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Depolarization gates spine calcium transients and spike-timing-dependent potentiation

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Timing-dependent long-term potentiation (t-LTP) is induced when synaptic activity is immediately followed by one or more back-propagating action potentials (bAPs) in the postsynaptic cell. As a mechanistic explanation, it has been proposed that the bAP removes the Mg^{2+} block of synaptic NMDA receptors, allowing for rapid Ca^{2+} entry at the active synapse. Recent experimental studies suggest that this model is incomplete: NMDA receptor-based coincidence detection requires strong postsynaptic depolarization, usually provided by AMPA receptor currents. Apparently, the brief AMPA-EPSP does not only enable t-LTP, it is also responsible for the very narrow time window for t-LTP induction. The emerging consensus puts the spine in the center of coincidence detection, as active conductances on the spine together with the electrical resistance of the spine neck regulate the depolarization of the spine head and thus Ca^{2+} influx during pairing. A focus on postsynaptic voltage during synaptic activation not only encompasses spike-timing-dependent plasticity (STDP), but explains also the cooperativity and frequency-dependence of plasticity.

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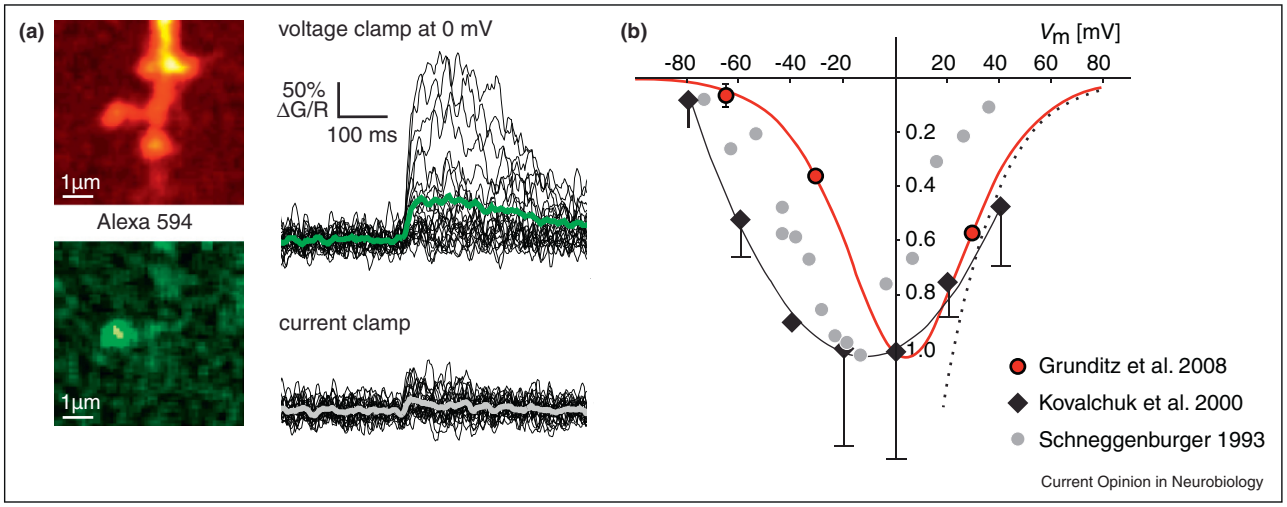
Introduction

Spike-timing-dependent plasticity (STDP) is often seen as an extended and more quantitative version of Hebb's learning rule, since both formalisms are based on the idea that only synapses that are causally related to postsynaptic spiking should be strengthened. However, the timing between synaptic activity and a postsynaptic spike is clearly not the only parameter affecting synaptic strength. The number of repeated pairings and their frequency, the number of activated synapses and their locations, the presence or absence of neuromodulators all have a major impact on plasticity induction, but do not appear in the STDP formalism [1,2•]. To arrive at a more general theory of synaptic plasticity, it is productive not to focus entirely on Na^+ spike timing, but more generally on the degree of postsynaptic depolarization [3,4••] and subsequent Ca^{2+} influx [5]. It has been shown that this approach can not only explain the timing-dependence and frequency-dependence of LTP but also predicts the development of bidirectional connectivity and localized receptive fields, characteristic features of neocortex [4••]. Which depolarizing mechanisms are most relevant for plasticity induction *in vivo* is a topic of intense debate. Here we review the role of back-propagating action potentials (bAPs) and AMPA receptor currents in timing-dependent long-term potentiation (t-LTP) and discuss the role of NMDA receptors and dendritic spines in coincidence detection.

