Signaling to the Nucleus by an L-type Calcium Channel-Calmodulin Complex Through the MAP Kinase Pathway

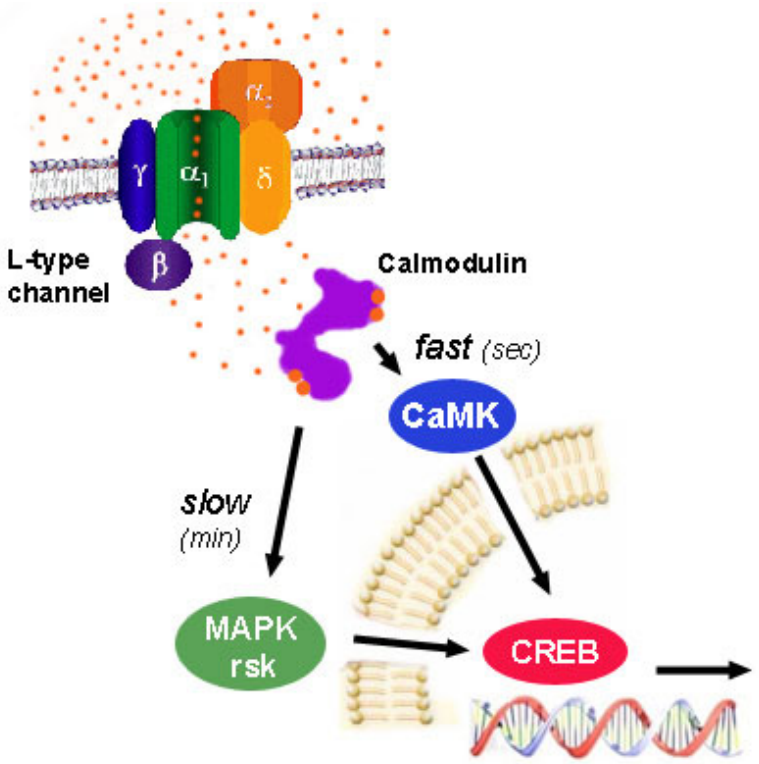
Ricardo E. Dolmetsch, Urvi Pajvani, Katherine Fife, James M. Spotts, Michael E. Greenberg

Science 294: 333 - 339 *(2001)*

Presented by Debbie Castillo

Ca^{2+} Signals in Biological Systems

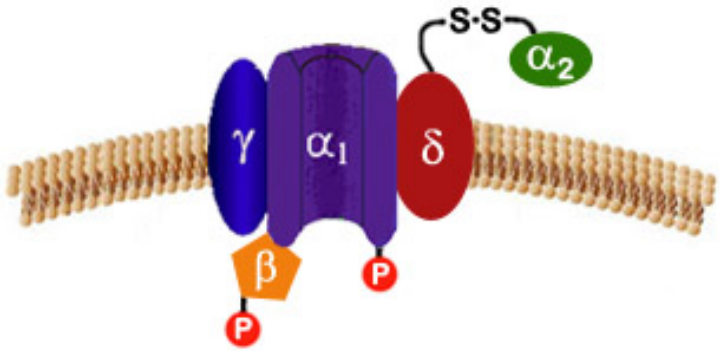
April 27, 2006



Introduction:

- Voltage-gated channels in general play a central role in neuronal function
- They convert electrical activity into biochemical events
- This includes pathways that lead to gene expression essential for dendritic development, neuronal survival, and synaptic plasticity among others.
  - CREB, MEF-2
- Neurons express at least 9 types of voltage gated Ca^{2+} channels, each carrying a different function
- Focus of this paper: L-Type Channels (LTC's)

Structure of LTC



P phosphorylation site

S-S disulfide bridge

- Oligomers:
  - α1:pore-forming subunit; responsible for binding to drugs
  - β, α2δ, γ(some cases)

LTC's role in Neurons

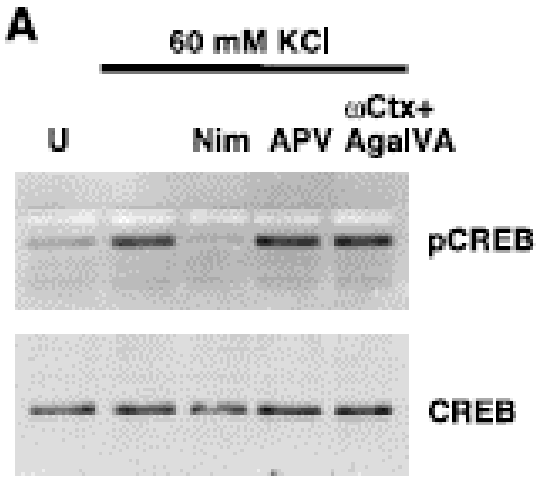
- Activates transcription factors such as CREB and MEF-2
- Which in turn increase expression of a group of Ca^{2+} genes such as c-fos, brain-derived neurotrophic factor (BDNF) and Bcl-2
- Important for neuronal survival, learning and adaptive responses
- Blocked by dihydropiridines (DHP's), diltiazem

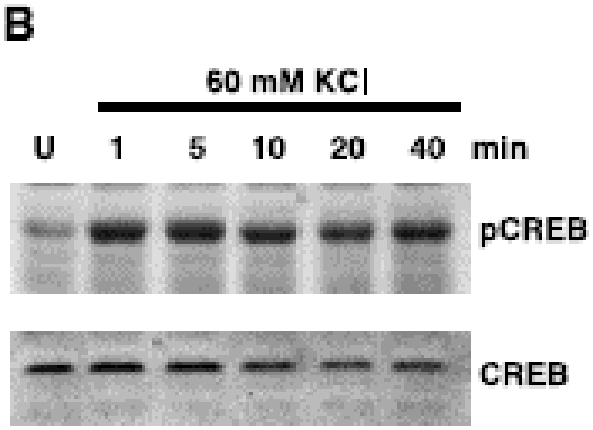
cAMP Response Element Binding Protein (CREB)

- Activated by phosphorylation of Ser 133
- Drives expression of a number of genes responsible for regulating neuronal survival and plasticity
- Allows recruitement of CREB binding protein (CBP) and initiates transcription

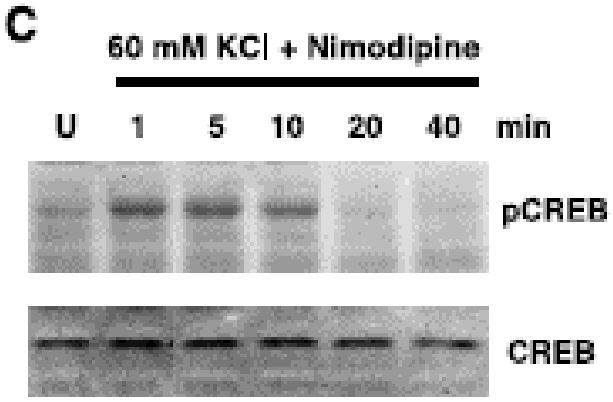
Is activation dependent of LTC's?

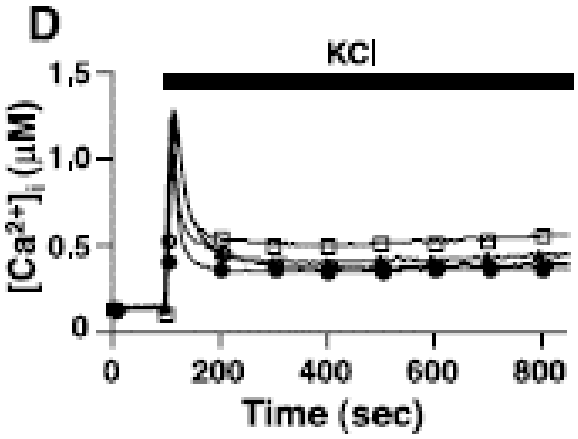
Prolonged Activation of CREB is dependent on LTC's





Prolonged activation of CREB is required for transcription.

