Correction

NEUROBIOLOGY. For the article “Calmodulin priming: Nuclear translocation of a calmodulin complex and the memory of prior neuronal activity,” by Paul G. Mermelstein, Karl Deisseroth, Neela Dasgupta, Ann L. Isaksen, and Richard W. Tsien, which appeared in number 26, December 18, 2001, of Proc. Natl. Acad. Sci. USA (98, 15342–15347; First Published December 11, 2001; 10.1073/pnas.211563998), Figs. 3 and 4 did not appear in color due to a printer’s error. The color figures and their legends appear on this page and the facing page.

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Fig. 3. Qualitative consistency of CaM translocation after depolarization in various neuronal populations. (A) After a 3-min, 90-mM K⁺ stimulation, significant increases in CREB phosphorylation were observed in hippocampal neurons taken from region CA3-CA1 (P < 0.001) or dentate gyrus (DG) (P < 0.001). (B and C) In granule cells from the dentate gyrus (DG), significant increases in nuclear CaM also were observed (P < 0.05), although the changes were less pronounced than in CA3-CA1 cells (P < 0.001). (D) Increased nuclear CaM in cerebellar granule cells (Cereb) (P < 0.001), similar in magnitude to that observed in CA3-CA1 pyramidal neurons.
Fig. 4. Translocation of CaM into the nucleus depends on its binding to a target protein in a Ca\(^{2+}\)-dependent manner. (A) Hippocampal neurons transfected with cDNA encoding for ECFP-CaM exhibit a depolarization-induced nuclear translocation of fluorophore-labeled CaM, indicated by a significant increase in the ratio of nuclear to cytoplasmic fluorescence (P < 0.05). Addition of the Ca\(^{2+}\)-CaM binding peptide M13 onto ECFP-CaM prevented translocation. Translocation of ECFP-CaM was restored by mutating the M13 region [W800A, R812A (amino acid residues correspond to the smMLCK sequence)] to prevent Ca\(^{2+}\)-CaM binding (34). NS, not significant. (B and C) The CaM kinase inhibitor KN-93 (1 μM) blocked both CREB phosphorylation and CaM translocation (10, 45), suggesting that a CaM kinase (e.g., a CaMKK) may be required for CaM translocation. NS, not significant. (D) How interaction of CaM with a kinase could hasten its translocation to the nucleus. Calculated rms diffusion radius in three dimensions, at room temperature, using a diffusion coefficient (2.5 × 10\(^{-9}\) cm\(^2\)/s) measured for free CaM in smooth muscle cytoplasm (39). Also plotted is the theoretical corresponding rms radius for the diffusion of a typical large soluble protein (5 × 10\(^{-8}\) cm\(^2\)/s; e.g., kinase-bound CaM) assuming no binding interactions with cytoplasmic structures, showing greater spread over the same time period.
Calmodulin priming: Nuclear translocation of a calmodulin complex and the memory of prior neuronal activity


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The neuronal nucleus plays a vital role in information processing, but whether it supports computational functions such as paired-pulse facilitation, comparable to synapses, is unclear. Ca2+–dependent movement of calmodulin (CaM) to the nucleus is highly responsive to Ca2+ entry through L-type channels and promotes activation of the transcription factor CREB (cAMP-responsive element binding protein) through phosphorylation by CaM-sensitive kinases. We characterized key features of this CaM translocation and its possible role in facilitation of nuclear signaling. Nuclear CaM was elevated within 15 s of stimulus onset, preceding the first signs of CREB phosphorylation in hippocampal pyramidal neurons. Depolarization-induced elevation of nuclear CaM also was observed in cerebellar granule cells, neocortical neurons, and dentate gyrus granule cells. Nuclear translocation of CaM was not blocked by disruption of actin filaments or microtubules, or by emptying endoplasmic reticulum Ca2+ stores with thapsigargin. Translocation of fluorescently tagged CaM was prevented by fusing it with the Ca2+/CaM binding peptide M13, suggesting that nuclear CaM accumulation depends on association with endogenous Ca2+/CaM binding proteins. To determine whether increased nuclear CaM might influence subsequent nuclear signal processing, we compared responses to two consecutive depolarizing stimuli. After a weak “priming” stimulus that caused CaM translocation, CREB phosphorylation caused by a subsequent stimulus was significantly faster, more sensitive to Ca2+ elevation, and less specifically dependent on Ca2+ influx through L-type channels. CaM translocation not only supports rapid signaling to the nucleus, but also could provide a “memory” for facilitatory effects of repeated neural activity, seen in altered phosphorylated CREB dynamics and Ca2+ channel dependence.