Measuring NMDA receptor properties at the synapse

The NMDA receptor, by virtue of its sensitivity to glutamate, glycine, D-serine, zinc [6], and membrane voltage, has been identified early on as a key player in the detection of coincident presynaptic and postsynaptic activity [7,8,9•,10••]. In fact, some textbooks imply that it can do the job of spike-timing measurement *alone*, a simplified view that we will try to deconstruct here. To understand and model synaptic coincidence detection, a realistic description of the voltage-dependence of NMDARs is most critical. In a classic study, excised patches pulled from hippocampal pyramidal cells have been used to determine the shape of the blocking curve with high precision [11], and most computational studies of STDP mechanisms are based on the parameters determined by Jahr and Stevens. While the excised patch technique allows for perfect voltage clamp, it has the disadvantage of sampling mostly extrasynaptic receptors. Extrasynaptic NMDARs have a high content of NR2A subunits and show pronounced Ca^{2+}-dependent rundown and desensitization [12]. Interestingly, synaptic NMDARs are protected from rundown *independent* of subunit composition, suggesting that interactions with specific signaling proteins in the PSD modify the gating properties of synaptic receptors [13]. Selective binding of the NR2B tail to postsynaptic proteins is also required for the induction of LTP [14]. Since receptors in excised patches are stripped of potentially important binding partners, it would be very desirable to quantify the current-to-voltage relationship of synaptic receptors *in situ*. In principle, optical measurements of calcium influx

Figure 1



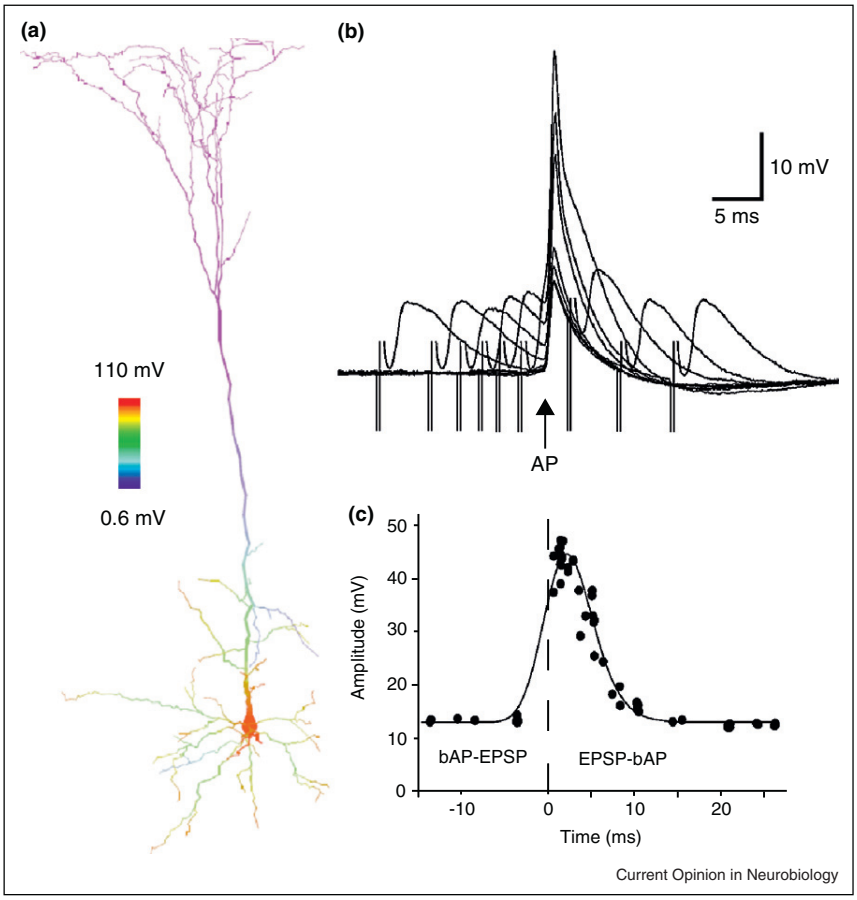
Voltage-dependence of NMDAR-mediated Ca^{2+} transients. **(a)** Spiny dendrite of CA1 pyramidal cell filled with Alexa-Fluor 594 (red image) and Ca^{2+}-sensitive dye (green image). Electrical stimulation of Schaffer collaterals triggers Ca^{2+} transients in active spine. **(b)** Amplitude of NMDAR-mediated Ca^{2+} transients depends on the holding potential. Under conditions of blocked AMPA receptors (red), space clamp is improved and curve is shifted to the right.
Data replotted from Refs. [15,16•,17].