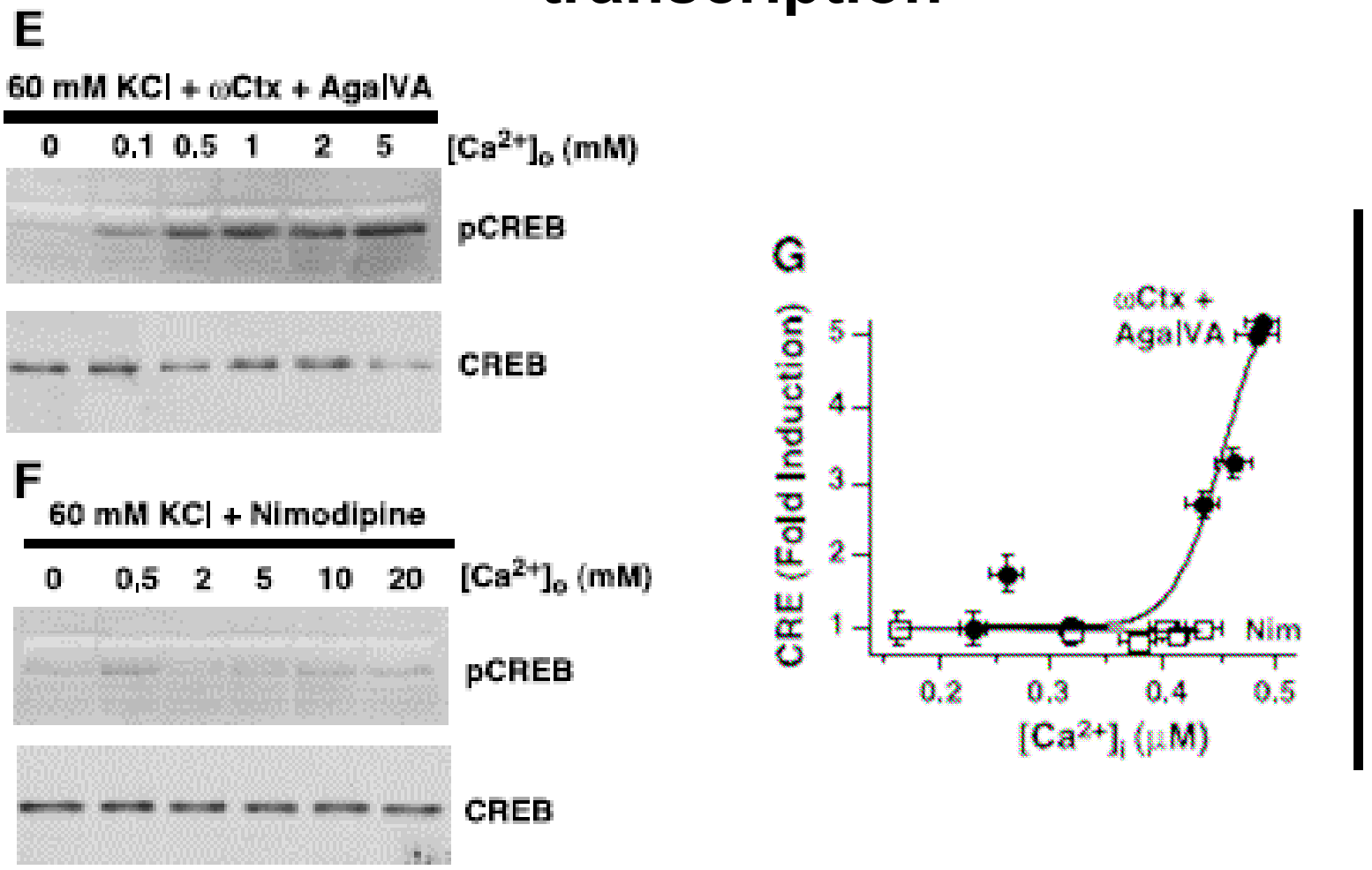




CREB-dependent transcription is most effectively activated by calcium influx via LTC's only

What about the Ca++ influx from R, N, and P/Q channels?

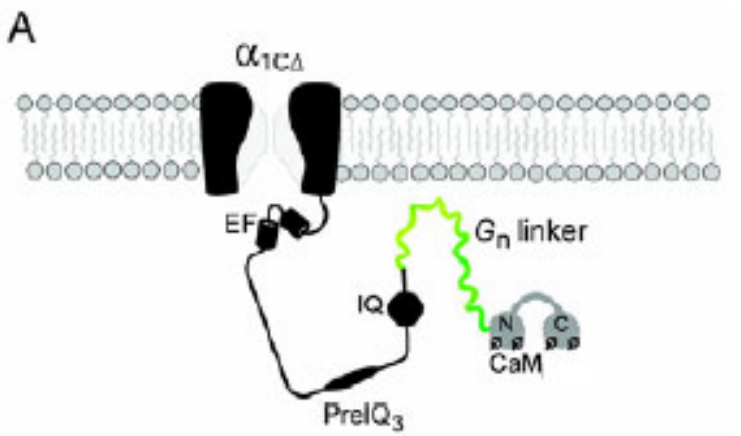
R, N and P/Q Calcium is not specific for CREB activation and CREB-dependent gene transcription



What Feature of LTC selectively couples it to regulation of genes?

- Ca^{++} binding protein bound to the channel (i.e. CaM) senses local $[Ca^{2+}]_i$ and selectively activates signal

el is open.



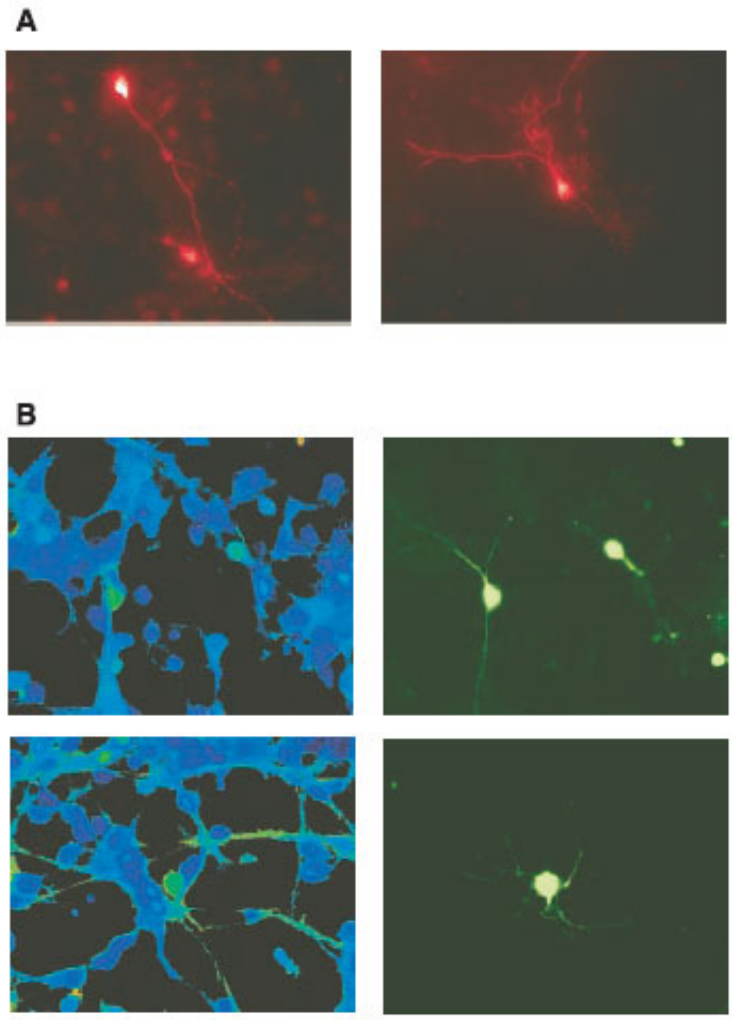
Mori et al., (2004), *Science*, 304:432-5

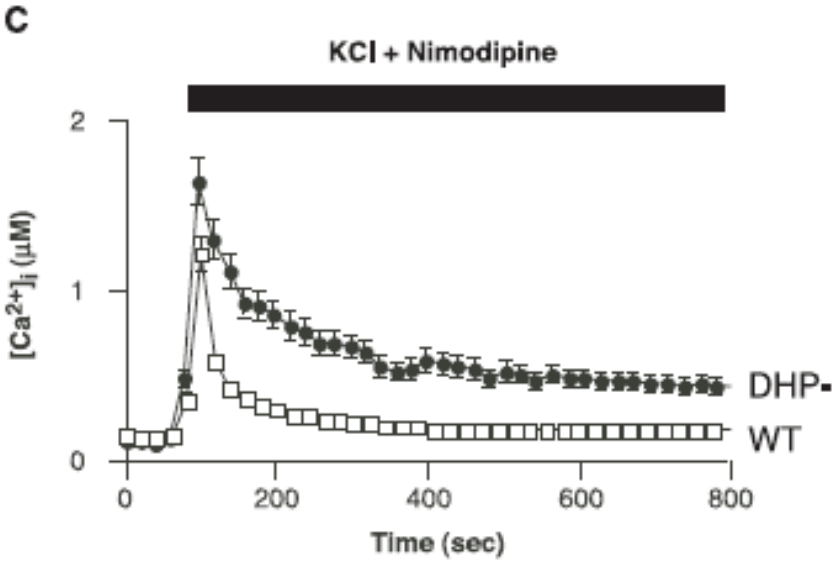
• Downstream pathway leads to activation of CREB via phosphorylation by MAPK or CaMK

Method: Knock-in Technique

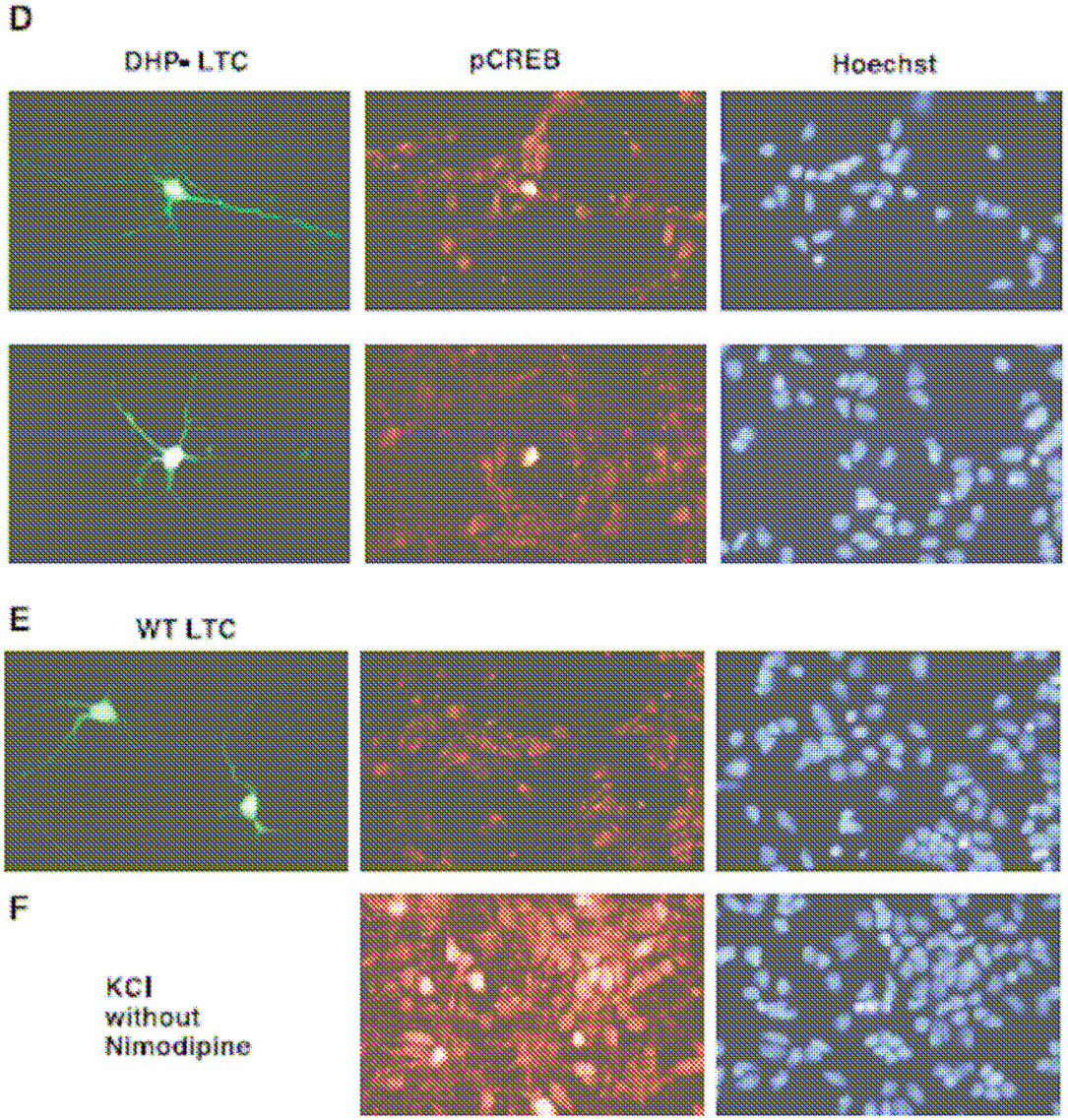
- Introduce exogenous mutant LTC's into primary neurons
- Mutations located in pore0subunit and cytoplasmic domains of LTC
  - α1C position 1039 [Thr→ Tyr] 100X less sensitive to DHP (DHP-LTC)
  - Cytoplasmic mutations on carboxyl terminus of LTC that binds to CaM (in particular: IQ domain)
- Treated with nimodipine, APV, ω-conotoxin MVIIC, ω-Agatoxin-IVA to block endogenous channels (and endo calcium rise)
- Depolarize neurons to -30mV (add. Of 60mM KCl)
- Observe influx of $[Ca^{++}]_i$ and activation of CREB produced (or not produced) by mutated LTC's