The information-processing repertoire of neurons would be greatly expanded if the nucleus was able to respond to successive stimuli in a supra-additive way. This capability would support recognition of multiple closely spaced inputs beyond simple integration, computations like those synapses perform (e.g., paired-pulse facilitation). Because of the speed required, a plausible candidate for such input pattern decoding is the especially rapid signaling cascade controlling the fast activation kinetics of the key transcription factor CREB (cAMP-responsive element binding protein). This pathway is initiated in hippocampal pyramidal cells by depolarization-induced opening of L-type Ca2+ channels (1–7) and calmodulin (CaM) mobilization to the nucleus (6, 8, 9), supporting activation of CaM kinase kinase (CaMKK) and CaM kinase IV-mediated CREB phosphorylation at Ser-133 (4, 10–23). The effects of the fast CaM kinase pathway can be prolonged sufficiently in vitro and in vivo to control gene expression (10, 20, 23), likely through cooperation with the calcineurin (10, 24) and mitogen-associated protein kinase (MAPK) (22, 25) pathways. But there are few obvious functional advantages for this signaling pathway coupling to gene expression to be so fast in onset (tens of seconds), particularly because only much more prolonged CREB phosphorylation (tens of minutes) suffices for effective gene expression (10, 25). We therefore considered that this pathway may in part be designed to participate in the fast nuclear processing of multiple closely spaced inputs.

What are the likely relevant patterns of neuronal activity in vivo that would serve as the input for the candidate fast nuclear processor? Multielectrode recordings from the hippocampus of awake, freely moving rats have revealed that the dominant cell type in the hippocampus (complex-spike cells corresponding to the pyramidal neurons) is inactive for the vast majority of the time (26). Only when the rat localizes to a cell’s place field is activity seen, often consisting of brief clusters of spikes separated by intervals with little appreciable activity (e.g., ~10-s bursts at 5–10 Hz separated by 45–90 s) (27). These experiments may be biased, if at all, toward recording from the more active cells because of the necessity of seeking active cells during positioning of the electrodes, and for practical purposes elimination of many common low-frequency firing neurons (28). The best information at present therefore indicates that the natural state of the average hippocampal pyramidal cell is inactivity (~<1 Hz), insufficient to drive strong nuclear signaling (4), except when the animal is moving in the vicinity of the cell’s place field, at which time activity comes in short separated bursts. An appropriate experimental protocol might then involve starting from a physiologically quiescent baseline and providing multiple (e.g., two) spaced mild stimuli.

What could be the signature of altered nuclear signaling that would be detectable experimentally? We might expect larger or smaller responses to the second pulse, by analogy with paired-pulse facilitation and depression. The signaling could also be faster or slower, although it would seem a challenge to make this fast signaling even faster. More interestingly, it is possible that the information content of the signaling pathway from membrane to CREB could be altered. The specific route of Ca2+ entry rather than bulk Ca2+ elevation is in many cases the relevant parameter in nuclear signaling (6, 7, 29), and one recent study has clearly implicated physical coupling between the L-type Ca2+ channel and CaM as part of the mechanism for generating this specificity in phosphorylated CREB (pCREB) formation (7). Signaling to CREB therefore can communicate information about activity of specific membrane channels and because L-type Ca2+ channels respond selectively to certain types of electrical activity (30), signaling to CREB also can carry information about activity of specific membrane channels and because L-type Ca2+ channels respond selectively to certain types of electrical activity (30), signaling to CREB also can carry

Abbreviations: CaM, calmodulin; CaMKK, CaM kinase kinase; CREB, cAMP-responsive element binding protein; pCREB, phosphorylated CREB; MAPK, mitogen-associated protein kinase; TTX, tetrodotoxin; CFP, cyan-fluorescent protein; ECFP, enhanced CFP.

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specific information about electrical activity (4). This specificity could, in principle, also be altered in an activity-dependent way.

One of the most striking characteristics of the fast activity-dependent CREB phosphorylation in hippocampal CA3–CA1 pyramidal neurons is its absolute dependence on L-type Ca\(^{2+}\) channels and N-methyl-D-aspartate receptors (4, 21, 31). When these channels are blocked, other channel types generate similarly robust increases in somatic and nuclear Ca\(^{2+}\) but consistently fail to give any significant CREB phosphorylation (6). This fact demonstrates that fast CREB phosphorylation, in these cells, simply cannot be driven by elevations in bulk nuclear Ca\(^{2+}\) alone. Still, in neocortical neurons in the presence of blockers of N-methyl-D-aspartate receptors and L-type channels a small fast component of pCREB can still be seen (7). These data indicate that, in principle, conditions can exist in which the strict channel-type dependence can be relaxed. It would be of computational significance if CA3–CA1 pyramidal cells could be temporarily converted to this mode by prior activity, as the resulting nuclear events would be more sensitive but less specifically attuned to surface membrane events.

