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Current Opinion in Neurobiology

Synaptic clustering by dendritic signalling mechanisms

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Dendritic signal integration is one of the fundamental building blocks of information processing in the brain. Dendrites are endowed with mechanisms of nonlinear summation of synaptic inputs leading to regenerative dendritic events including local sodium, NMDA and calcium spikes. The generation of these events requires distinct spatio-temporal activation patterns of synaptic inputs. We hypothesise that the recent findings on dendritic spikes and local synaptic plasticity rules suggest clustering of common inputs along a subregion of a dendritic branch. These clusters may enable dendrites to separately threshold groups of functionally similar inputs, thus allowing single neurons to act as a superposition of many separate integrate and fire units. Ultimately, these properties expand our understanding about the computational power of neuronal networks.

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Current Opinion in Neurobiology 2008, **18**:321–331

This review comes from a themed issue on
Signalling mechanisms
Edited by Carlos Ibanez and Michael Häusser

Available online 4th October 2008

0959-4388/$ – see front matter

DOI 10.1016/j.conb.2008.08.013

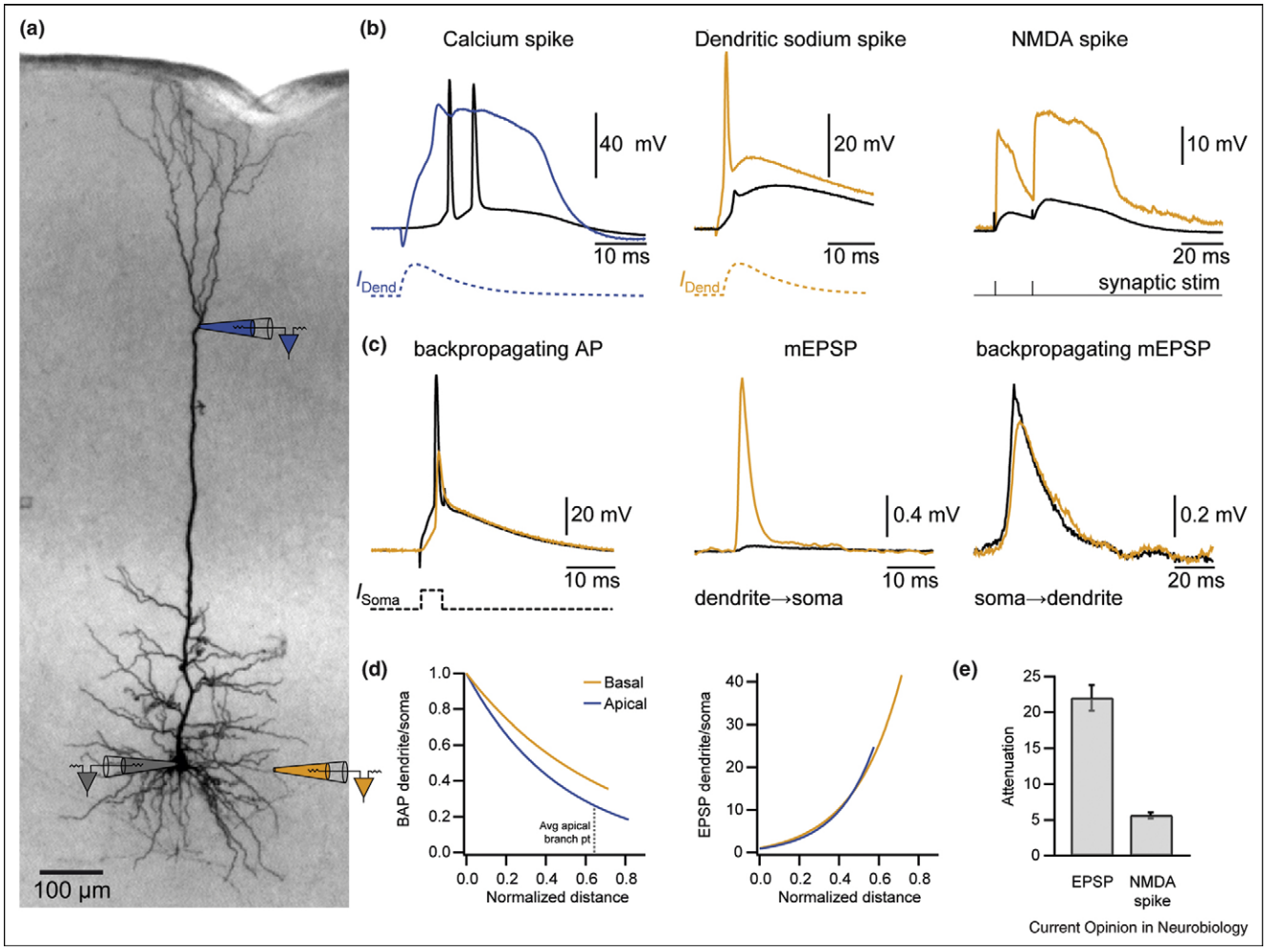
Introduction

The pattern of action potential (AP) discharge within a neural network constitutes the 'neural code' of information processing [1]. It is now widely considered that the transformation of synaptic input into AP output on the cellular level is strongly influenced by the properties of the dendritic tree in virtually all neurons. Indeed, it has been evident for some time now that dendrites possess active conductances that shape signal integration and that have the ability to generate local spikes in a highly nonlinear fashion [2].