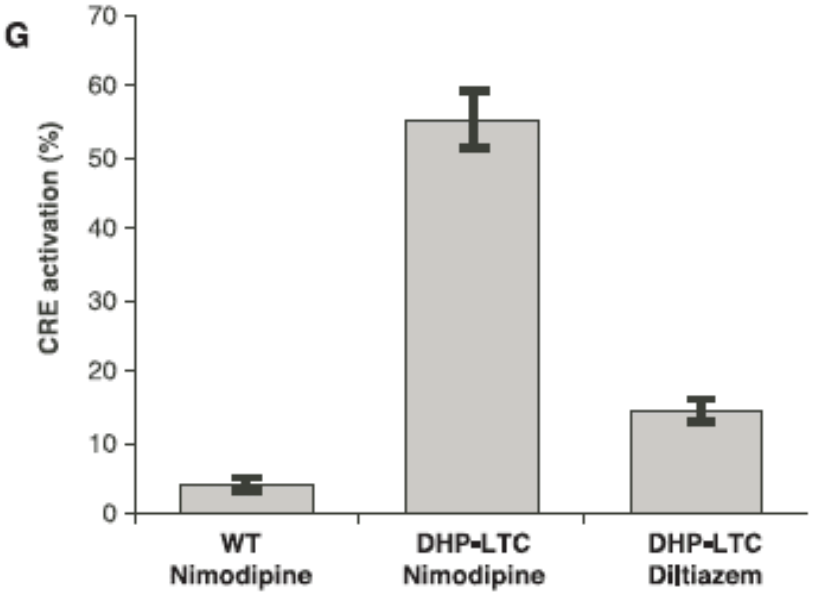
DHP-LTC is expressed in Neurons and elevates intracellular calcium



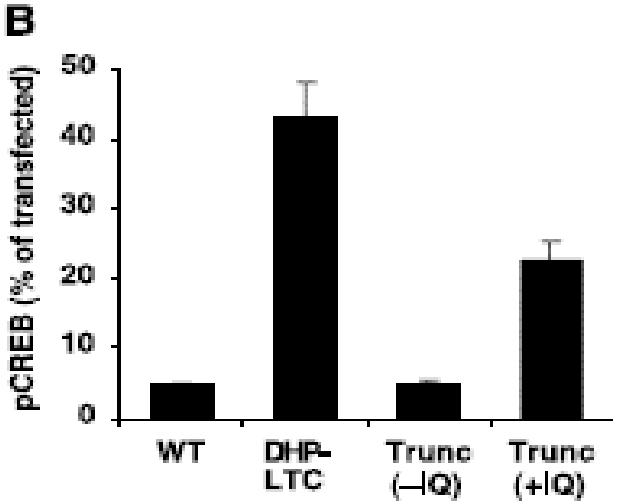


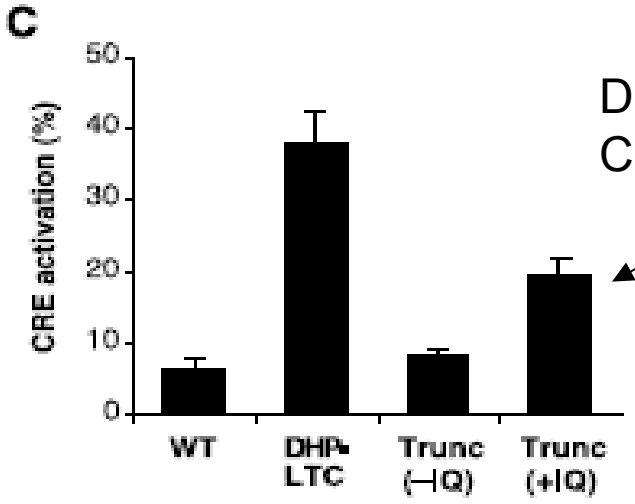
DHP-LTC Activates CREB



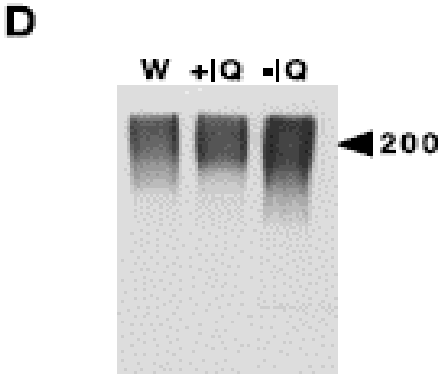


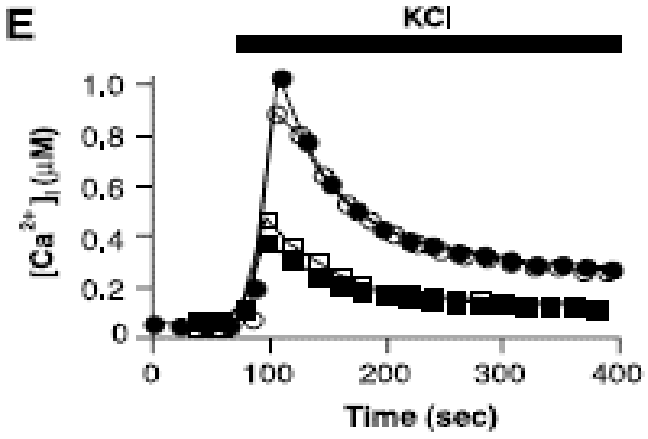
Complete Removal of IQ in Carboxyl Terminus Eliminates signaling to CREB





Diminished activation of CREB and CRE





Closed circles: DHP-LTC +IQ

Open circles: DHP-LTC

Closed square: DHP-LTC –IQ

Open Squares: WT LTC

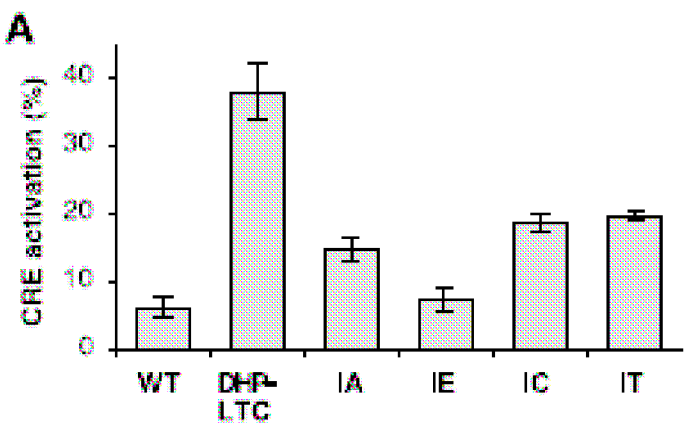
* In presence of channel blockers

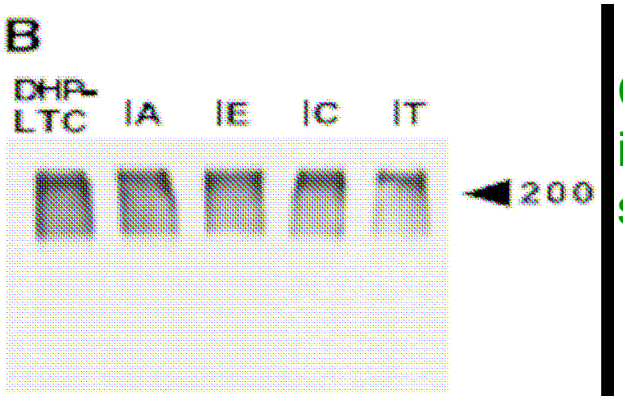
Why are +IQ mutations defective in prolonging activation of CREB?