**Methods**

Neurons were taken from the CA3–CA1 region of the hippocampi of 1- to 2-day-old rat pups as described (4). Cells were similarly cultured from the hippocampal dentate gyrus, cerebellar cortex, and neocortex (6–8 days in vitro for dentate gyrus, 8–10 days in vitro for all other neurons). Because neurons are highly active because of extensive recurrent connections in dissociated culture and both pCREB formation and CaM translocation take ~2 h to reverse (6), to obtain a physiological baseline neurons were preincubated ~2 h at room temperature in Tyrode solution containing 129 mM NaCl, 5 mM KCl, 2 mM Ca\(^{2+}\), 1 mM MgCl\(_2\), 30 mM glucose, 25 mM Hepes, 0.1 mM glycine, 0.001 mM tetraoxotoxin (TTX), pH 7.35. Where indicated, solutions also contained nifedipine (5 \(\mu\)M) or D-APS (50 \(\mu\)M).

Neurons were then washed twice with PBS containing 100 \(\mu\)M glycine and permeabilized for 5 min in block solution (PBS with 4% goat serum) containing 0.1% Triton X-100. We have also used an overnight permeabilization protocol with 0.4% saponin (Sigma) with comparable results. The cells were then washed and stained for 1–2 h in block solution containing anti-pCREB polyclonal [a marker of CREB phosphorylation, a downstream process to CaM translocation, is unaffected by the composition of the fixation solution, other channel types may give similar CREB phosphorylation (8)]. Pyramidal neurons were depolarized with 90 mM K\(^{+}\). However, without EGTA in the fixation solution, nuclear CaM was elevated even in the “unstimulated” neurons, occluding the stimulus-evoked increase (Fig. 1). As expected, CREB phosphorylation was unaffected by the composition of the fixation solution, because it is a slower, downstream process. Increasing the temperature of the fixation solution from 4°C to room temperature also increased apparent basal levels of nuclear CaM, particularly when Ca\(^{2+}\) chelators were not present (data not shown).

**Results**

Establishing Experimental Conditions for Studying Fast Signaling. To explore fast nuclear paired-pulse processing, we characterized the onset of the CaM translocation (Figs. 1 and 2). CaM translocation is so fast that fixation conditions may play a role in these experiments (Fig. 1). Previous reports have demonstrated that aldehyde fixation causes an immediate and massive cytosolic Ca\(^{2+}\) increase (35), thus allowing fast Ca\(^{2+}\)-dependent processes to proceed during fixation (36). We buffered external Ca\(^{2+}\) in the fixative with 4 mM EGTA to prevent the paraformaldehyde solution from acting as a trigger for CaM translocation. Under these conditions, CaM translocation and CREB phosphorylation were clearly observed in cultured CA3–CA1 hippocampal pyramidal neurons depolarized with 90 mM K\(^{+}\). However, without EGTA in the fixation solution, nuclear CaM was elevated even in the “unstimulated” neurons, occluding the stimulus-evoked increase (Fig. 1). As expected, CREB phosphorylation was unaffected by the composition of the fixation solution, because it is a slower, downstream process. Increasing the temperature of the fixation solution from 4°C to room temperature also increased apparent basal levels of nuclear CaM, particularly when Ca\(^{2+}\) chelators were not present (data not shown).

Paired-Pulse Facilitation of Nuclear Signaling to CREB. Using the appropriate Ca\(^{2+}\) chelation during fixation, we next characterized the very early kinetics of this pathway (Fig. 2A). Pyramidal neurons were depolarized with 90 mM K\(^{+}\) for 15 s, then fixed at various time points. Significant increases in nuclear CaM were seen at 15 s, when pCREB did not yet significantly increased (consistent with the idea that CaM translocation can participate in even the fastest CREB activation). We reasoned that because elevation of nuclear CaM slowly reverses over tens of minutes to hours after a single stimulus (6), translocation of CaM after an initial stimulus might influence signal processing of a subsequent stimulus. To test this hypothesis, based on the reasoning given earlier, we applied a mild paired-pulse protocol using 20 mM K\(^{+}\) and a spacing of 45 s. As found with the stronger stimulus (Fig. 2A), CaM translocation, but not pCREB, was detectable within 15 s of a single weak stimulus (Fig. 2B). However, after this first stimulus, a marked increase in pCREB was observed within 15 s of the onset of a second stimulus. This extremely fast pCREB increment was not attributable to augmented Ca\(^{2+}\) influx during the second stimulus (Fig. 2C). Rather, it appeared to be a novel form of nuclear paired-pulse facilitation whereby one input primes the next.