In order to fully understand the rules of local signal processing and plasticity in dendrites, electrical recordings and functional imaging of the resulting signalling cascades have to be integrated. Recent advances in high resolution voltage [3] and Ca^{2+} imaging [4], focal stimulation of single or multiple synapses by glutamate uncaging [5••,6] and patch-clamp recordings from submicron sized dendrites [7••] have revealed important details about their electrical and biochemical signalling properties. Nevertheless it is largely unknown how these dendrites are contacted by their presynaptic partners and what rules determine their connectivity pattern [8]. Evidently, the electrical properties of the dendritic arborisation coupled to the biochemical machinery involved in activity-dependent refinement of neuronal circuitry determine the self-organisation of the neuronal network architecture. It is generally believed that memory traces are stored by long-term changes in the synaptic connection strength, dendritic excitability and eventually in the synaptic connectivity pattern [9,10]. On the basis of the electrical properties and the resulting plasticity mechanisms of fine dendritic structures we propose that synaptic cluster formation is a general building principle of the connectivity pattern of neuronal networks. Our general argument is that the impact of individual synapses on the generation of a somatic AP is rather weak owing to the constraints of the dendritic tree [7••,11,12]. On the contrary, locally generated spikes that can be evoked by synchronous and clustered inputs to a dendritic sub-branch can nonlinearly increase this impact [7••,13,14,15•]. This enables active dendrites, when compared with purely passive dendrites to exhibit a greater specificity of spiking responses following different spatio-temporal patterns of synaptic activity. Therefore, single neurons with active dendrites are able to perform transformations of synaptic input into AP output that would otherwise require more than one neuron with passive dendrites. Thus, active dendritic properties expand the computational capacity of the individual neuron [16••,17,18,19]. Furthermore, there exist plasticity mechanisms that can result in the clustering of inputs [5••,16••,20]. Therefore, nonlinear dendritic integration of localised inputs might be favoured as compared to linear summation of distributed inputs. A similar idea supporting our argument was recently suggested on the basis of the biochemical machinery of translation-dependent synaptic plasticity [21••].

Here we review the most recent work on the properties of dendrites. These studies were mainly focused on the pyramidal neurons of cortical layer 5 and hippocampal area CA1. We describe their electrical properties and the generation of a variety of active dendritic events and Ca^{2+} signals that are important for the modification of synaptic strength and dendritic excitability. We summarise the evidence for our hypothesis that synapses are functionally clustered on dendritic sub-

Figure 1



Phenomenology of dendritic spikes. **(a)** Image of a biocytin stained layer 5 pyramidal neuron. Electrodes depict different recording positions at the soma (grey), apical dendrite (blue) and basal dendrite (orange). **(b)** Recordings of dendritic spikes and the corresponding somatic waveform. EPSP-like current injection to the apical dendrite results in the generation of a Ca^{2+} spike (blue trace, left) that evokes a short burst of APs at the soma. A similar current injection to a basal dendrite can evoke a local Na^{+} spike (orange trace, middle) that is strongly attenuated towards the soma. Focal, extracellular synaptic stimulation to the basal dendrites can evoke a NMDA receptor mediated regenerative dendritic spike (NMDA spike, orange trace, right) that is less attenuated towards the soma as compared to a subthreshold EPSP (see (c and e)). **(c)** An AP evoked by somatic current injection backpropagates into the basal dendritic tree. The AP amplitude decreases with the distance from the soma (orange trace, left). Miniature EPSPs (mEPSPs) generated near the site of dendritic recording have large local amplitudes in the basal dendrite (orange trace, middle) but they are strongly attenuated towards the soma. By contrast, mEPSPs originating close to the soma propagate well to the distal basal dendritic site (orange trace, right). **(d)** Attenuation of BAP amplitude as the function of normalised dendritic length in basal (orange) and apical (blue) dendrites (left graph). When compared to the normalised dendritic length attenuation in the short basal dendrites and the much longer apical dendrite are very similar. Attenuation of EPSPs towards the soma in apical and basal dendrites is identical when compared to the normalised dendritic length (right graph). These comparisons suggest that despite the relatively small physical size of basal dendrites their electrical dimensions are equivalent to that of the much larger apical dendrite. **(e)** Attenuation of EPSPs compared to the attenuation of NMDA spikes. NMDA spikes are much less attenuated as compared with subthreshold EPSPs originating from the same dendritic location, suggesting that these generative dendritic events increase the dendritic coupling efficacy. Adapted from references [7••,14].

branches. We discuss how the properties of dendritic spike generation combined with the local biochemical signalling machinery should favour the formation of these clusters. This nonrandom connectivity pattern resulting in nonlinear summation should greatly expand the computational properties of a neural network [17,19,21••,22].

Phenomenology of dendritic spikes

We begin our argument by characterizing the variety of dendritic spikes that have been described so far and their impact on AP generation (Figure 1). Dendritic spikes add computational capacity to neurons; however, in addition to specialised active mechanisms, they require synaptic input that is synchronised and spatially clustered. The

dendritic membrane contains a large number of ionic conductances that can generate active dendritic events. A detailed description of their properties and consequences for neuronal integration can be found in a number of recent reviews [2,23,24]. Direct dendritic voltage recordings from the apical dendrites of cortical and hippocampal pyramidal neurons have revealed the presence of voltage-gated channels that are permeable to Na^+, K^+ and Ca^{2+} supporting the backpropagation of somatically initiated APs [25], and the generation of local regenerative dendritic events [26].