Possibilities:

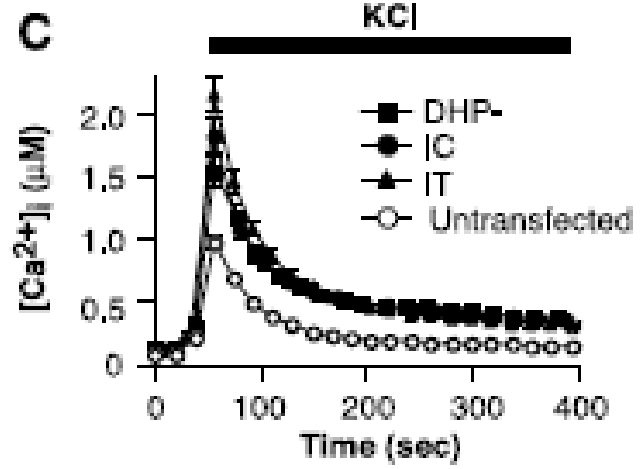
1. **Eliminates binding signaling proteins to carboxyl terminus** (further mutations downstream of IQ→ this half of COOH terminal not needed; channels as effective as DHP-LTC's)
2. **Disrupts the binding of CaM to the IQ motif**
   - Point Mutations of isoleucine 1627 in IQ motif to prevent CaM:
     - **(IA):** alanine
     - **(IE):** glutamate
     - **(IC):** cysteine
     - **(IT):** threonine

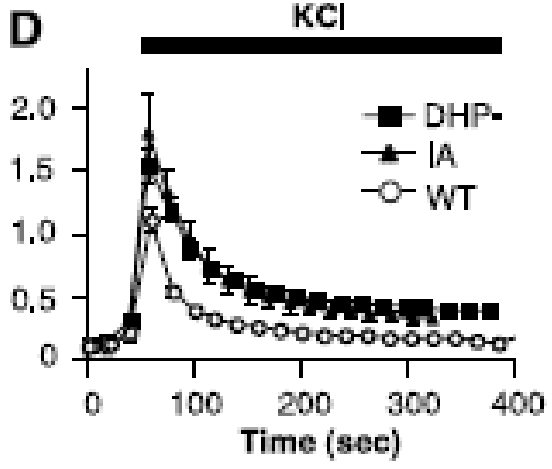
Point Mutations Not able to activate CRE or prolong CREB phosphorylation, but most able to sustain $[Ca^{++}]_i$ rise

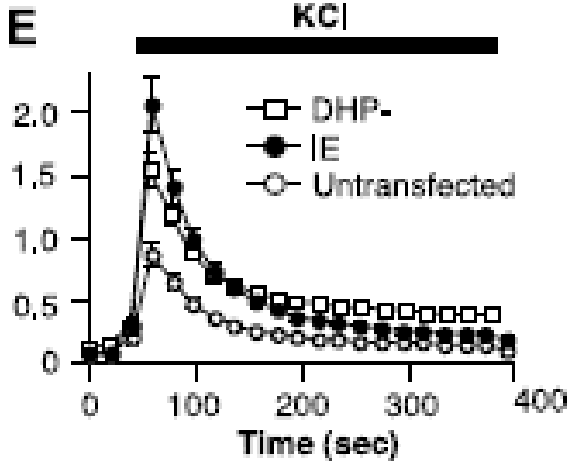




CaM binding to LTC is important for signaling to CREB

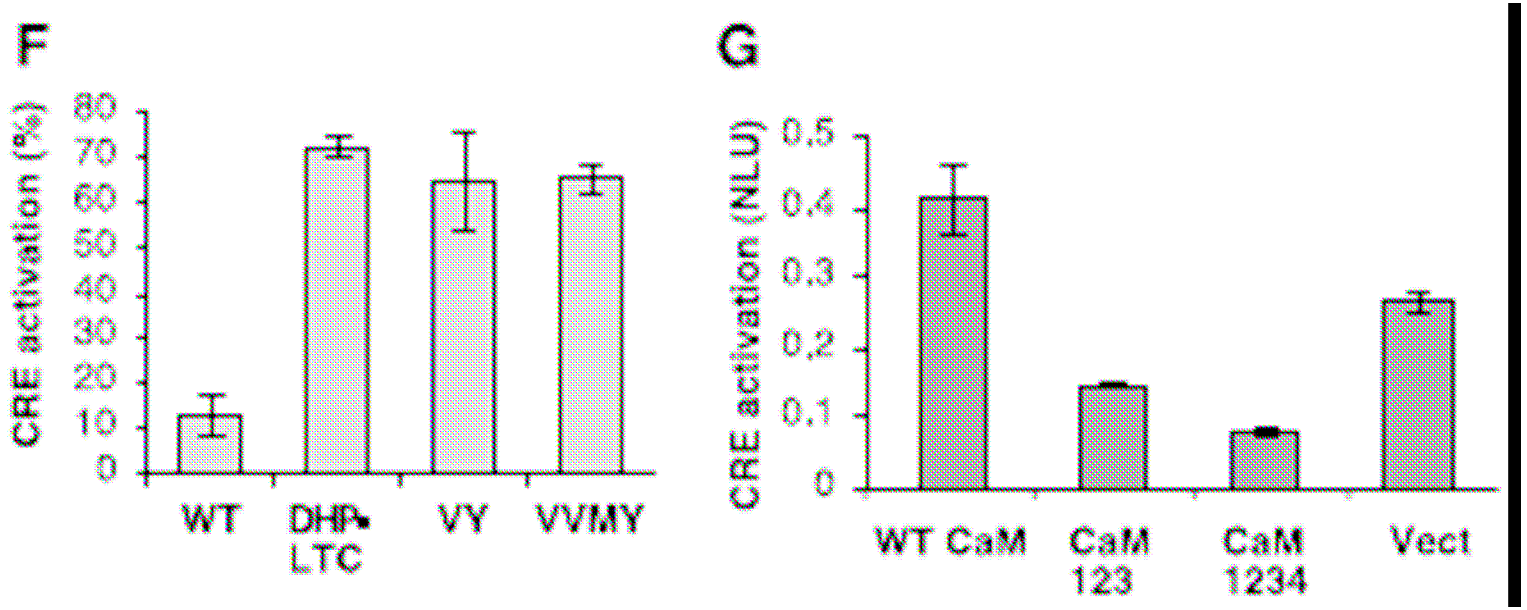






LTC's with point mutation are defective in Ca-dependent channel inactivation

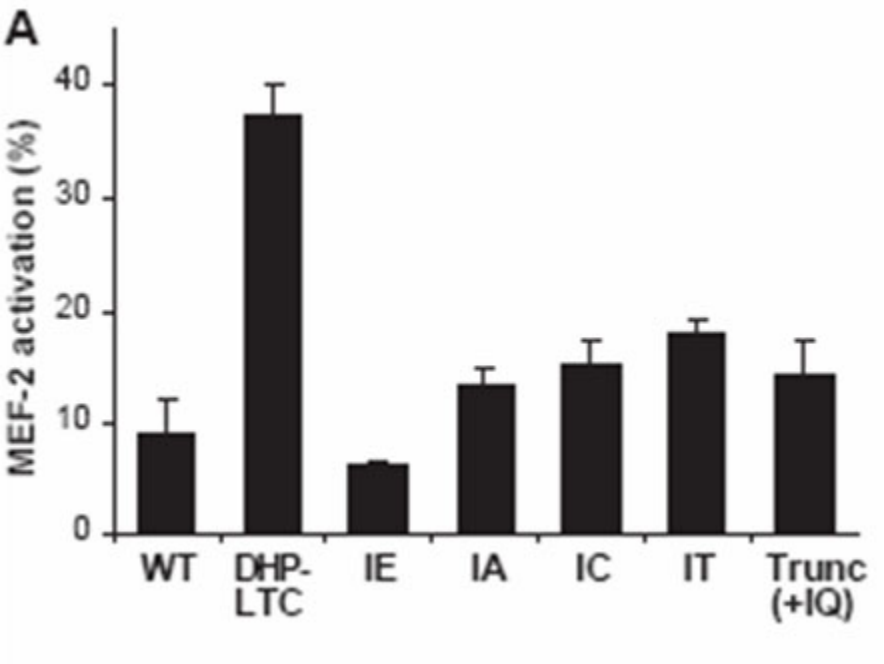
Is it require for LTC signaling to CREB?

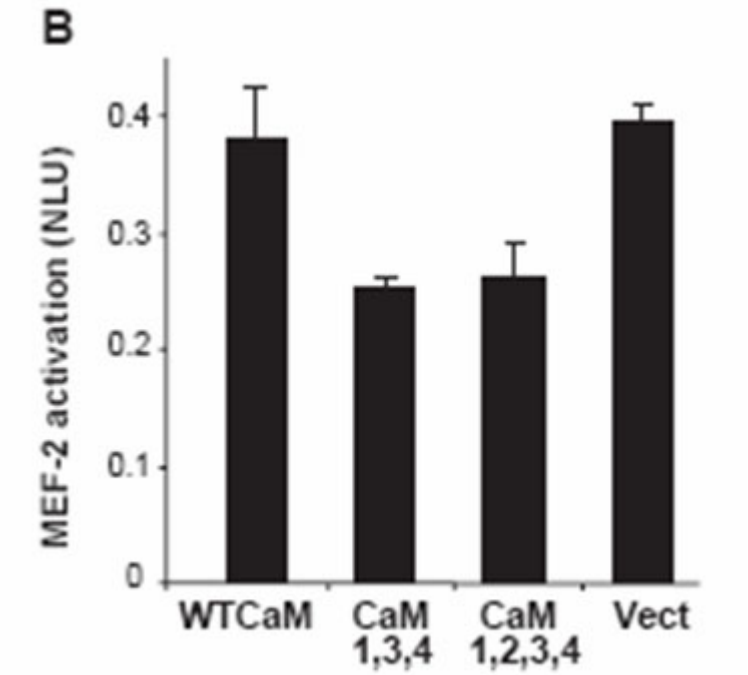


Mutations in EF hands: cause defect in Ca-dep inact., but intact IQ motif→ bind CaM