at different holding potentials can be used to extract the voltage-dependence of the Mg^{2+} block (Figure 1a). In an early Ca^{2+} imaging study, cholinergic neurons that lack a large dendrite were chosen to ensure good voltage clamp [15] (Figure 1b, gray markers). However, stimulation by NMDA iontophoresis activated both extrasynaptic and intrasynaptic receptors. In later studies, synaptically evoked Ca^{2+} transients in dendritic spines were quantified at different holding potentials [16•,17]. Although both studies recorded from CA1 pyramidal cells in 1 mM Mg^{2+}, the first study reported the point of half-maximal calcium influx at −60 mV, the second study at −20 mV, a very large discrepancy (Figure 1b). An important experimental difference was that Grunditz *et al.* performed their voltage-clamp experiments under AMPA receptor blockade. Does that mean active AMPA receptors flux Ca^{2+} ions into the spines of CA1 pyramidal cells? The pharmacology of spine calcium signals and the linear IV curve of synaptic AMPARs suggest that this is not the case [16•]. The most likely explanation for the discrepancy is that in spite of somatic voltage clamp, the voltage inside the spine head escapes during the AMPA-EPSP, thus shifting the apparent voltage-dependence of NMDAR-mediated calcium influx to the left. Indeed, the magnitude of the shift (~40 mV) could be taken as a rough estimate of the AMPA-EPSPs inside the spine head. Given the key importance of the NMDA receptor's voltage-dependence for our understanding of coincidence detection, it is worrisome that there is so little agreement in the experimental data. To put future modeling studies on a more solid footing it will be essential to repeat and refine measurements of synaptic NMDA currents.

Attenuation and boosting of bAPs

For STDP to work, the bAP must propagate from the soma to the synapse. The general morphology of dendrites, strongly tapering from the soma to the distal tips, supports efficient back-propagation. However, active dendritic conductances such as A-type K^+ channels actively depress the amplitude of bAPs in the distal dendrite. At a distance of 350 µm, the calcium signal amplitude triggered by a bAP in a L5 pyramidal cell is only 10% of the amplitude measured close to the soma [18]. The changing amplitude and waveform of the AP as it propagates back into the dendrite results in very different learning rules at proximal and distal synapses [19,20]. Interestingly, strong dendritic depolarization by correlated synaptic input (or current injection) promotes back-propagation of APs, in L5 pyramidal cells mainly though the activation of Na^+ channels [21], in CA1 pyramidal cells though inactivation of A-type K^+ channels [22,23]. Thus, interactions between bAPs and NMDARs at distal synapses will be enhanced during periods of intense synaptic activity, creating another layer of coincidence detection on the dendritic level (Figure 2). One interesting prediction of bAP boosting is that clustered excitatory inputs should improve back-propagation into the 'causal' dendritic branch but not into inactive branches, providing selective feedback to synapses on the active branch [24]. While synchronized activity of many closely spaced synapses is necessary to enable dendritic coincidence detection, NMDA receptors are able to detect the timing of bAPs at individual active synapses. At distal synapses, both mechanisms are likely to contribute to t-LTP. Whether the synapse-specificity