Calcium spikes

Depolarisation of the distal apical dendrite results in the activation of Ca^{2+} conductances that generate a Ca^{2+} spike [14,26]. These Ca^{2+} spikes can be evoked by synaptic stimulation to the tuft region, which suggests that the summation of inputs at the specialised Ca^{2+} initiation zone, located in the distal apical dendrite and exhibiting a high density of voltage-dependent Ca^{2+} channels (VDCCs), can cause a local spike [26]. The result of the Ca^{2+} spike is to switch the firing behaviour of a cortical pyramidal neuron to bursting mode [27]. The interaction with backpropagating APs (BAPs) can lower the threshold for the initiation of a Ca^{2+} spike and thus it has been proposed that these regenerative events constitute a coincidence detection mechanism for inputs arriving in different cortical layers (feedback input to the tuft region and feed forward input to the basal region) [14]. The locally confined elevation in dendritic Ca^{2+} concentration during a Ca^{2+} spike can involve an extended region that employs a particular Ca^{2+}-dependent synaptic plasticity rule that influences a number of synaptic contacts [28–30].

Sodium spikes

In contrast to the locally confined zone for the generation of Ca^{2+} spikes, local Na^+ spike generation appears to be possible in most regions of the pyramidal cell dendritic tree [7••,13,27,31,32]. These spikes are similar to somatically evoked APs with a fast rise and short duration but their propagation to the soma is less reliable and can be strongly attenuated at the soma particularly when generated in fine dendrites [6,7••,32]. Nevertheless dendritic Na^+ spikes still tend to increase the temporal accuracy of somatic AP output owing to the speed of the rising phase of the depolarisation at the soma [13,32]. Activation of local Na^+ spikes requires short local depolarisation with large amplitude [27]. This implies that multiple synchronous inputs are required to be activated in a short length of dendrite. Indeed, synchronous and clustered activation of synapses by two-photon uncaging of glutamate on a radial oblique dendrite in a CA1 pyramidal neuron can generate a Na^+ spike in a single branch that can therefore be seen as a single integrative compartment [6]. Synaptically evoked Na^+ spikes are accompanied by Ca^{2+} influx through NMDA receptors and VDCCs at the activated dendritic region that has implications for the spatial distribution of plasticity.

NMDA spikes

The most prominent regenerative event in basal dendrites is the *N*-methyl-D-aspartate (NMDA) spike. It is also triggered by synchronous and clustered input to a basal dendritic sub-branch activating NMDA receptor channels [33]. This causes the regenerative activation of NMDA receptors as a result of the voltage-dependent relief of Mg^{2+} block for a 10–20 μm stretch of dendrite [15•,22]. NMDA spikes result in a nonlinear response to synaptic inputs with a step-like increase in dendritic and somatic depolarisation that cause plateau potentials lasting 20 to hundreds of milliseconds. These are much more effectively transmitted to the soma than corresponding subthreshold EPSPs owing to their prolonged time course. Although a single NMDA spike rarely reaches threshold for somatic Na^+ spikes, they amplify the somatic impact of synchronously activated inputs to the basal dendrites by generating a large local response and thereby increase the electrical coupling to the soma (Figure 1e) [7••]. In this case, the local dendritic integration of synaptic inputs follows a sigmoidal nonlinearity. This suggests that each basal dendritic compartment can perform a complex integration of synaptic inputs, the result of which is then transmitted to the axo-somatic integration point for AP generation [19]. In this respect the basal-somatic compartments can be seen as a two-layer network of a number of nonlinear dendritic subunits feeding onto the AP initiation zone at the axon hillock (Figure 3d) [17,19]. The concept of a single dendritic branch serving as a computational subunit expands the computational properties of a neuron substantially [15•,16••]. It was even suggested that a single dendrite could correspond to a cascade of multiple cooperating dynamic decision making units with varying properties depending on the position of NMDA spike initiation [15•].

Backpropagating action potentials

BAPs signal the activity state of the neuron back to sites of synaptic input via transient increases in dendritic membrane potential, an elevation in Ca^{2+} through VDCCs and interaction with NMDA receptors. The coincidence of BAPs and synaptic inputs is the basis for synaptic modification by spike-timing-dependent plasticity (STDP) [34]. But does the well-studied phenomenon of BAPs contribute to the formation of clustered synaptic inputs? In this part we summarise the basic properties of BAPs that have important implications for synaptic plasticity mechanisms described later.

Clearly, the extent of backpropagation into the dendritic arborisation is a key parametre for STDP that depends on morphology and the distribution of ionic conductances within the dendritic arborisation [35] as well as the

depolarisation state of the dendrite [36]. In the apical dendrites of pyramidal neurons the amplitude of the BAP varies considerably with distance from the soma [36,37]; however, there is no detectable Ca^{2+} influx in the distal parts of the apical dendrite and the tuft with either a single BAP or trains of BAPs until they reach a certain critical frequency [14]. Principles of passive dendritic cable properties predict that BAPs would propagate worse in thinner dendrites like the basal dendrites [38]. Interestingly, although it was found by several groups that BAPs decrement stronger as a function of distance in the basal dendrites than in the apical dendrite, there is broad consensus that the BAP is able to cause Ca^{2+} influx into most if not all of the basal arborisation [3,7••,39]. Indeed, the amplitude of the BAP appears to be quite similar in the basal and apical dendrites as a function of the proportional length along the dendrite travelled (Figure 1d) [7••]. It might therefore be speculated that a general building principle for the dendrites of large cortical pyramidal neurons is to equalise their electrical properties.