**Relaxed Channel Specificity in the Primed State.** The increase in speed of CREB phosphorylation might be caused by an event such as the reuse of recently translocated CaM and/or associated molecules. If so, the known dependence of fast pCREB formation on specific Ca\(^{2+}\) entry pathways (L-type channels and N-methyl-D-aspartate receptors) also might be changed, because this strict dependence is likely caused by the unique ability of
these channel types to mobilize a CaM complex to the nucleus in pyramidal neurons. Accordingly, we compared the effects of the L-type channel inhibitor nifedipine on ‘unprimed’ and ‘primed’ responses. As expected (6), the increment in pCREB associated with the first stimulus was largely ablated by blockade of L-type channels (Fig. 2D, c). In contrast, the rapid primed CREB phosphorylation caused by the second stimulus remained in the presence of nifedipine (Fig. 2D, d); this finding was particularly striking because L-type channel blockade greatly reduced the second Ca²⁺ transient, leaving only a small residuum of Ca²⁺ entry caused by other pathways (Fig. 2C). Further, we have found that in primed neurons, as in neocortical neurons (7), rapid CREB phosphorylation can still proceed at reduced levels where both L-type Ca²⁺ channels and N-methyl-D-aspartate receptors were blocked (P.G.M., unpublished data). From these results we can conclude that priming greatly increased the Ca²⁺ sensitivity of the CREB phosphorylation and likely broadened its acceptance of Ca²⁺ sources. This influence of past activity on nuclear signal processing is consistent with a model in which Ca²⁺ interacts with recently translocated apo-CaM or CaM complexes to activate the fast CaM kinase IV cascade.

**Cellular Diversity of CaM Translocation.** Ca²⁺-dependent CaM translocation to the nucleus has now been reported by using a variety of techniques in hippocampal pyramidal neurons (6) as well as in cortical neurons (8), sensory ganglion neurons (37), neuroblastoma cells (9), pancreatic acinar cells (38), smooth muscle cells (39, 40), rat basophilic leukemia cells (41), and human embryonic kidney cells (9), indicative of a widely used nuclear signaling pathway. The results in Fig. 2 were obtained with hippocampal pyramidal neurons in cultures from the CA3 region.
cases Ca\(^{2+}\) caused by cells experiencing high recent levels of activity during
suggesting that variations in the degree of CaM translocation do
can result from either variations in CaM translocation char-
acteristics in different cell types, or persistent
variation in CaM translocation dependence of nuclear processes

CaM. In contrast, granule cells from cerebellum showed CaM
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Pronounced, albeit significant (\(P<0.001\)). (G and H) Increased nuclear
CaM in cerebellar granule cells (Cereb) (\(P<0.001\)), similar in magnitude to that
observed in CA3–CA1 pyramidal neurons.

CA1 regions. In contrast, little CaM translocation was reported
in a study of total hippocampal cultures, likely to include a
proportion of dentate granule cells as well as pyramidal neurons
(42). Accordingly, we compared dentate gyrus cells with CA3–
CA1 pyramidal neurons (Fig. 3 A–C). With similar depolarization-
induced CREB phosphorylation (Fig. 3A), CaM transloca-

tion in dentate granule cells (Fig. 3 B and C) was less
pronounced, albeit significant (\(P<0.05\)). One contributory
factor to the difference with CA3–CA1 pyramidal neurons was
that the granule cells displayed higher basal levels of nuclear
CaM. In contrast, granule cells from cerebellum showed CaM
translocation as large as in hippocampal CA3–CA1 neurons,
suggesting that variations in the degree of CaM translocation
do not strictly depend on cell size. It is interesting to speculate that
variations in “basal” levels of nuclear CaM and subsequent
variation in CaM translocation dependence of nuclear processes
could result from either variations in CaM translocation char-
acteristics in different cell types, or persistent “CaM priming”
causd by cells experiencing high recent levels of activity during
dissection or in culture. In fact, it appears likely that in some
cases Ca\(^{2+}\) entry route specificity can be completely relaxed
through one of these mechanisms, as Ca\(^{2+}\) alone was found to
generate detectable pCREB in a preparation of partially lysed
cells (42).