Figure 2



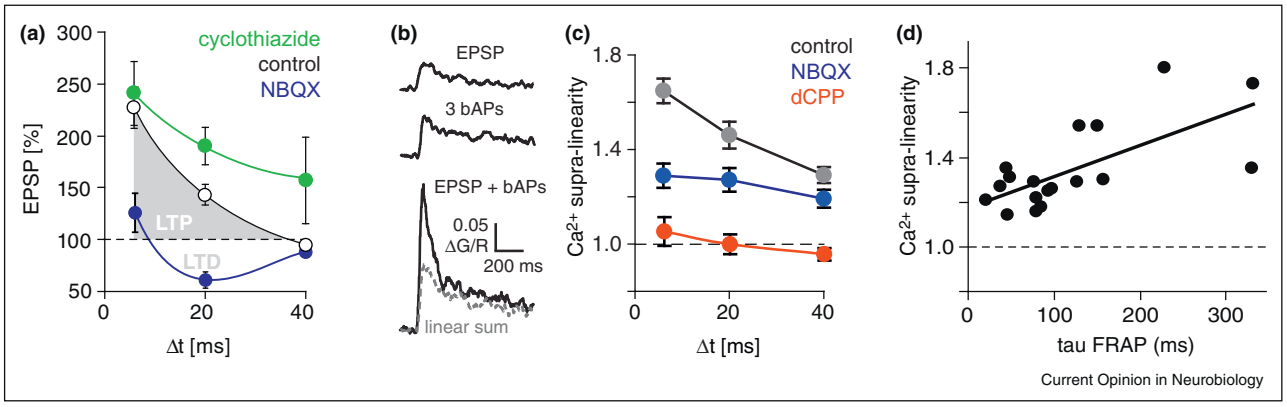
bAP boosting after dendritic depolarization. **(a)** A single AP triggered by somatic current injection does not efficiently back-propagate into the dendrite (simulation, colors indicate membrane voltage). **(b)** With the dendrite depolarized by clustered input, the bAP propagates more efficiently into the active dendrite (dendritic recording, 480 μm from soma). **(c)** bAP boosting occurs for positive (EPSP–bAP) timings that are typically associated with LTP, but not for the reverse sequence (bAP–EPSP). Thus, bAP amplification is likely to contribute to t-LTP by enhancing postsynaptic depolarization during pairing.
Reproduced from Ref. [21].

of LTP, which has been demonstrated at spine synapses relatively close to the soma [25•,26,27••], breaks down at distal synapses remains to be seen.

The spine as electrical compartment and amplifier

Although the properties of NMDA receptors are of crucial importance for STDP, they are not sufficient to understand (or model) coincidence detection. Rather, the entire spine acts as a coincidence detector, making use of an asymmetric effect of the spine neck resistance: dendritic events such as bAPs efficiently invade even highly isolated spines with little attenuation, since only a minimal current has to flow through the spine neck to depolarize the tiny spine [28,29]. The situation is different in the other direction: the EPSP generated in a highly isolated spine is attenuated when it enters the parent dendrite, a low impedance compartment [30,31••]. The most dramatic effect of the spine neck resistance, however, is on the local EPSP amplitude inside the active spine head. Since the spine head membrane is full of depolarization-activated cation channels such as Ca^{2+} channels and NMDA receptors, the EPSP is likely to get amplified inside the spine head. In a biophysical model of a spine with high neck resistance, it is relatively easy to generate a regenerative event (a 'spine spike'). The high trial-to-trial variability of spine calcium signals, on the other hand, argues against regenerative 'all-or-nothing' events [32]. Spine spikes might be prevented by a negative feedback loop that keeps spine depolarization in check: Ca^{2+} activated K^+ channels (SK channels) on the spine head terminate the spine EPSP by shunting the synaptic current [31••,33••]. Cholinergic input interferes with this feedback loop and thus boosts EPSP amplitude and spine Ca^{2+} transients [34]. It is likely that additional homeostatic mechanisms such as Ca^{2+}-dependent receptor

Figure 3



Non-linearity of spine calcium signaling is enhanced during the AMPA-EPSP. **(a)** When AMPARs are blocked during pairing (NBQX), t-LTP cannot be induced. Boosting AMPA currents by cyclothiazide prolongs the t-LTP window. **(b)** If bAPs arrive just after an uncaging-evoked EPSP (Δt = 6 ms), the Ca^{2+} transient in the spine shows a sharp peak that is not predicted by a linear summation of the Ca^{2+} transients caused by the EPSP and bAPs alone. **(c)** With AMPARs blocked (NBQX, blue markers), the fast peak disappears and Ca^{2+} signals become more linear. **(d)** With the first AP arriving 6 ms after synaptic activation, supra-linearity is most pronounced in spines that are diffusionally isolated from the dendrite. This correlation is not seen when AMPARs are blocked.
Reproduced from Ref. [38••].

endocytosis are employed to keep spine voltage in the optimal range, outside the 'spine spike' region, but sufficiently depolarized to sensitize NMDA receptors for coincidence detection [16•,35••]. In summary, to appreciate the full complexity of spine signaling, the spine has to be studied as a functional unit (current clamp).

