is massive and highly convergent. CerebCortex.
52. Thomson AM (2010) Neocortical layer 6, a review. Front Neuroanat 4: 13.