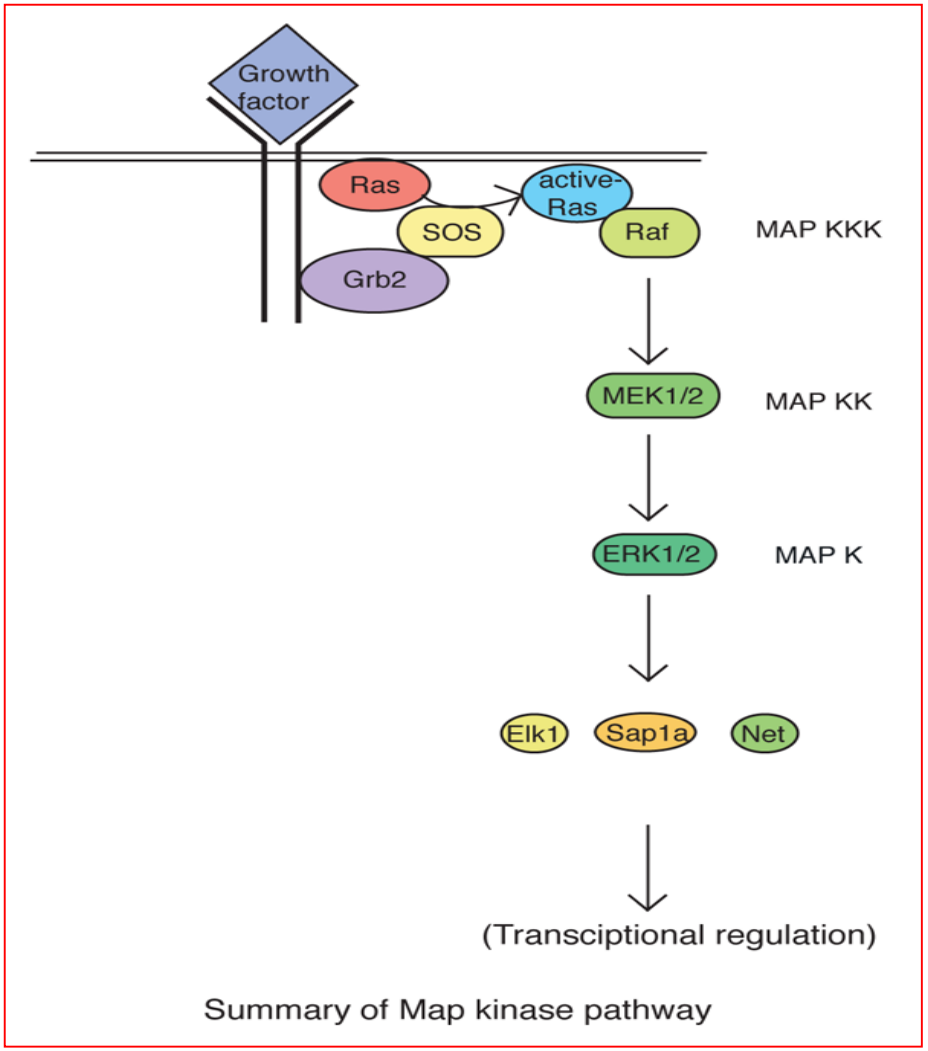
CaM binding to LTC's IQ motif is important for signaling to CREB

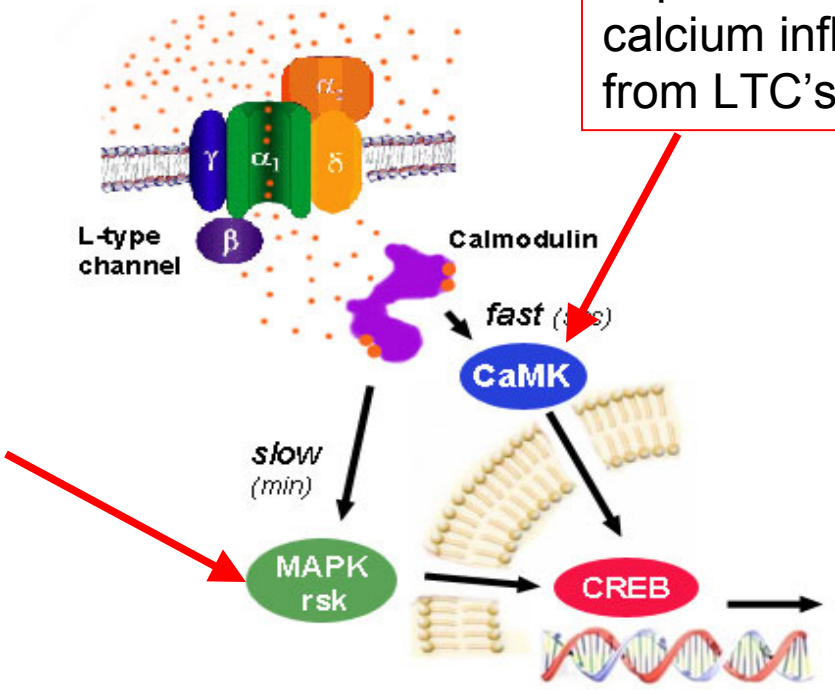
CaM Binding to LTC IQ motif Also Required for Activation of MEF-2





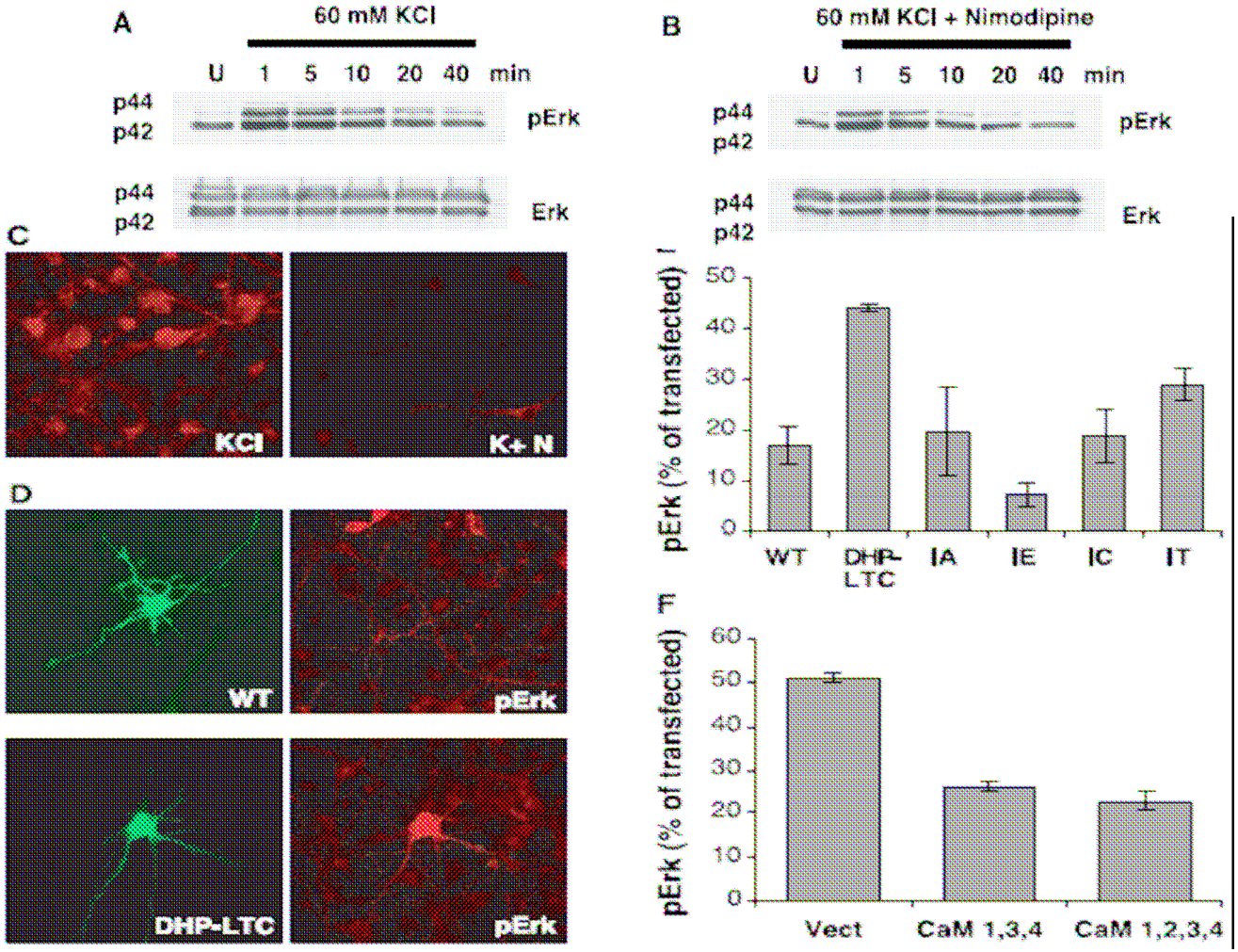
CaM Binding to LTC activates CREB via MAPK pathway





Not directly dependent on calcium influx from LTC's

MAPK Erk1/2 activation also LTC Calicum-CaM-IQ Dependent



Summary

- Prolong activation of CREB is LTC dependent
- Using Knock in technique, can show that the IQ motif is essential for CaM binding
- This CaM-IQ binding is dependent on Ca influx from LTC
- The Ca-CaM-IQ association of the LTC, signals to CREB via MAPK (either conformational change in LTC or via other signaling proteins.)

Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity

Giles E. Hardingham, Fiona J. L. Arnold and Hilmar Bading

Nature Neuroscience 4, 261 - 267 *(2001)*

Presented by Debbie Castillo

Ca^{2+} Signals in Biological Systems

April 27, 2006

Opposing Views

Vs.