CaM Interacts with a CaM-Binding Molecule for Translocation. The
speed and robustness of CaM translocation raises interesting
mechanistic questions. At face value, free diffusion is the
simplest explanation, but translocation would be greatly re-
tarded by vigorous CaM buffering within the cytoplasm (39). This
finding suggests CaM translocates while complexed with
another protein. Such a complex has been previously hypoth-
esized (6) to be of additional theoretical value in stabilizing the
Ca\(^{2+}\)-bound form of CaM, because thermodynamic cycle
analysis dictates that high affinity binding to a Ca\(^{2+}\)/CaM-
selective protein must greatly increase the stability of Ca\(^{2+}\)/
CaM and thereby allow the signaling to proceed even in the
presence of high affinity Ca\(^{2+}\) buffers. Using various con-
structs of CaM fused to enhanced CFP (ECFP), we examined
whether translocation of CaM would be impaired if Ca\(^{2+}\)-
dependent interactions were disrupted. In control experi-
ments, ECFP-CaM showed a stimulus-induced increase in
nuclear localization (Fig. 4A Left). In contrast, addition of the
CaM-binding peptide M13 to the C terminus of the ECFP–
CaM eliminated the nuclear increase (Fig. 4A Middle). This
inhibition was not found with a modified version of M13
containing mutations at key positions involved in Ca\(^{2+}\)-
dependent binding to CaM (34) (Fig. 4A Right). Thus, CaM
translocation is prevented by a specific interaction with M13
(40), suggesting that normally, Ca\(^{2+}\)-dependent association
with an endogenous CaM binding protein is necessary to drive
nuclear CaM translocation.

Among the many possible candidates that may translocate
with CaM to the nucleus, we have already excluded certain
Ca\(^{2+}\)/CaM-dependent kinases (6) and calcineurin (see addi-
tional text and Fig. 5, which is published as supporting informa-
tion on the PNAS web site, www.pnas.org), but ras/MAPK
pathway messengers such as the CaM-binding Ras-GRF (43),
and isoforms of CaMKK remain viable possibilities. We explored
the latter possibility by use of KN-93, a small molecule that
specifically binds to and inhibits CaM kinases and CaMKKs
solely by interfering with CaM binding (44). Interestingly, KN-93
not only blocked CREB phosphorylation (Fig. 4B) but also
completely prevented CaM translocation (Fig. 4C). The inactive
homolog KN-92 did neither (10, 45) (Fig. 3C). These data
suggest that CaM may require binding to a kinase to move to the
nucleus. This signaling pathway, however, does not require an
intact cytoskeleton, as disruption of microtubules with nocodo-
zole or actin filaments with cytochalasin D had no effect on CaM
translocation or CREB phosphorylation. Intracellular Ca\(^{2+}\)
stores were also not required (see additional text and Figs. 6 and
7, which are published as supporting information on the PNAS
web site).

Because of the high CaM-binding capacity in cytoplasm (39),
formation of a CaM complex would promote CaM transloca-
tion to the cell body simply by speeding its diffusion (Fig. 4D).
It is interesting to note that based on previous diffusion
coefficient measurements in cytoplasm (39), the rms diffusion
radius for free CaM would correspond to \(<5\) \(\mu\)m at 15 s; in
contrast, a radius of \(>20\) \(\mu\)m can be estimated for a typical
soluble protein with no retardation by binding. Thus, on the
rapid time scale of CREB phosphorylation, a complex con-
sisting of Ca\(^{2+}\)/CaM and another protein could readily read-
ch the cell body, although full translocation into the nucleus may
involve an additional nondiffusive, strongly temperature-
derpendent step (6, 40, 46).

Discussion
A Role for the CaM Pathway in Fast Temporal Processing by the
Nucleus. In hippocampal pyramidal cells, CREB phosphory-
lation is normally observed only with CaM translocation into
the nucleus, a process primarily dependent on activation of
L-type calcium channels. However, in the wake of an appro-
riate priming stimulus, further stimulation causes robust,
faster formation of pCREB apparently without the need for
further CaM translocation. Further, the restriction for Ca\(^{2+}\)
to enter a cell by means of L-type Ca\(^{2+}\) channels for CREB
phosphorylation to occur is relaxed. This effect of neuronal
priming represents a fast temporal integration of intracellular
signals by the nucleus, resulting in altered nuclear signaling
(more sensitive and less specific) that could play an important
role in the processing underlying memory formation in the hippocampus.