Furthermore, the exact extent of AP backpropagation is subject to regulation from a number of factors including activation of K^+ conductances [39], inhibition [40] and neuromodulation [41]. The decrement of BAP amplitude in fine dendrites might even be sub-branch or pyramidal type specific [3,7••,39]. The activation of K_A voltage-dependent K^+ channels and an inhomogeneous distribution of voltage-dependent Na^+ channels have been implicated to be important factors of AP backpropagation in basal dendrites [7••,39]. The heterogeneity in dendritic excitability might support the idea that individual dendrites constitute separate computational compartments.

Summation and propagation of EPSPs

Impact of excitatory postsynaptic potentials on somatic action potential generation

An important observation with regard to the importance of clustered inputs is that excitatory postsynaptic potentials (EPSPs) significantly attenuate towards the soma as a consequence of the passive membrane properties of the dendritic arborisation (Figure 1c) [7••,11,12,38]. Nevertheless, just as the backpropagation of APs covers the same proportional dendritic length in basal dendrites compared with the apical dendrite, the attenuation of synaptically evoked miniature EPSPs (mEPSPs) towards the soma appears to be proportionally similar in the two structures (Figure 1d) [7••,12]. The corollary of this finding is that the amplitude of the depolarisation caused by the synaptic input is also a function of the distance from the soma and this is mostly determined by the local input resistance. In contrast, inputs to the apical dendrite of CA1 pyramidal neurons show distance-dependent scaling of EPSP amplitude by an increasing number of synaptic AMPA receptors with distance [11]. The local input resistance is sensitive to the morphology and local conductances. Thus, channels such as I_h can have an enormous influence on local amplitude of EPSPs and their temporal summation [42,43]. I_h, which is present particularly in the distal dendrites of layer 5 and CA1 pyramidal neurons, significantly decreases the temporal summation of nearly synchronous inputs. This implies that local depolarisations have a greater amplitude in the basal dendrites (here, miniature EPSPs can have amplitudes of up to 8 mV), compared with the apical trunk. The large local depolarisation by EPSPs together with their small impact on somatic depolarisation suggests that clustered and synchronous inputs to basal dendrites can more readily evoke a local dendritic spike than a somatic AP.

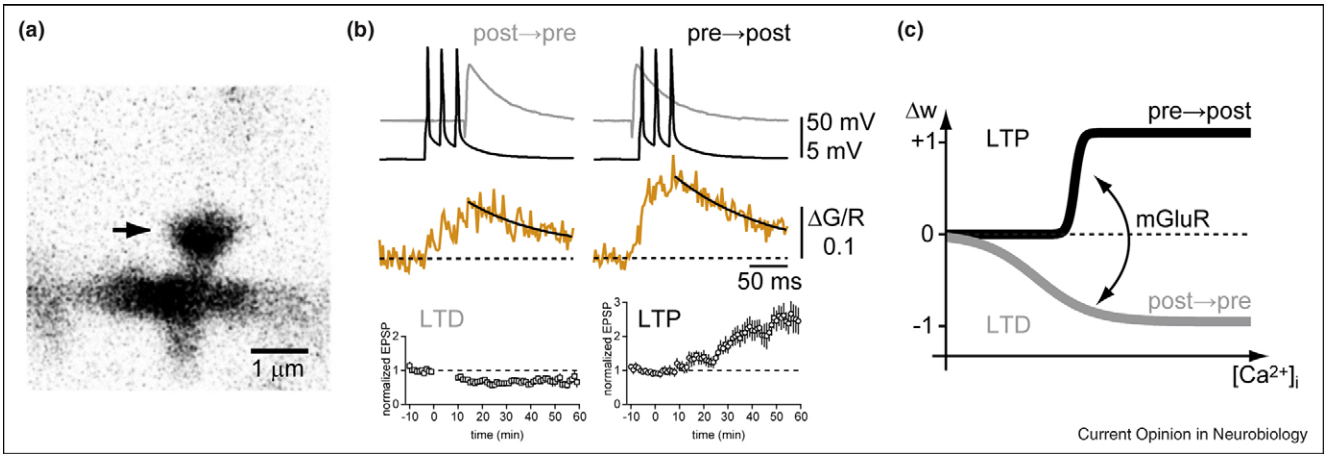
The influence of dendritic mEPSPs at the soma (amplitudes 0.05–0.2 mV; depending slightly on synapse location) is consistent with the reported size of EPSP amplitudes determined from paired recordings of layer 5 pyramidal neurons taking the number of synaptic contacts into account [44]. These spatially distributed inputs will sum linearly at the soma giving rise to the observed unitary EPSP size. This suggests that a large number of inputs have to summate to reach threshold for action potential generation if inputs are distributed.