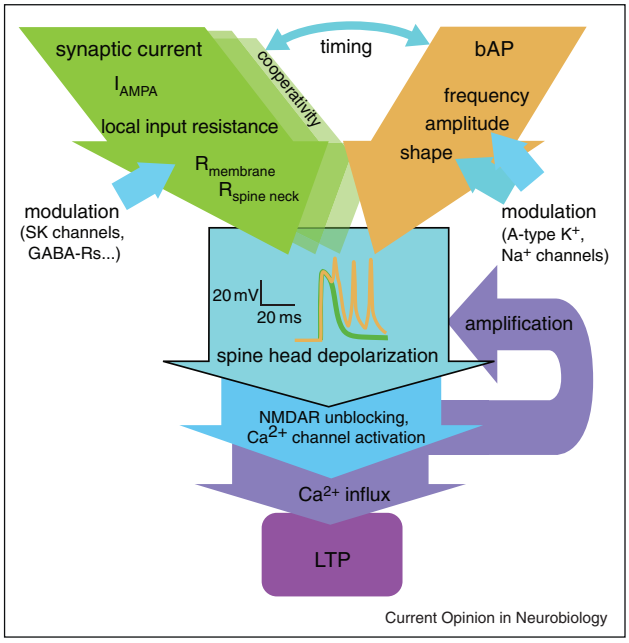
The brief AMPA-EPSP, not the NMDA receptor, is responsible for the narrow t-LTP window

Calcium influx into the spine is necessary for the induction of long-term plasticity and determines the magnitude of synaptic potentiation [36•]. The spine is most sensitive to additional depolarization (e.g. by bAPs) at the steepest part of the IV curve for Ca^{2+} (Figure 1b). The AMPA-EPSP can thus be seen as a sensitizing stimulus, depolarizing the spine membrane to a point where NMDA receptors and high-threshold VDCCs will react strongly to coincident bAPs. Four experimental observations support the idea that AMPAR-mediated depolarization enables coincidence detection by NMDARs: first, AMPAR currents are necessary for the induction of t-LTP [37••,38••]. Under conditions of AMPAR block, t-LTP can be rescued by somatic current injection [37••]. Second, prolonging synaptic AMPAR currents by cyclothiazide extends the temporal window for t-LTP induction [35••,37••,38••] (Figure 3a). Third, blocking AMPAR currents prevents rapid Ca^{2+} influx during the EPSP [17] and significantly reduces supra-linear Ca^{2+} signals during pairing [38••] (Figure 3b,c). Fourth, supra-linearity of Ca^{2+} influx during pairing is strongest in spines which are diffusionally isolated from the dendrite [38••], suggesting that AMPA-EPSPs have the highest amplitude in these well-isolated spines (Figure 3d). This could be due to a higher number of active AMPARs in diffusionally isolated spines or due to the electrical resistance of the spine neck.

The great unknown: spine neck resistance

Depolarization of the spine head during the EPSP is a function of the number of active AMPARs and the input resistance. Since the resistance of the spine membrane is extremely high at rest (but not if K^+ channels are active [33••]), the input resistance of the spine is dominated by the resistance of the spine neck (Figure 4). Estimates of spine neck resistance based on cable theory suggest weak electrical compartmentalization [39], which is not consistent with the strong effect of AMPAR blockade on spine Ca^{2+} signals. So where does the hidden resistance come from? Estimating ohmic resistance from spine neck length and diameter rests on the assumption of a homogenous cytoplasm, or more specifically, homogenous resistivity everywhere inside the neuron. However, the spine neck is not simply a very thin part of the dendrite. In contrast to dendritic cytoplasm, it is filled with a dense network of actin fibers and actin-binding proteins [40,41]. In addition, the negative membrane curvature of spine necks might attract specific proteins containing I-BAR-domains or other curvature sensors to the submembrane space [42,43]. An important open question is whether the gel-like substance in the spine neck only affects diffusion of macromolecules, or also slows down the main charge carrier in the cell, K^+ ions. If it does, the cable theory approach might systematically underestimate spine neck resistance. It would be very interesting to use correlative light microscopy and electron microscopy of identified

