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The role of dendritic spines: comparing the complex with the simple

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Abstract

Since Cajal first observed dendritic spines as 'small thorns' projecting from the dendrites of cerebellar Purkinje cells over a hundred years ago, there has been continued debate on the role of these structures. Over 90% of excitatory synapses connect dendritic spines within the central nervous system, implying their functional importance. No fewer than 20 hypotheses have been proposed for the function of dendritic spines. These range from a simple mechanism for increasing the surface area of excitatory synaptic contacts, to a neuroprotective role. This review compares the two morphologically distinct spine types found on CA3 pyramidal neurons. We compare the similarities and differences displayed by these spines in compartmentalising Ca^{2+} and discuss a potential role for the morphologically complex spines found on CA3 pyramidal neurons.

Keywords: Dendritic spine; Ca^{2+} compartmentalisation; Mossy fibre synapse; CA3 pyramidal neuron

1. Spines as biochemical compartments

A number of functions have been proposed for dendritic spines (for recent reviews see: Harris and Kater, 1994; Shepherd, 1996; Yuste and Majewska, 2001; Nimchinsky et al., 2001). The distinctive morphology of many spines, in which a relatively large 'head' is connected to the dendritic shaft by a thin 'neck', led to a popular hypothesis that spines function as compartments of synaptic response. Modelling studies have caused the initial concept of spines as electrical compartments to lose favour in recent times (Koch and Zador, 1993; Svoboda et al., 1996), but the application of new imaging techniques has provided compelling evidence that spines can indeed act as biochemical compartments. Through the use of intracellular fluorescent Ca^{2+} indicators, it is possible to demonstrate postsynaptic Ca^{2+} transients restricted to the spine (Fig. 1; Müller and Connor, 1991; Malinow et al., 1994; Yuste and Denk, 1995; Emptage et al., 1999; Mainen et al., 1999). These studies have focused on morphologically simple spines, such as those formed by the commissural/associational and recurrent collateral inputs contacting CA1 and CA3 pyramidal neurons in the hippocampus. However, not all spines are similar, and certain dendritic spines have a much more complex morphology. The best known examples of 'complex spines' are the so-called 'thorny excrescences' where mossy fibre axons of dentate granule cells contact the soma and proximal (mainly apical) dendritic segments of hippocampal CA3 pyramidal neurons. Do these far larger and more complex spines also compartmentalise Ca^{2+}? In a recent study, we demonstrated that sub-threshold stimulus-dependent postsynaptic Ca^{2+} transients occur at thorny excrescences, and found that such spines can act as discrete units of Ca^{2+} response (Fig. 1; Reid et al., 2001). Therefore, both structurally simple spines and complex spines compartmentalise Ca^{2+}. This similarity between the two structurally distinct spines suggests that a primary function of dendritic spines is to act as a biochemical compartment, in particular for Ca^{2+} responses. What then is the function of such compartmentalisation?

2. Ca^{2+} compartmentalisation and synaptic plasticity

The ubiquity of Ca^{2+} compartmentalisation by spines would seem to imply that it serves a similar function at all synapses. An attractive possibility is that the biochemical compartmentalisation offered by spine morphology facilitates the synapse-specific induction of plasticity. A consequence of compartmentalisation is that Ca^{2+} influx due to synaptic stimulation is limited to the active spine. As the

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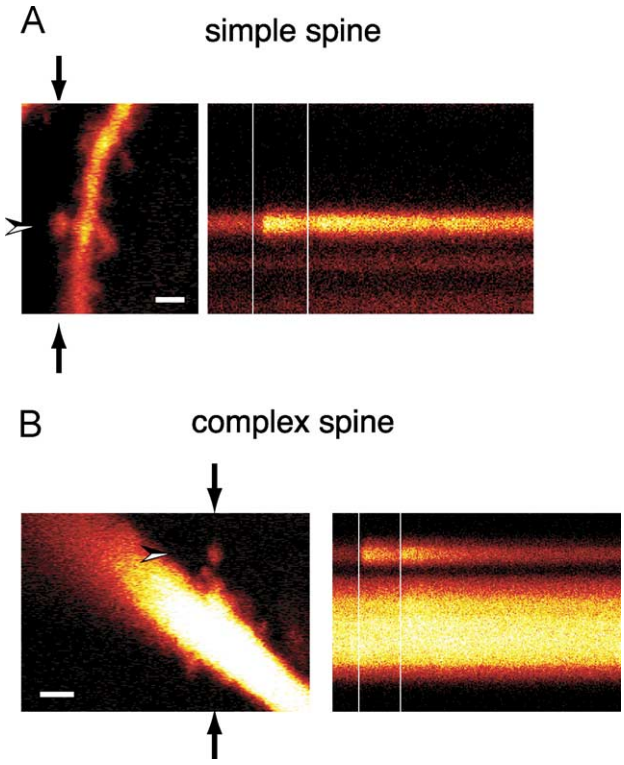