Linear and nonlinear summation of synaptic inputs

Linear summation of subthrehold EPSPs can be observed when synapses onto the same dendrite are asynchronously activated [6]. Excitatory inputs to neighbouring spines can add linearly owing to the properties of spine necks acting as electrical isolators to prevent input interactions [45]. Activation of synapses on different dendritic sub-branches also results in linear summation [19]. It has been proposed that linear summation is the main purpose of the dendritic arborisation [46]. Nevertheless, recent evidence suggests that fine dendritic branches like the basal dendrites of layer 5 pyramidal neurons can work in two modes of integration: subthreshold, weakly location-dependent summation of subthreshold EPSPs and local amplification of spatiotemporally clustered information by their nonlinear dendritic properties [7••]. The summation of a few nonlinear dendritic events (e.g. NMDA spikes) could be a much more efficient way to generate AP-output as compared to the summation of many distributed inputs [15•,17,19,22]. Which mode of integration is prevalent in the *in vivo* situation has yet to be determined.

In summary, three important points emerge: (1) EPSPs can cause far more depolarisation at their site of origin than at the site of integration in the axon, (2) most dendrites are equipped with the necessary machinery for the generation of local spikes. Thus, it can be assumed that clustering of inputs in space and time on a dendrite is likely to lead to local electrogenesis and (3) these local

Figure 2



Calcium signalling in spike-timing-dependent plasticity. **(a)** Two-photon fluorescence image of a dendritic spine on a basal dendrite of a layer 2/3 pyramidal neuron. The arrow points to the active spine. **(b)** Electrical activity patterns for the induction of STDP (upper traces), the corresponding Ca^{2+} transients (orange, middle traces) and the resulting averaged and normalised change in synaptic efficacy (lower graphs). Post–pre pairing with a time delay of 10 ms results in the linear summation of the Ca^{2+} transients and the expression of LTD. Pre–post pairing with a time delay of 10 ms results in supralinear summation of the Ca^{2+} transients and the expression of LTP. **(c)** Change in synaptic weight (Δw) as a function of peak Ca^{2+} concentration. The peak Ca^{2+} transient amplitude is not a good predictor for the direction of change in synaptic strength. A mGluR-dependent signalling mechanism that is coactivated if the postsynaptic APs precede the synaptic activation (post–pre) is required for the induction of LTD (solid grey line). The large Ca^{2+} influx through the NMDA receptor that is evoked if the APs follow the synaptic activation (pre–post) results in the expression of LTP (solid black line). Therefore, two coincidence detectors are required for the differential expression of STDP. Modified from reference [47].

potentials are likely to have a larger impact on the axo-somatic integration point that generates AP-output.

Plasticity mechanisms favouring synaptic clustering

In recent years, several different plasticity mechanisms have been discovered that can support functional clustering of synapses along dendrites. These mechanisms bias the local dendrite to potentiate synaptic inputs that share a similar pattern of activation.

Ca^{2+} signalling in spike-timing-dependent plasticity suggests a dispersed learning rule

If clustered inputs were to exist, there should be factors controlling their formation. So an important question is, how could the formation of clustered synapses that might code for similar information develop? STDP has been suggested to be an important mechanism for experience-dependent refinement of neuronal circuits, but is it an adequate learning rule that could lead to synaptic cluster formation?

Each depolarisation of the neuronal membrane results in a brief Ca^{2+} transient either locally or globally. The biochemical machinery that translates the rise in Ca^{2+} into a change in synaptic weight is quite complex and is tightly regulated [23]. Several signalling cascades can converge to differentially decode the evoked Ca^{2+} transients. It is becoming evident now that a rise in Ca^{2+} alone is not a good predictor for the direction of change in synaptic efficacy evoked by STDP (Figure 2). The timing-dependent interaction of synaptic input with postsynaptic spikes results in LTP if the postsynaptic spikes follow synaptic activation within a defined time window, whereas the reversed order results in LTD [34]. The differential rise in Ca^{2+} evoked by these patterns of activity alone is necessary but not sufficient to account for the bidirectional change in synaptic strength [47]. A large rise in Ca^{2+} due to the activation of the NMDA receptor for pre–post pairings is the trigger for the induction of LTP as expected. The induction of LTD in contrast requires the activation of a second coincidence detector that has been identified as a metabotropic glutamate receptor (mGluR) [47,48]. Activation of the mGluR triggers the Ca^{2+}-dependent synthesis of endocannabinoids that act as a retrograde messenger to decrease the presynaptic release probability [47–49]. Nevertheless, there might be other mechanisms for timing-dependent LTD employed by different sets of synapses [50]. The rise in Ca^{2+} evoked by STDP protocols is synapse-specific and can be localised to individual spines thus it can ensure input-specific modification of synaptic strength [51]. It was suggested that in oblique dendrites of CA1 pyramidal neurons, the EPSP can modulate the BAP to maximise the Ca^{2+} current through the NMDA receptor. This would result in a uniform elevation of Ca^{2+} in all activated synaptic spines for LTP inducing stimuli [52]. Nevertheless, STDP depends

on the location of the synaptic contact along the dendritic tree owing to the properties of the BAP resulting from the morphology and distribution of ionic conductances [29,30]. STDP can be considered as a dispersed learning rule because the plasticity at a synapse is not influenced by the activity at nearby synapses. In this way, the learning rule is evenly applied across the entire dendritic tree with no local interactions. When the BAP simply interacts with subthreshold synaptic potentials (i.e. not local spikes) this does not result in the clustering of inputs even though the distance-dependence of the STDP learning rule could result in some differences between proximal and distal synapses.