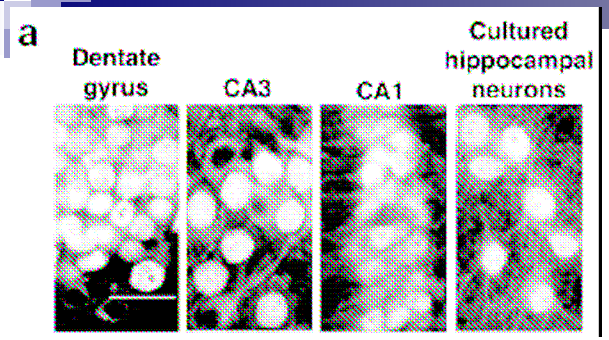
Tsien

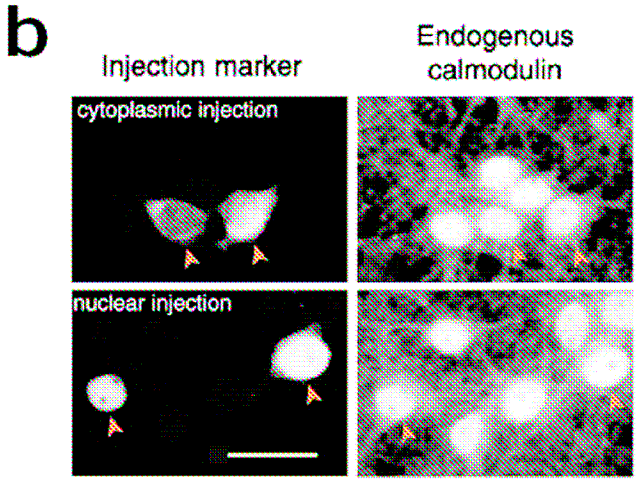
- Translocation of CaM into nucleus is necessary for CREB activation
- CaM translocation is linked to L-type Ca++ entry

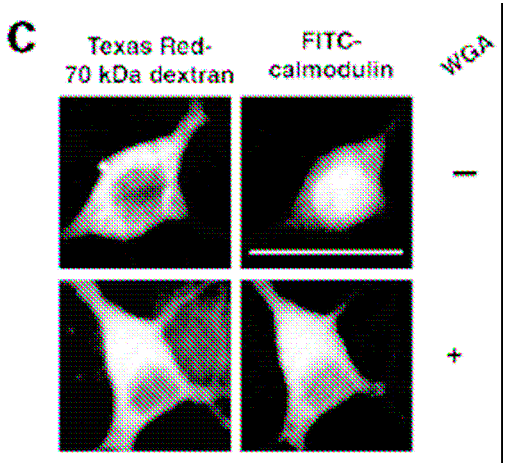
Hardingham

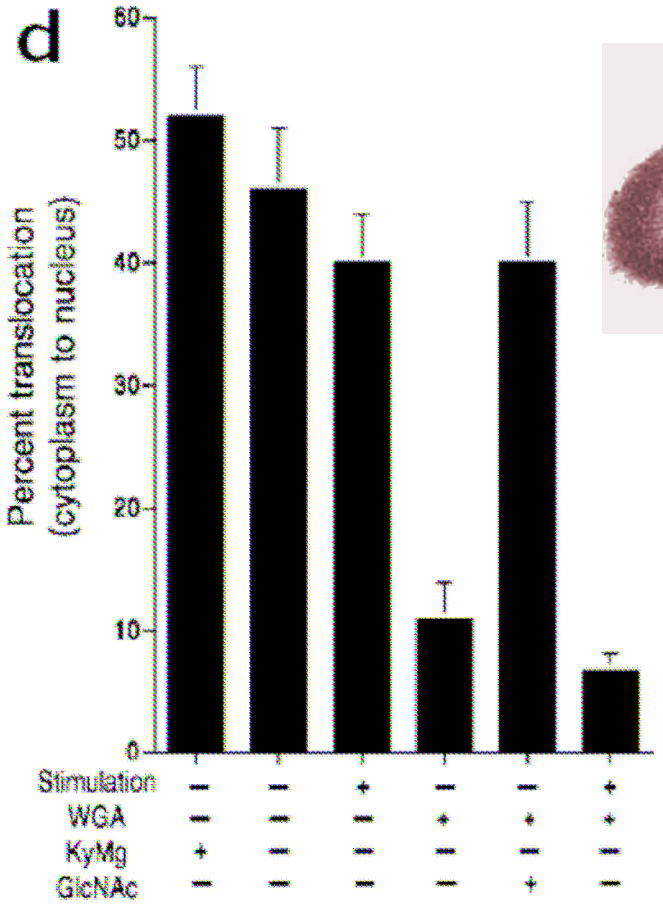
- Synapse-to-CREB signalling can occur in absence of nuclear import of CaM
- Independent Ca++ signal processing in neuronal nuclei couples synaptic activity to CREB-mediated gene transcription (i.e. intranuclear Ca++ stores, and intranuclear CaMK)
- CBP activation signal dependent on nuclear Ca++ and CaMK.

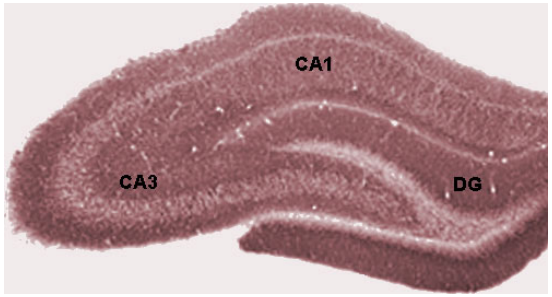
Sub-cellular localization of CaM is mostly found in nucleus: A intrinsic property of hippocampal neurons







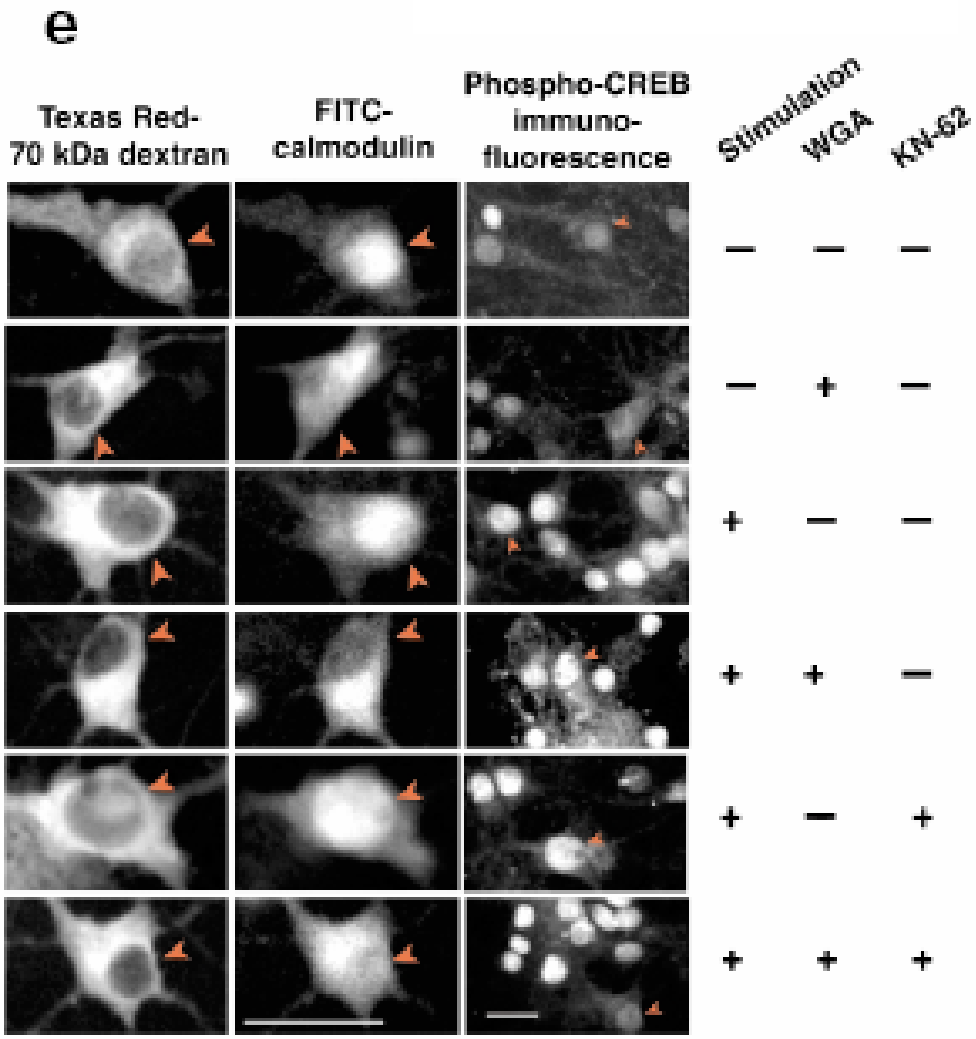


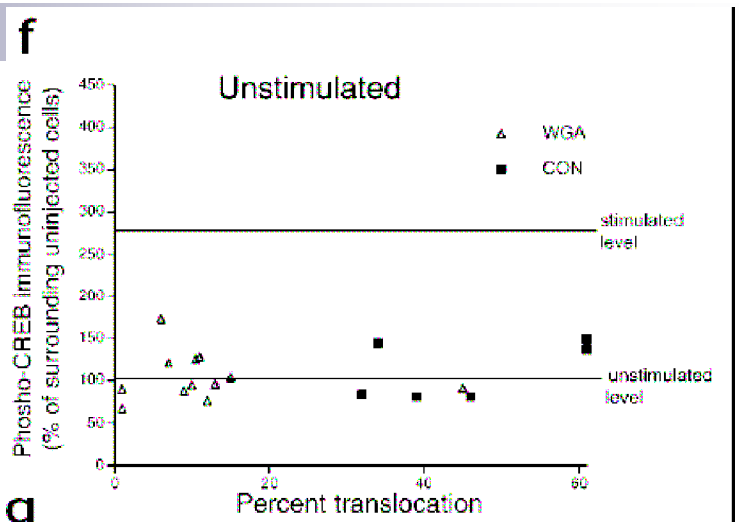


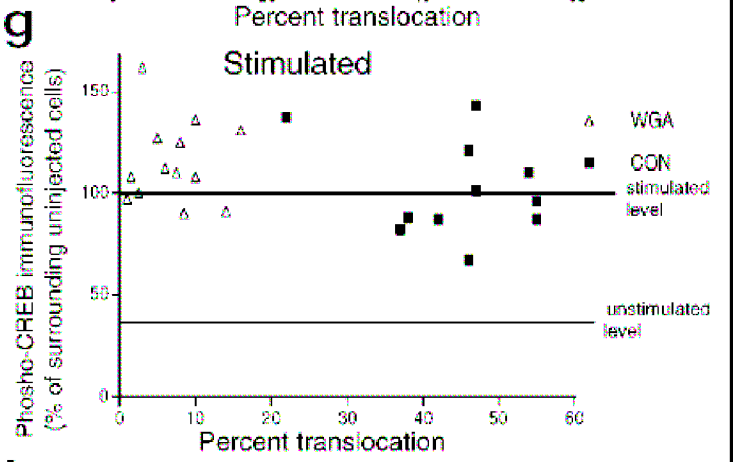
Thus, CaM can not be used to signal to nucleus via translocation, because at rest, CaM exists in the nucleus already.