Fig. 1. Confocal imaging of Ca^{2+} transients in individual spines, elicited by subthreshold synaptic stimulation suggests that both 'simple' and 'complex' spines compartmentalise Ca^{2+}. (A) Left panel: Ca^{2+} indicator-filled distal dendritic segment of a CA3 pyramidal neuron showing a morphologically 'simple' spine. Right panel: A line scan shows an evoked postsynaptic Ca^{2+} transient (EPSCaT) that is restricted to the 'simple' spine. The arrows in the left panel indicate the trajectory of the line scan. (B) Left panel: Ca^{2+} indicator-filled proximal dendritic segment of a CA3 pyramidal neuron (soma is upper right) illustrating a morphologically 'complex' spine. Right panel: A line scan shows an EPSCaT that is restricted to one 'complex' spine. The arrows in the left panel indicate the trajectory of the line scan. In the false colour 'thermal' lookup table used in these confocal images, increasing fluorescence is denoted by colours from black through red to yellow and white. The vertical white lines ('time stamps') in the line scans mark the delivery of a single stimulus. The interstimulus interval between the two time stamps is 75 ms for both (A) and (B). Scale bars represent 1 μm in (A) and 10 μm in (B).

induction of long-term potentiation and depression are known to depend upon postsynaptic Ca^{2+} transients (Bliss and Collingridge, 1993), this could provide a mechanism for imparting input specificity to activity-dependent modification of synaptic strength, in keeping with the classical model of Hebb (1949). Are such mechanisms universal? The complex spines at hippocampal mossy fibre synapses also compartmentalise Ca^{2+}, but the mechanism underlying the induction of plasticity at this synapse is unclear. The introduction of the high affinity buffer 1,2-bis-(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) into CA3 pyramidal neurons has been found to block the induction of mossy fibre long-term potentiation by some investigators (Williams and Johnston, 1989; Yeckel et al., 1999), but not by others (Zalutsky and Nicoll, 1990; Mellor and Nicoll, 2001). This controversy not only clouds the question of mossy fibre long-term potentiation induction, but also raises questions about the universality of spine Ca^{2+} compartmentalisation as the basis for input-specific plasticity. If the induction of plasticity at the mossy fibre → CA3 synapse occurs at a presynaptic locus, then spine Ca^{2+} compartmentalisation would, at least at these synapses, presumably play another role. The observation that synaptically evoked Ca^{2+} fluxes in spines of different morphology on the same neuron arise from different sources of Ca^{2+} suggests that this may be the case.

3. Different Ca^{2+} influx mechanisms underlie the Ca^{2+} signal at morphologically distinct dendritic spines

To date, the only direct comparisons between morphologically distinct classes of spine have been carried out in organotypic hippocampal slices using the sharp intracellular electrodes. Since commissural fibres degenerate in hippocampal organotypic cultures, stratum radiatum stimulation in these preparations predominantly activates associational axons (e.g., projecting from CA3 to CA3 fields) as recorded from a CA3 pyramidal cell. The comparison, therefore, between morphologically 'complex' and 'simple' spines in the context of this review is a comparison between mossy fibre and associational inputs to CA3 cells. We have recently shown that excitatory postsynaptic Ca^{2+} transients (EPSCaTs; Fig. 1) are blocked by 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX), a broad-spectrum antagonist of the AMPA/kainate ionotropic receptors in both spine types (Fig. 2; Reid et al., 2001; Emptage et al., 1999). This suggests that the EPSCaTs generated both at the mossy fibre → CA3 and at CA3 → CA3 synapses are dependent on the release of glutamate and the activation of AMPA/kainate receptors. However, mechanisms underlying the EPSCaTs differ considerably. At the simple spines (CA3 → CA3), the transient arises from NMDA receptor-mediated Ca^{2+}-induced Ca^{2+} release (CICR), with minimal contribution from voltage activated Ca^{2+} channels (VACCs) (Fig. 2; Emptage et al., 1999). This is in contrast with the complex spines (mossy fibre → CA3) where the Ca^{2+} comes mainly from VACCs, less from NMDA receptors, and not at all from CICR (Fig. 2; Reid et al., 2001). However, there is controversy regarding the mechanism underlying the generation of EPSCaTs at 'simple' spines. Although there is general consensus that these Ca^{2+} transients appear largely dependent on NMDA receptor-mediated glutamatergic transmission (e.g. Yuste et al., 1999; Emptage et al., 1999), there is disagreement about the role played by internal Ca^{2+} stores in amplifying the Ca^{2+} signal (Kovalchuk et al., 2000). The basis for the inconsistencies remains unclear, although the different model systems (cultured vs. acute slice) and electrophysiological recording methods (sharp vs. whole cell patch clamp) may contribute to the divergent results. The considerable difference between sources of the synaptic Ca^{2+} transient at thorny excrescences in the same CA3 pyramidal cells suggests a