Figure 4



Spine head depolarization is the central variable gating LTP. The amplitude of the EPSP in the spine is determined by the synaptic current (I_{AMPA}) and the spine neck resistance. The resistance of the spine head membrane is very high at rest, but can drop dramatically when SK channels or GABAergic inputs are activated on the spine (shunting). Synchronous activation of many synapses may trigger LTP in the absence of bAPs (cooperativity). In t-LTP, additional depolarization is provided by bAPs (yellow arrow). Amplitude and shape of bAPs are subject to modulation (see Figure 2). When bAPs closely follow synaptic activity, the EPSP in the spine head is substantially broadened (yellow trace, simulation from Ref. [16•]). Strong spine head depolarization leads to unblocking of NMDARs and activation of VGCCs, Ca^{2+} influx, and further depolarization (feedback arrow). If this process is repeated several times, the synapse is strengthened (LTP).

synapses [46] to compare live cell diffusion measurements [44•] with spine ultrastructure. This way, it might be possible to determine the local viscosity inside the spine and to estimate ion mobility in this compartment.

In addition to the importance of AMPAR function for t-LTP induction, the following observations support the idea of spines as electrical compartments: two-photon glutamate uncaging has been used to show correlations between spine neck length and EPSP amplitude in the head [30]. NMDA receptors in the spine head are unblocked by AMPA receptor co-activation on the spine head, but not by local dendritic depolarization of identical amplitude [31••]. High-threshold Ca^{2+} channels are activated in spines during unitary EPSPs that have amplitudes of ~1 mV at the soma [16•,31••]. These findings suggest that the input impedance, at least in a subset of spines, is very high. A caveat of Ca^{2+} imaging studies using 'blind' electrical stimulation is the selection bias towards spines that do produce sizable Ca^{2+} signals. In uncaging experiments, this bias is reduced, but the ratio of AMPA to NMDAR activation depends strongly on the precise location and power of the uncaging pulse [31••]. Optogenetic approaches provide an unbiased, jet physiological way to stimulate identified synapses, and can be combined with Ca^{2+} imaging to investigate presynaptic and postsynaptic mechanisms of plasticity [45]. These approaches could also be used to follow the fate of stimulated synapses for more than the typical one or two hours.

Conclusions

A major criticism of STDP has been the fact that in most experiments, bAPs are triggered by somatic current injection, while *in vivo* APs are the result of massive excitatory input [47••]. To 'naturalize' STDP, it will be important to focus on the multiple natural sources of synaptic depolarization rather than on artificially induced spikes. The local AMPA-EPSP clearly has a strong impact on Ca^{2+} influx [31••,38••] and t-LTP [35••,38••], but it will be equally important to study how massive dendritic events such as NMDA spikes and Ca^{2+} spikes [48] influence the strength of synapses that are active before, during, and after the dendritic spike [49].

The idea that the structure of spines could have an important impact on synaptic function has seen a revival in recent years. While several studies emphasize the role of the spine neck in restricting diffusion of Ca^{2+} *out of* the spine [50,51•], the spine neck's ohmic resistance might be even more important in controlling Ca^{2+} *influx* [16•,31••,38••]. The full complexity of spine signaling and electrical amplification can only be investigated in a cell that is free to depolarize (current clamp experiments). Under these conditions, however, blocking a particular type receptor or ion channel will influence the currents through all other channels. Thus, computer modeling is frequently employed to interpret the data from current clamp experiments and to test various ideas about coincidence detection and downstream signaling. To obtain a more quantitative description of the various regulatory mechanisms inside spines, it would be an important step forward to work collectively on a 'canonical' spine model which could serve as an intuition pump. The NEURON framework comes to mind as a good starting point for open source collaborations [52], but at present, inbuilt mechanisms are too primitive to provide a realistic simulation of, for example, Ca^{2+} influx during coincident activity and downstream reaction–diffusion systems [53•]. More sophisticated models have been published [54•], but are not easily accessible for modification. We agree that spine voltage is the central variable governing synaptic plasticity (Figure 4) [4••], but only after fleshing out the molecular details will we be able to understand the changes in synaptic properties underlying learning, aging, and disease [55]. Once

experimentalists and theorists start working together we will have a chance to decode the intricate network of feedback regulations inside spines.

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- •• of outstanding interest

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