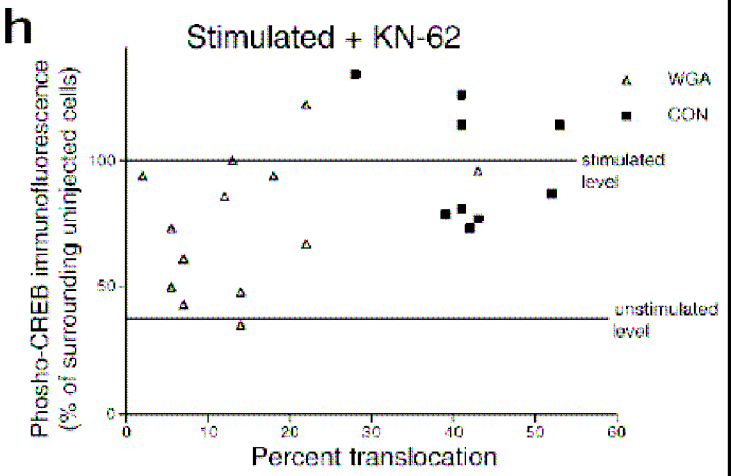
Is Nuclear import of any protein necessary for Ca++ Signaling to CREB?

Ca++ signaling to CREB is not dependent on any protein import





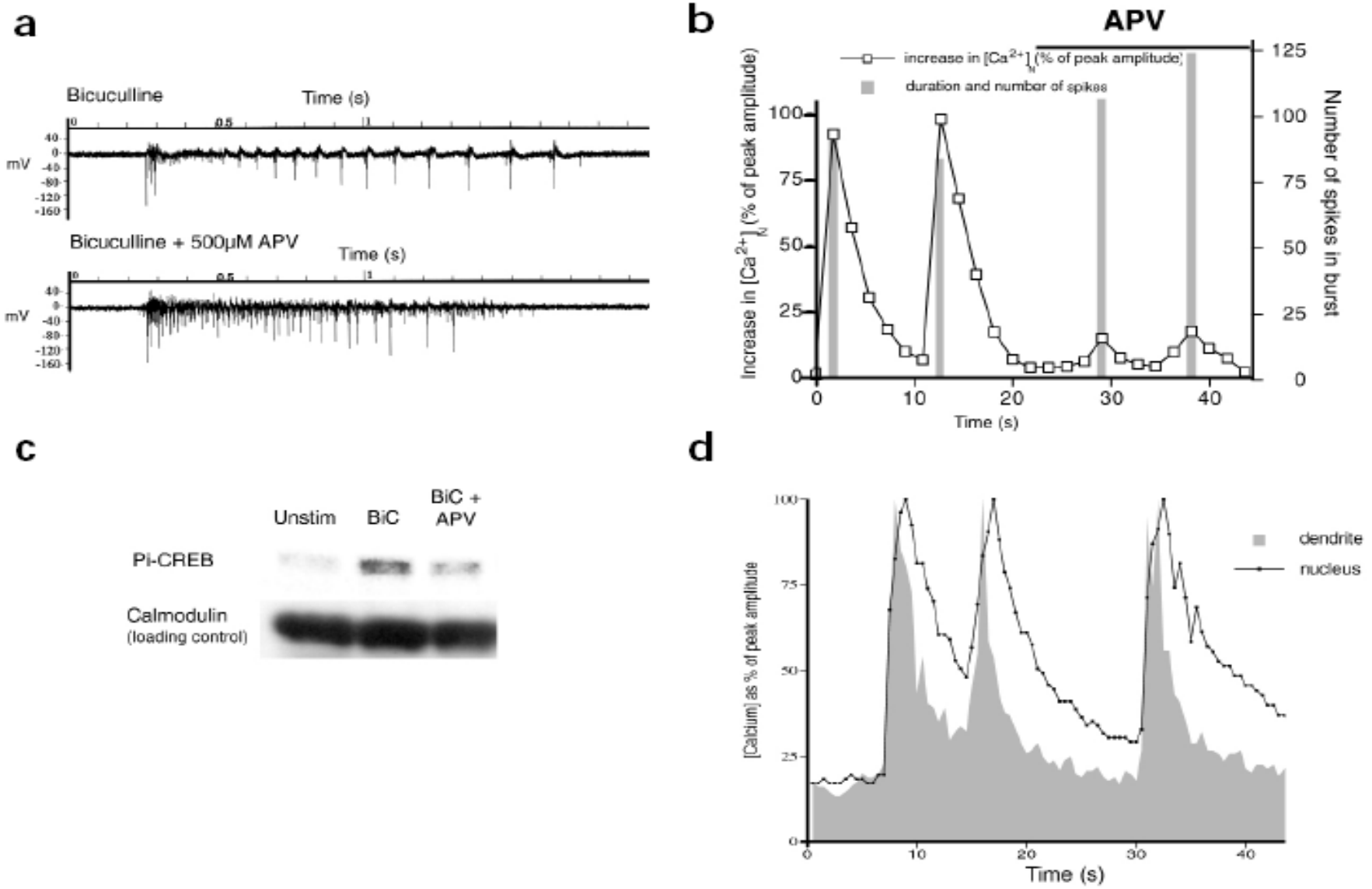




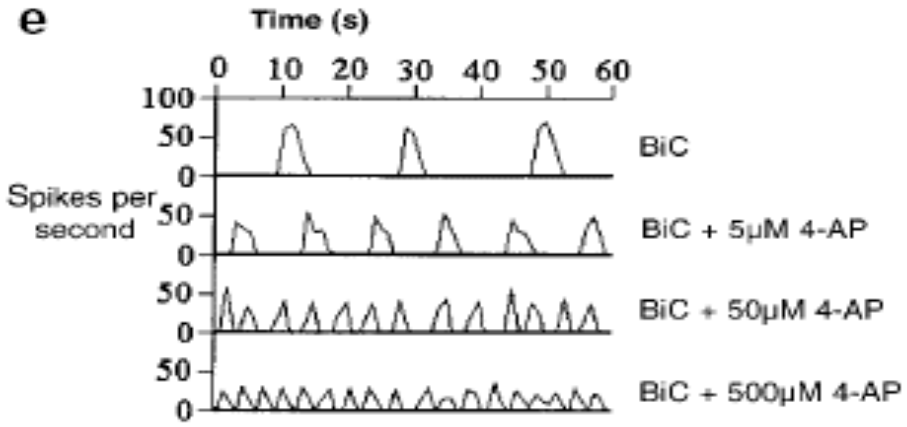
Calcium Wave Propagates to Nucleus

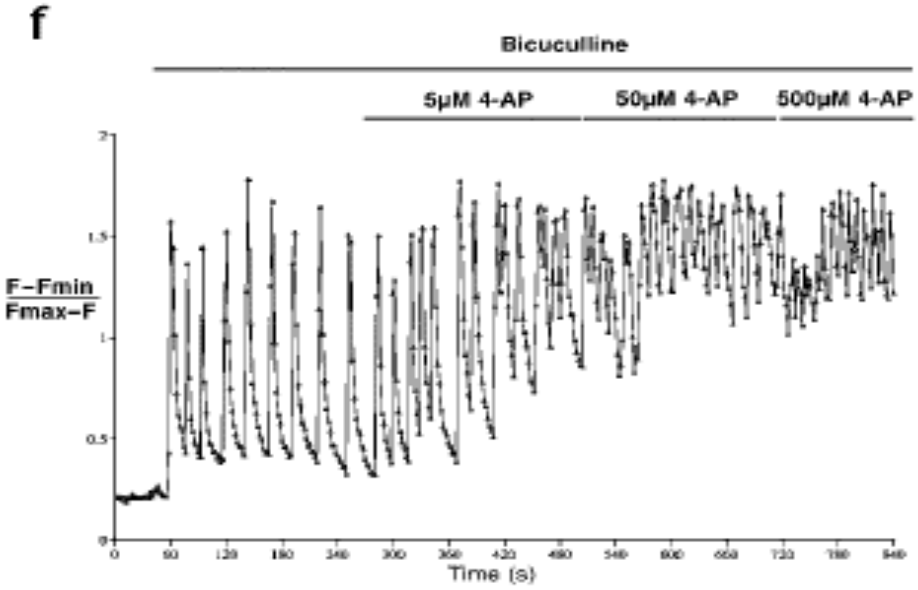
- Bicuculline: Blocks GABA-A receptors
- Produced burst of AP's firing
- Burst produced NMDA receptor-dependent Ca++ transients that lasted longer than the detectable electrical activity
- Applying Bicuculline also resulted in phosphorylation of CREB; triggered by NMDA receptors

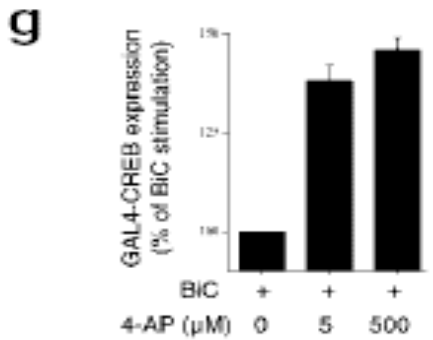
Calcium Wave Propagates to Nucleus

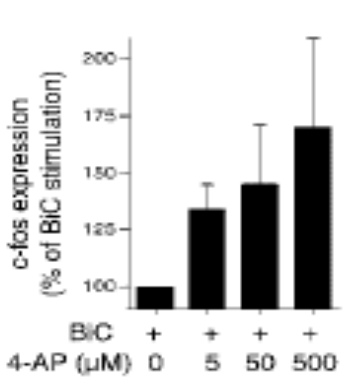


Conversion of Burst Frequency coded electrical signal to nuclear Ca++ amplitude-coded signal