Cross talk of synaptic plasticity

A recent report by Harvey and Svoboda suggests that local learning rules can be dynamically modifiable [5••]. They found that LTP at one synaptic contact lowers the threshold for the induction of LTP at neighbouring synapses at a stimulation strength that did not cause any plastic changes under control conditions (Figure 3b). The extent of this sensitised plasticity zone spans about 10 μm of dendrite and lasts for 10 min and is presumably due to the diffusional spread of the Ca^{2+} activated small GTPase Ras to neighbouring spines [53]. This cross talk of synaptic plasticity is still synapse-specific and thus differs from mechanisms of heterosynaptic plasticity. This novel mechanism of cross talk might be fundamental to the establishment of clusters of synapses on the same dendritic branch. About 20 synapses are influenced by the induction of LTP at one of them. The subsequent activation of neighbouring synapses will eventually bind inputs that are processing information of the same behavioural epoch [53]. By nonlinear dendritic summation these clustered synapses can greatly increase the storage capacity of the neuron [16••,18].

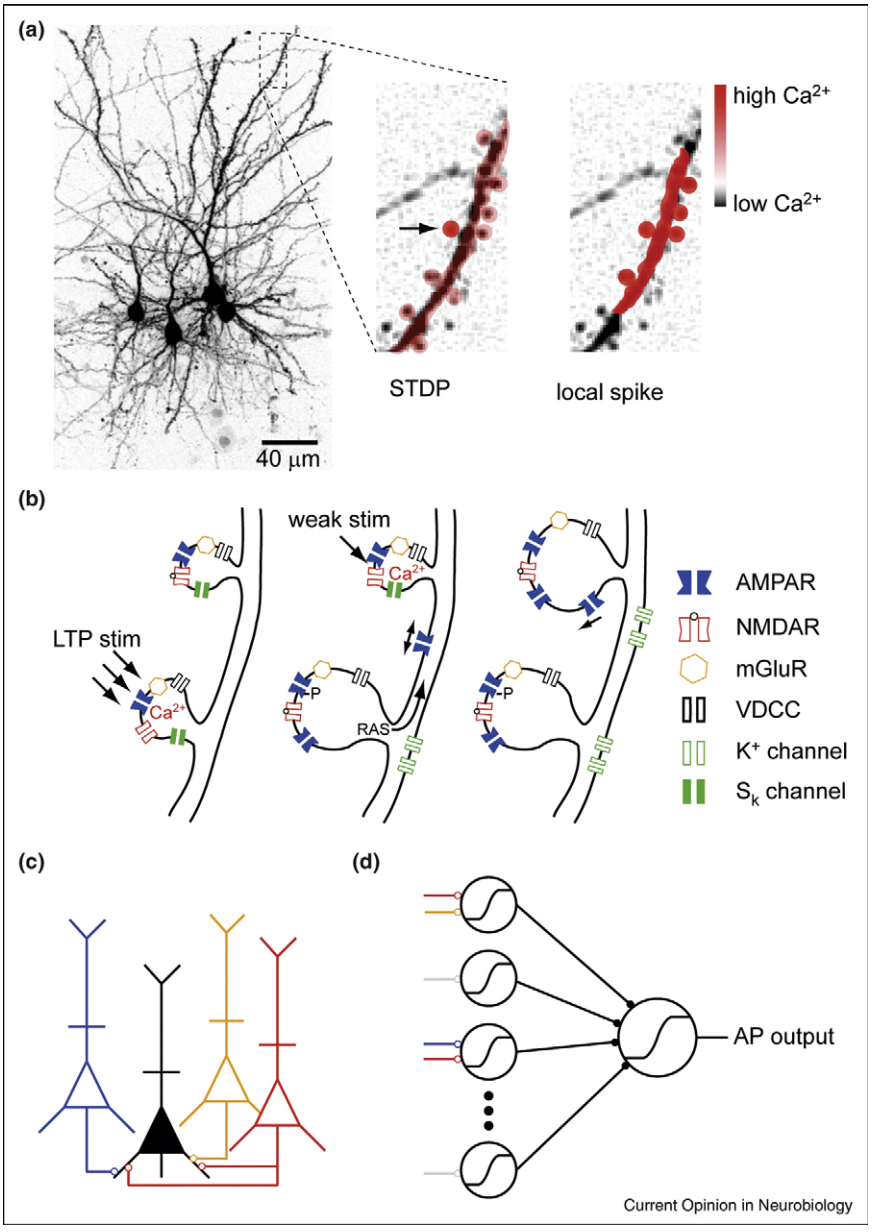
Local spikes and synaptic plasticity

What influence do active regenerative events in dendritic sub-branches have on the local plasticity rules? Local spikes can extend for some 10 μm and evoke substantial elevations in dendritic Ca^{2+} [15•,26,54]. The cooperative activation of synapses generating a local spike can indeed result in the modification of the contributing synapses. For example, a theta burst pairing protocol in hippocampal CA1 pyramidal neurons is most effective if a local complex spike is generated [55]. This local spike can result in potentation without the requirement for BAPs [54]. LTP can even be induced by a single local dendritic spike in contrast to most plasticity induction protocols, prominently STDP protocols, which require multiple pairings [56]. A decrease in synaptic efficacy induced by a single dendritic spike was observed in cortical pyramidal neurons [57]. Thus, cooperatively activated synapses generating a local spike rapidly undergo the same change in synaptic efficacy, again arguing for the formation of synaptic clusters on a dendritic sub-branch with similar synaptic properties. A reason for the difference in the number of required pairings for STDP as compared to local spike plasticity might be the spread of the evoked Ca^{2+} signal (Figure 3a). Whereas STDP protocols result in spine-specific increases in Ca^{2+} concentration, local spikes show an additional large Ca^{2+} response in the dendritic shaft [57,58]. This could recruit Ca^{2+}-dependent signalling cascades within the dendritic shaft.