**Quantitative Considerations in CaM Signaling.** Because some CaM is constitutively present in the nucleus (6), why might additional CaM be important for controlling nuclear events like CREB phosphorylation? The nuclear CaM kinase cascade of CaMKK and CaM kinase IV steeply depends on CaM, so that even a 2-fold change in free CaM would likely be significant. In turn, stimulus-induced increases in free nuclear CaM are likely to be much greater than 2-fold; because of constitutive interactions with nuclear entities such as histones (47), free CaM represents only a small fraction of total CaM [estimates range from 1/20 to 1/1,000 (48, 49)]. Therefore, simple translocated CaM could greatly stimulate the CaM kinase cascade, during both unprimed and primed responses. Furthermore, if the CaM-binding molecule critical for nuclear CaM translocation is itself a CaMKK, the net effect of the translocated complex on initial CREB phosphorylation would be even more substantial. Needless to say, different cell types in different preparations could show varying dependence on CaM translocation depending on their "basal" free CaM levels, which in turn would likely depend in part on their recent history.

**Nature and Significance of CaM Priming.** But what is the priming event itself? Because free nuclear CaM is likely to be limiting, simple persistence of free apo-CaM could greatly enhance the speed and sensitivity (although reduce the specificity) of subsequent responses by responding to nuclear Ca^{2+}, whatever the source. The key priming event also could be an increase in nuclear CaMKK or even the CaMKK-mediated phosphorylation of CaM kinase IV itself. On the other hand, if the CaM-binding molecule in the translocating complex plays a role in MAPK signaling (e.g., Ras-GRF) the priming event also could involve activation of the MAPK pathway to CREB and could underlie reported L-type channel dominance (7, 22) and CaM involvement (7, 22) in slow MAPK signaling to CREB.

A rapidly translocating CaM complex appears to have appropriate quantitative properties to act as a mediator of both rapid nuclear signaling and nuclear memory. Moving Ca^{2+} sensors from the neighborhood of L-type channels to the nucleus can be thought of as priming the neuron in response to a weak stimulus, altering its response to future stimuli. The consequences for pCREB formation and ultimately, gene expression are both quantitative (sensitizing the system to small Ca^{2+} signals) and qualitative (allowing participation of

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**Fig. 4.** Translocation of CaM into the nucleus depends on its binding to a target protein in a Ca^{2+}-dependent manner. (A) Hippocampal neurons transfected with cDNA encoding for ECFP–CaM exhibit a depolarization-induced nuclear translocation of fluorophore-labeled CaM, indicated by a significant increase in the ratio of nuclear to cytoplasmic fluorescence (P < 0.05). Addition of the Ca^{2+}–CaM binding peptide M13 onto ECFP–CaM prevented translocation. Translocation of ECFP–CaM was restored by mutating the M13 region [W800A, R812A (amino acid residues correspond to the smMLCK sequence)] to prevent Ca^{2+}–CaM binding (34). NS, not significant. (B and C) The CaM kinase inhibitor KN-93 (1 μM) blocked both CREB phosphorylation and CaM translocation (10, 45), suggesting that a CaM kinase (e.g., a CaMKK) may be required for CaM translocation. NS, not significant. (D) How interaction of CaM with a kinase could hasten its translocation to the nucleus. Calculated rms diffusion radius in three dimensions, at room temperature, using a diffusion coefficient (2.5 × 10^{-8} cm²/s) measured for free CaM in smooth muscle cytoplasm (39). Also plotted is the theoretical corresponding rms radius for the diffusion of a typical large soluble protein (5 × 10^{-8} cm²/s; e.g., kinase-bound CaM) assuming no binding interactions with cytoplasmic structures, showing greater spread over the same time period.
a broader range of Ca\textsuperscript{2+} sources). It appears that the selective communication between L-type channels and CaM not only enables preferential coupling between synaptic depolarizations (as opposed to action potentials) and CREB phosphorylation during an initial round of Ca\textsuperscript{2+} entry (30), but through priming, also could promote increased recognition of other forms of electrical activity (50) over a subsequent period. It will be of great interest to test for altered primed responses to low frequency or brief electrical stimuli, map out the temporal characteristics (onset and decay) of the priming, determine the types of prepulse stimuli capable of providing the priming, and correlate these findings with the known in vivo patterns of hippocampal pyramidal cell activity.

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