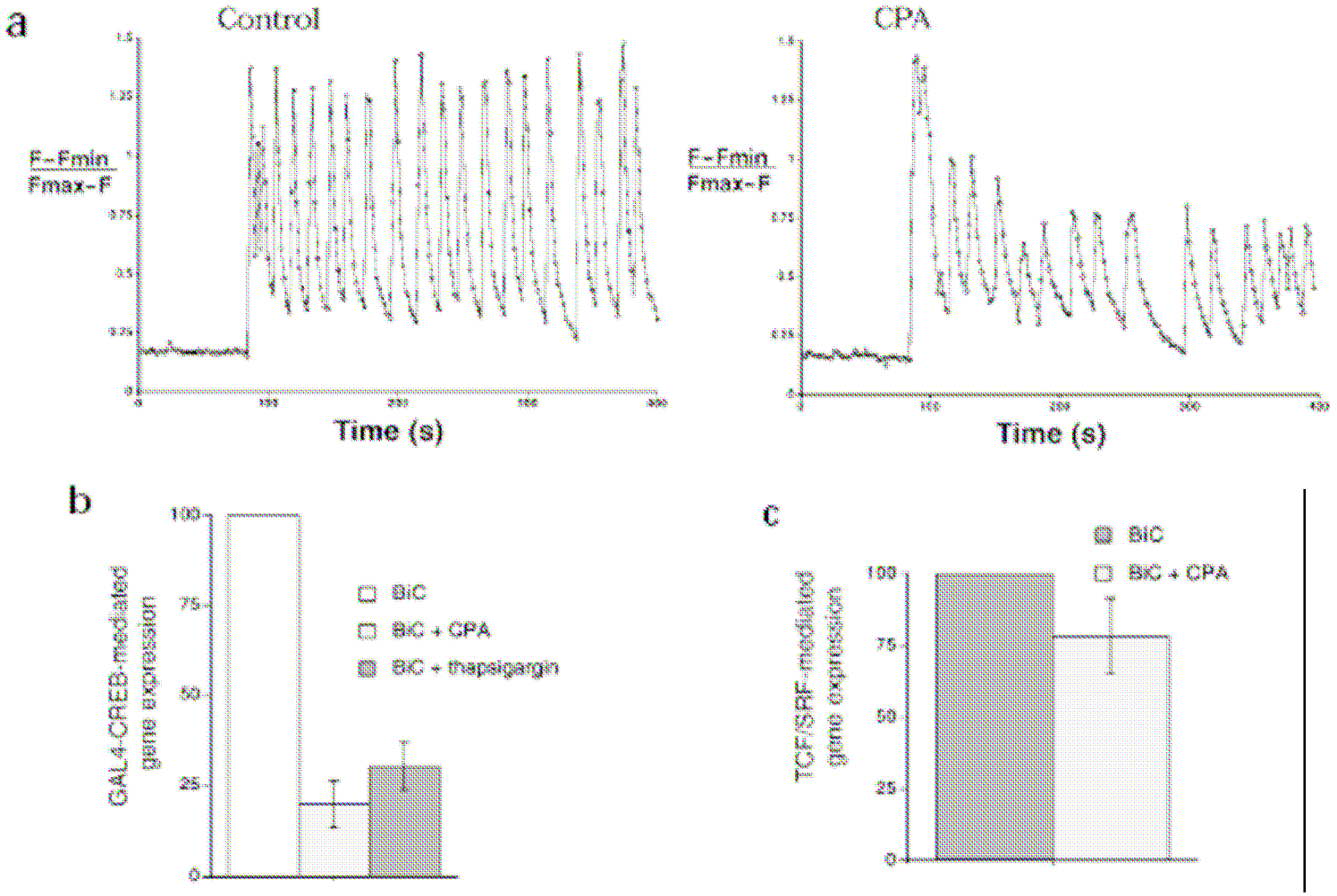








Depleting nuclear Calcium stores compromising synaptically evoked nuclear calcium transients



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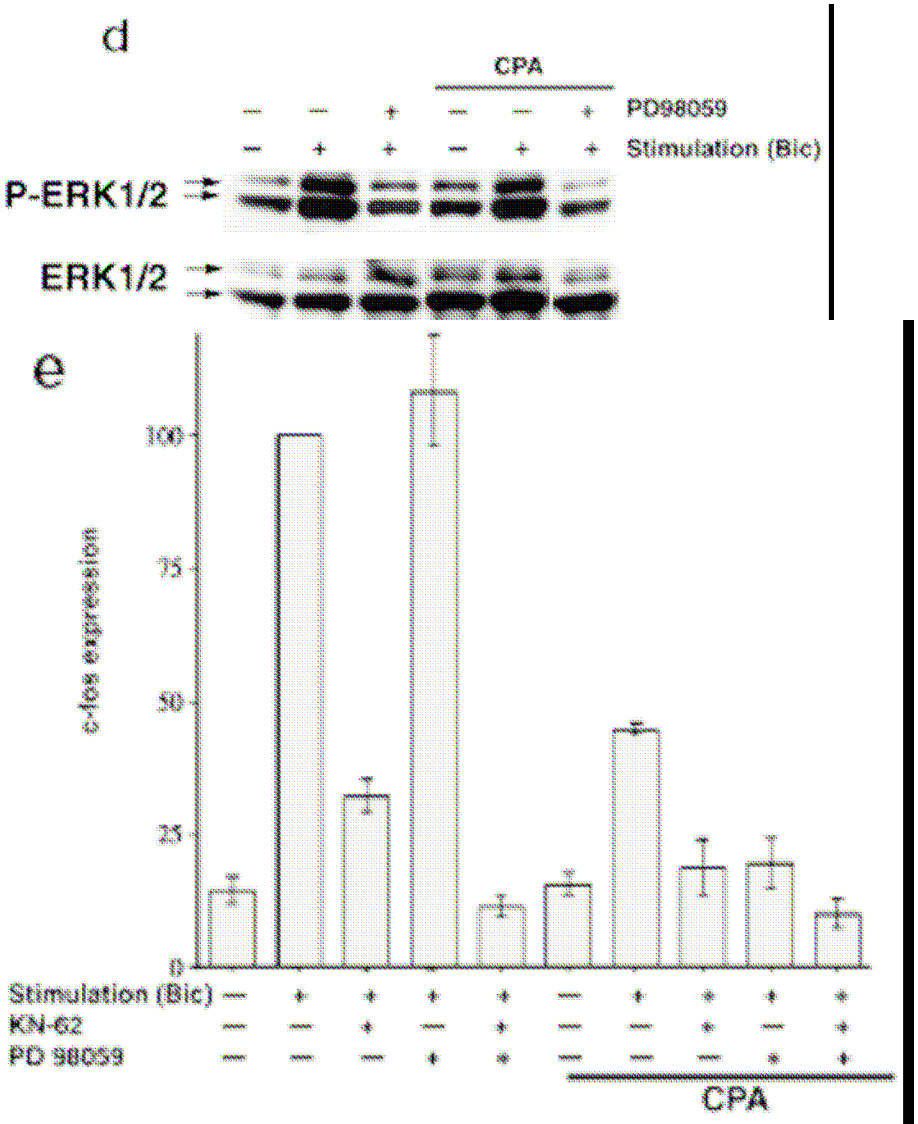
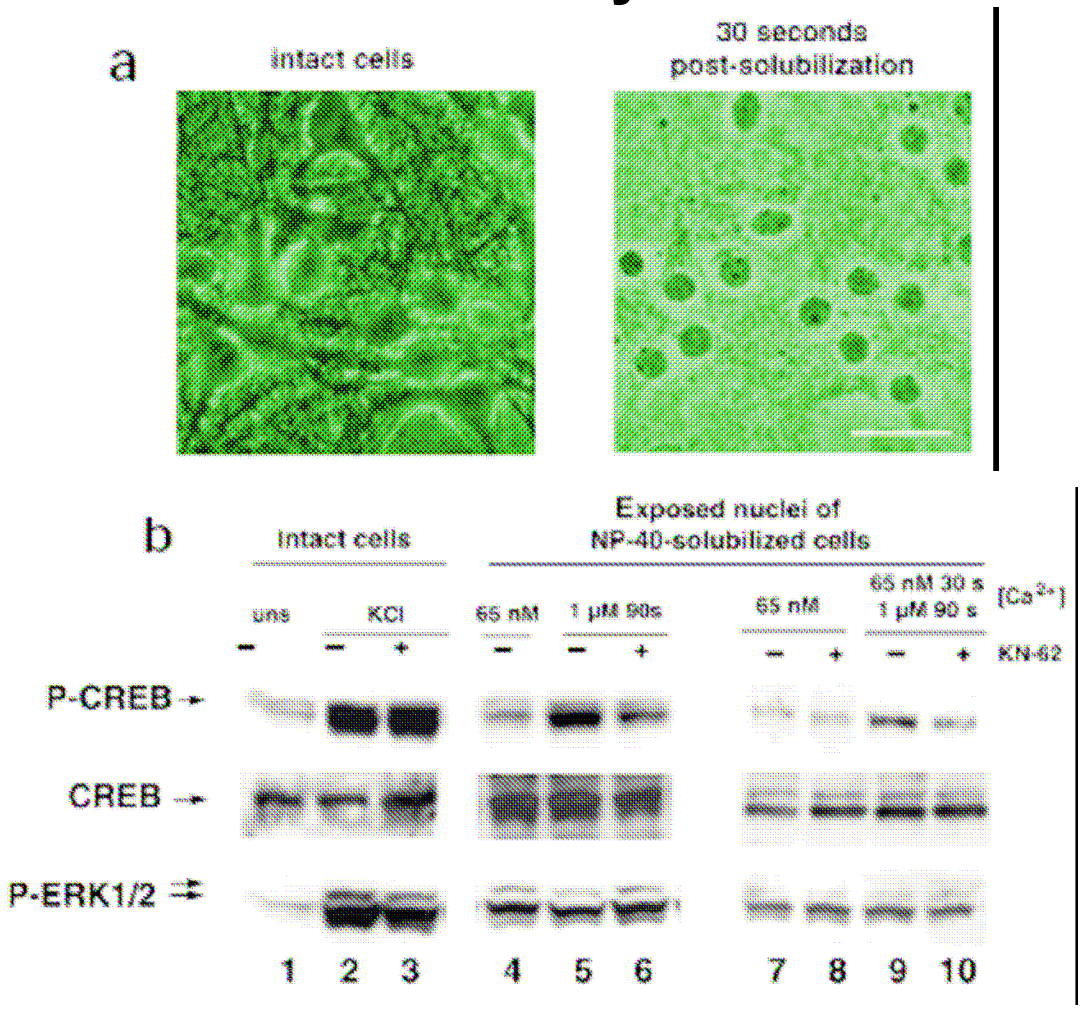


Isolating Nuclei and bombarding them with Ca++: Ca++ is needed for CREB phosphorylation mediated by CaMK



THE END!