Nevertheless not all regenerative dendritic events are triggers of synaptic plasticity [16••,20]. Furthermore, as explained above, an increase in intracellular Ca^{2+} concentration is necessary but not a sufficient determinant for the sign or magnitude of changes in synaptic efficacy. Additional factors such as neuromodulation or the sequence of events have to be taken into account. Striking examples for this argument are NMDA spikes that result in large increases in Ca^{2+} in the basal dendrites of layer 5 pyramidal neurons but they do not result in a change in the synaptic strength [20]. One can argue that these spikes contribute to signal integration in the dendrites rather than triggering synaptic plasticity. However, in the presence of BDNF acting as a neuromodulator NMDA spikes result in the induction of LTP. Strikingly, the induction of LTP has a broad time window with strong potentiation occurring if the NMDA spike precedes subthreshold synaptic activation by 100 ms. This mechanism could therefore recruit more local synapses to the cluster that generates an NMDA spike. Due to the nonlinear nature of NMDA spike generation, the potentiation of a few synapses in the same dendritic compartment could have a much greater impact on the input–output function of a neuron as compared to the modification of the same number of synapses distributed on separate sites along the dendritic tree.

There is evidence from Ca^{2+} and voltage sensitive dye imaging that some form of Ca^{2+} regenerativity, reminiscent of an apical dendrite Ca^{2+} spike [59], can be triggered in the distal tips of basal dendrites by bursts of high frequency APs [39]. This regenerative Ca^{2+} event in the distal basal dendrites has an important effect on synaptic plasticity in these regions [28]. Pairing of synaptic inputs with bursts of APs can trigger LTP with a broad time window. AP bursts preceding synaptic activation trigger LTP, where a simple STDP learning rule would predict the induction of LTD. Furthermore low frequency bursts that do not elicit the Ca^{2+} event in the distal dendrites do not induce changes in synaptic strength. The burst of APs presumably results in a long-lasting afterdepolarisation of the dendritic membrane that is sufficient to relieve the Mg^{2+} block of subsequently activated NMDA receptor channels thus resulting in a large Ca^{2+} transient and the induction of LTP. Thus, inputs to this region following a high frequency burst of APs can be cooperatively potentiated.

Figure 3



Mechanisms and computational implications of clustered synaptic plasticity. **(a)** Two-photon fluorescence image of four cortical pyramidal neurons illustrating the elaborate dendritic arborisation. The images on the right show an enlargement of a region of dendrite with synaptic spines and schematically illustrate changes in Ca^{2+} concentration for activity patterns corresponding to STDP (left) and local spike plasticity (right). STDP protocols result in the input-specific large increase in Ca^{2+} that is restricted to the activated spine (arrow) [47,51]. A smaller increase in Ca^{2+} concentration is additionally evoked by the BAPs throughout most of the dendritic tree. A local spike originating from the activation of a number of adjacent synapses results in a locally restricted elevation of Ca^{2+} in the activated spines and the adjacent dendritic shaft [57]. Shades of red depict differences in Ca^{2+} concentration. **(b)** Summary of events following the induction of LTP at a single synaptic spine. Following LTP induction by Ca^{2+} influx through NMDA receptors (red) AMPA receptors (blue) are phosphorylated (P) and/or new AMPA receptors are inserted into the synaptic membrane by exocytosis. Concomitantly, the spine head increases in volume. Additionally, ionic conductances are inserted (here unspecified K^+ channels; light green [60]) or removed (e.g. S_k channels; solid green [62]) locally resulting in the modification of dendritic excitability in the vicinity of the activated synapse. Cross talk between spines by a diffusible factor, recently suggested to be RAS, can lower the threshold for the stimulation intensity required for the induction of LTP at a neighbouring spine [5••,53]. This spine subsequently also increases in size and synaptic strength. Mobile AMPA receptors (depicted by arrows) can get immobilised in activated spines [72]. **(c)** Hypothetical wiring diagram of the four cells depicted in (a). The red cell contacts a postsynaptic cell shown in black on two separate dendritic branches and could therefore function in different clusters and contexts. **(d)** Abstract representation of the black cell shown in (c). Each dendritic compartment is represented as a nonlinear integration unit that connects to the axo-somatic integration point that generates AP-output. Clustered and synchronised inputs can activate individual computational subunits [17,19].

Plasticity of dendritic excitability

Changes in synaptic efficacy can also be accompanied by changes in dendritic excitability resulting from the modification or insertion of dendritic ionic conductances [10,60]. These changes can contribute to the change in synaptic strength by shaping the postsynaptic potential [61,62]. In addition, they can facilitate the backpropagation of APs and thus lower the threshold for subsequent potentiation in this region. Therefore, changes in dendritic excitability can locally modify the rules for synaptic plasticity on long time scales. It was recently shown that the coupling of local dendritic spikes to the soma of hippocampal pyramidal neurons can be modified in a branch-specific manner [16••]. This branch strength potentiation depends on a local NMDA receptor-dependent downregulation of A-type K^+ channels by associative pairing of synchronous input patterns to the dendritic branch with BAPs at theta frequency or cholinergic input. This could be a potential storage mechanism for information about the spatio-temporal correlation of synaptic activity patterns.