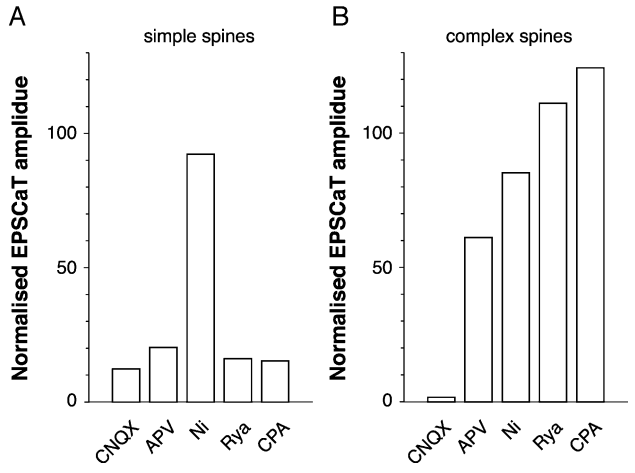

Fig. 2. Different Ca^{2+} influx mechanisms underlie the Ca^{2+} signal at morphologically distinct dendritic spines. (A) Summary histogram adapted from Emptage et al. (1999) of the mean EPSCaT amplitude generated at morphologically simple spines on CA3 and CA1 pyramidal neurons after the application of different treatments (normalised to initial baseline EPSCaT amplitude). These results suggest that the transients for simple spines mainly arise from NMDA receptor-mediated Ca^{2+} induced Ca^{2+} release, with minimal contribution from voltage activated Ca^{2+} channels. (B) Summary histogram adapted from Reid et al. (2001) of the mean EPSCaT amplitude of morphologically complex spines after the application of different treatments (normalised to initial baseline EPSCaT amplitude). These results suggest that the EPSCaT for complex spines are generated mainly from voltage activated Ca^{2+} channels, less from NMDA receptors, and not at all from Ca^{2+} induced Ca^{2+} release. Abbreviations: 6-cyano-7-nitroquinoxaline-2,3-dioine (CNQX), D-(−)-2-amino-5-phosphonovaleric acid (APV), Nickel (Ni), Ryanodine (Rya) and cyclopiazonic acid (CPA).

genuine difference between these types of synapse rather than a peculiarity of organotypic cultures (Kovalchuk et al., 2000). If these differences are real, why should spines on the same cell generate EPSCaTs by such different means?

4. The Ca^{2+} signal: analogue vs. digital

The Ca^{2+} signals generated at simple and complex spines not only differ in their source, but also in their dynamic characteristics. The all-or-none nature of the EPSCaT at simple spines is clearly demonstrated by the addition of sub-saturating concentrations of CNQX. Despite a significant reduction in the excitatory postsynaptic potential (EPSP) amplitude, and thus the synaptically evoked depolarisation at the spine, the EPSCaT amplitude remained (unpublished observations). This is consistent with an all-or none signal that is greatly amplified by internal stores. The Ca^{2+} signal in a simple spine therefore acts as a digital readout of synaptic activation. In contrast, EPSCaTs at complex spines result from Ca^{2+} influx through VACCs and NMDA receptor channels, both voltage-dependent processes. The application of CNQX at sub-saturating concentrations reduced both the synaptic amplitude and EPSCaT amplitude. Furthermore, the changes in EPSCaT amplitude brought about by different concentrations of CNQX were well correlated with the resulting changes in EPSP amplitude (unpublished observation). Thus the Ca^{2+} signal generated at the thorny excrescence synapse provides an analogue read-out of mossy fibre → CA3 synaptic strength. The dynamic range of synaptic strength at a single mossy fibre → CA3 synapse is much greater than that of a morphologically simple synapse, because it often contains multiple release sites. This also makes the dynamic range of the Ca^{2+} signal potentially large. What could be the role of graded postsynaptic Ca^{2+} responses at the mossy fibre → CA3 synapse?