Molecular events involved in synaptic plasticity and clustering

Postsynaptic modifications of synaptic strength resulting in LTP are accompanied by the phosphorylation of existing AMPA receptors [63] or the insertion of novel receptors via exocytosis [64]. This results in the growth of the synaptic spine [65]. Similarly, LTD can result in the opposite events that are dephosphorylation of AMPA receptors, removal by endocytosis, or as has been found recently, the insertion of novel, newly synthesised, receptors with different single channel properties [66]. Local protein synthesis that is triggered by plasticity inducing stimuli is therefore an important component in the late phase of long-term changes in synaptic strength [67]. Locally synthesised proteins can serve as an activity-dependent tag that labels activated synapses [68]. These synaptic tagging proteins can spread to neighbouring synapses within the same dendritic compartment thus increasing or decreasing their likelihood of undergoing synaptic changes by following stimuli [69]. This translation-dependent plasticity can integrate the transient electrical and biochemical activity patterns described above over timescales of minutes. Therefore, it has been proposed to be a major biochemical mechanism for the formation of the clustering of synapses that act cooperatively and associatively on a specific dendritic branch. The underlying biochemical machinery and its importance for cluster formation are reviewed elsewhere [21••].

Recent advances in tracking individual AMPA receptors in the postsynaptic membrane showed that synaptic activity can immobilise AMPA receptors containing the GluR1 subunit at an individual synapse whereas mobility and escape from the synaptic contact was high in neighbouring inactive synapses [70]. LTP in hippocampal CA1 pyramidal neurons is thought to require the insertion of GluR1 containing AMPA receptors into the postsynaptic membrane by exocytosis [64]. Concomitantly, the pool of extrasynaptic AMPA receptors increases significantly [71]. These receptors might be able to diffuse to neighbouring synapses and be stabilised there if these synapses get activated following the initial LTP inducing event [72]. This model of the spread of LTP could facilitate the formation of clusters of synapses. A schematic summary of the local plastic changes after the induction of LTP described here is given in Figure 3b.

Do clustered inputs exist?

Paired recordings from cortical neurons and subsequent morphological reconstruction show that the synaptic contacts between two cells are distributed over different dendritic branches [44]. However, the finding that synapses from one neuron to another are not clustered does not mean that individual synaptic inputs do not participate in functional clusters with other neurons. Indeed, the notion of clustering is enhanced by the possibility that different synapses from the same connection participate in different clusters (Figure 3c and d). However, definitive proof for the existence of clustered inputs is still elusive. A recent study reports cluster formation of axo-dendritic contacts after learning related modification of neuronal circuitry in the barn owl auditory system [73••]. Investigation of the evolution of the spatial distribution of synaptic inputs in a compartmental model of a layer 2/3 pyramidal neuron with a nonlinear local spike learning rule suggested that the correlated afferent inputs from two independent groups of cells result in spatial and mutually exclusive clustering for each group [74••]. It will be important to determine experimentally if neighbouring synapses transmit similar and related information to the postsynaptic cell. This formidable task has to be investigated in the intact brain. Network mapping by uncaging of glutamate [75], light activated ion channels [76], labelling of all presynaptic partners of a neuron [77] and genetic labelling of neuronal networks [78] will probably be important tools to answer this question. Together with advances in *in vivo* imaging of dendritic [79] and network function [80], these studies will yield detailed insight into the structure–function relationship of neuronal circuits. The clustering of synapses onto a dendritic sub-branch that we suggest here would represent a general design principle of cortical networks. This hypothesis might be testable with the recent efforts to simulate cortical columns [81,82].

Conclusion

Taken together, there is presently no conclusive evidence that synapses are functionally clustered. Nevertheless, we believe that there are good indications for the existence and the functional relevance of synaptic clusters. Firstly, from a theoretical point of view, clusters of synapses on a dendritic sub-branch can significantly

increase the computational power of a single neuron [16••,17–19]. Secondly, there exist plasticity mechanisms that favour the potentiation of synapses along a dendrite that share a similar pattern of activation [5••,16••,21••,53]. Thirdly, active dendritic mechanisms exist that cause the synchronous and clustered activation of groups of synapses to elicit larger axo-somatic voltage changes than would occur if the same inputs were distributed over several dendrites [15•,37]. Fourthly, dendritic spikes are observed *in vivo*, which strongly suggests that synchronous and clustered activation of synapses occur *in vivo* [83].

One main task for the future is to determine whether neighbouring synapses transmit similar information, or are activated by similar stimuli. In analogy to the topic organisation of cortical columns and micro-columns on the network level, one could speculate that this organisation principle also exists on the level of a single neuron. Each dendritic branch may be sensitive to a specific feature of a sensory stimulus. This would allow for a rich variety of information processing on the single neuron level, and thus correct a tremendous underestimation of the computational power of neuronal networks.

Acknowledgements

MEL is supported by the Swiss National Science Foundation (SNF) Grant No. PP00A-102721/1). TN is supported by SNF Grant No. 3100A0-118395.

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