5. Complex spines as electrical 'sinks' on the proximal dendrites of CA3 pyramidal neurons

Ca^{2+} acts as an activator or modulator of a number of second messenger cascades. Ca^{2+} can also gate cation chan-

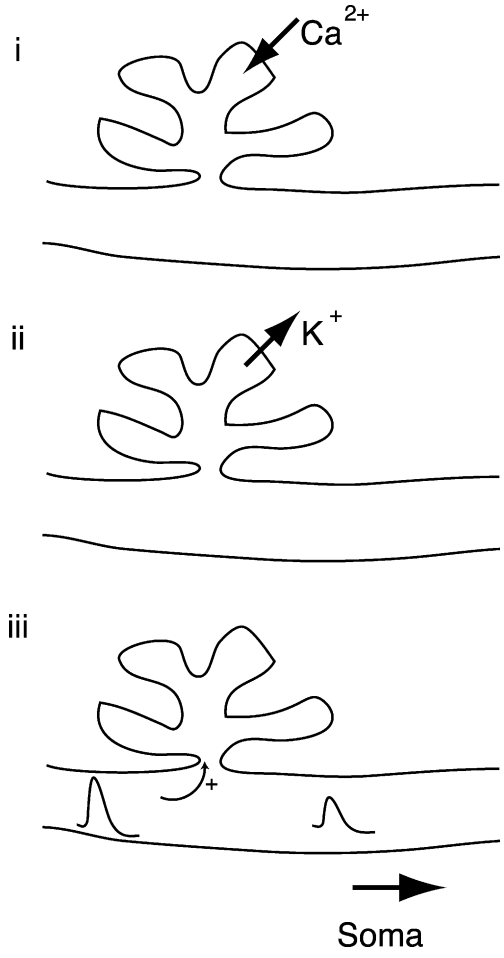

Fig. 3. A cartoon illustrating the principle of the electrical 'sink' hypothesis for complex spines on CA3 pyramidal neurons. (i) The release of glutamate from mossy fibre terminals results in an AMPA receptor-gated Ca^{2+} influx through voltage activated Ca^{2+} channels and NMDA receptors limited to the complex spine. (ii) The activation of Ca^{2+}-activated K^+ channels causes a short-lived localised hyperpolarisation of the complex spine. (iii) Because the spine is not electrically isolated from the proximal dendrite, it acts as an electrical 'sink' reducing the amplitude of EPSPs generated in the distal apical dendrites propagating towards the soma.

nels, such as Ca^{2+}-activated K^+ channels. The role of the compartmentalised Ca^{2+} signal at the mossy fibre → CA3 synapse may therefore be to activate or modulate ion channels localised to the complex spine. Modelling studies have suggested that spines are unlikely to be electrically isolated from the dendritic compartment (Koch and Zador, 1993; Svoboda et al., 1996). The proximal location of mossy fibre synapses on dendrites of places them in an ideal position to interact with synaptic potentials generated on the distal dendritic branches. The hypothesis we propose is that the complex spines could act as electrical 'sinks'. For example, the opening of Ca^{2+}-activated K^+ channels restricted to the complex spine would generate a low resistance 'sink' upon mossy fibre stimulation (Fig. 3). Excitatory potentials generated in the distal dendritic arbour propagating towards the soma would be attenuated if temporally linked to mossy fibre stimulation. Consistent with this hypothesis, a study exploring the temporal summation of mossy fibre and perforant path inputs to CA3 pyramidal cells observed that the summation of the EPSPs was sublinear if the mossy fibre was activated prior to the perforant path (Urban and Barrionuevo, 1998). This phenomenon was blocked by voltage clamping the cell at − 75 mV suggesting that the sublinear summation was voltage dependent and not just a consequence of shunting due to a mossy fibre synaptic conductance change. Additionally, the sublinear summation was blocked by 4-aminopyridine (5 mM) suggesting that the process was a consequence of the activation of transient voltage-dependent K^+ channels. However, the selectivity of high concentrations of 4-aminopyridine for voltage-dependent K^+ channels is questionable (Andreasen, 2002) raising the possibility that Ca^{2+}-activated K^+ channels may be involved. It will be of interest to investigate the effect of selective antagonists of Ca^{2+}-activated K^+ channels on this phenomenon. In addition, Urban and Barrionuevo only explored the effect of the summation of single pulses; given the large frequency facilitation observed at the mossy fibre synapse it would also be of interest to examine the effects of mossy fibre trains on temporal summation of mossy fibre and perforant path inputs. In summary, we suggest that in addition to the classical view of spines conferring synapse-specificity to the induction of plasticity, biochemical compartmentalisation at complex spines of the mossy fibre → CA3 synapse may confer synapse-specific modulation of the proximal dendritic membrane of CA3 pyramidal neurons.

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