Long-Distance Integration of Nuclear ERK Signaling Triggered by Activation of a Few Dendritic Spines
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Long-Distance Integration of Nuclear ERK Signaling Triggered by Activation of a Few Dendritic Spines

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The late phase of long-term potentiation (LTP) at glutamatergic synapses, which is thought to underlie long-lasting memory, requires gene transcription in the nucleus. However, the mechanism by which signaling initiated at synapses is transmitted into the nucleus to induce transcription has remained elusive. Here, we found that induction of LTP in only three to seven dendritic spines in rat CA1 pyramidal neurons was sufficient to activate extracellular signal–regulated kinase (ERK) in the nucleus and regulate downstream transcription factors. Signaling from individual spines was integrated over a wide range of time (>$30$ minutes) and space (>80 micrometers). Spatially dispersed inputs over multiple branches activated nuclear ERK much more efficiently than clustered inputs over one branch. Thus, biochemical signals from individual dendritic spines exert profound effects on nuclear signaling.

Activity-dependent gene transcription is essential for the maintenance of long-term potentiation (LTP) and memory consolidation (1, 2). Induction of LTP in single dendritic spines activates signaling that can either be restricted to the stimulated spine or spread into the parent dendrite over 5 to 10 $\mu$m (3–5). However, it is not known whether signaling initiated at single dendritic spines can be transmitted into the nucleus to regulate gene transcription. Extracellular signal–regulated kinase (ERK) is important, both for signaling within the stimulated spine and adjacent dendrites (3, 6, 7) and also for activating transcription factors in the nucleus during LTP (2, 8–11). Thus, ERK signaling may play an important role in relaying signals from the stimulated spines to the nucleus.

To monitor the activity of ERK in the nucleus, we ballistically transfected cultured organotypic hippocampal slices of rats with nuclear-targeted ERK activity reporter (EKAR$_{nuc}$) (12) and imaged CA1 pyramidal neurons with two-photon fluorescence lifetime imaging microscopy (2pFLIM). The expression of EKAR$_{nuc}$ was highly localized to the nucleus (12). Using the weak EKAR expression in the cytosol, we employed fluorescence intensity measurements to monitor structural plasticity of dendritic spines on secondary and tertiary apical dendritic branches (Fig. 1).

When a single spine was stimulated with a low-frequency train (1Hz, 60 s) (Fig. 1A) of two-photon glutamate uncaging pulses in the absence of Mg$^{2+}$, the spine volume increased rapidly by $275 \pm 18\%$ in 1 to 2 min (transient phase), which decayed to a sustained level $61 \pm 4\%$ larger than the original volume that lasted more than 1 hour (sustained phase) (Fig. 1, C and E), as expected (3–5, 13). The volume increase was similar to that induced in neurons expressing monomeric enhanced green fluorescence protein (mEGFP) (Fig. 1E). This structural LTP (sLTP) of spines is known to be associated with electrophysiological LTP (3–5, 13). Repeating this protocol in different spines one by one (at ~60-s intervals) (Fig. 1A), we induced sLTP sequentially in seven spines on three to five different dendritic branches (Fig. 1, B and C). After the seven-spine stimulation, we observed a slow and sustained elevation of ERK activity in the nucleus, as indicated by a gradual (over ~30 min) shortening of the fluorescence lifetime of EKAR$_{nuc}$ (~0.02 ns) that was maintained for at least the following 40 min (Fig. 1, D and F). Pharmacological inhibition of ERK with ERK inhibitor FR180204 (50 $\mu$m) completely prevented the fluorescence lifetime decrease respectively (Fig. 1, F and G). Inhibition of the classical upstream molecules Ras and mitogen-activated protein kinase kinase (MEK) (14) with dominant-negative Ras (dnRas) expression and MEK inhibitor U0126 prevented the fluorescence lifetime decrease, respectively (Fig. 1J and fig. S5). Thus, the change in fluorescence lifetime of EKAR$_{nuc}$ acted as a reliable reporter of ERK activation in the nucleus.

We further confirmed that our seven-spine stimulation protocol activates nuclear ERK by two methods independent of 2pFLIM imaging of EKAR$_{nuc}$. First, we performed immunostaining of phosphorylated ERK in CA1 neurons expressing mEGFP. Consistent with the EKAR$_{nuc}$ results, the level of phosphorylated ERK in the nucleus was persistently elevated after seven-spine stimulation (1J) (Fig. 1H and fig. S1). Second, we performed live imaging of mEGFP-tagged ERK2 in organotypic slices. Under basal conditions, mEGFP-ERK2 was localized predominantly to the cytoplasm but slowly translocated into the nucleus after seven-spine stimulation (Fig. 1I and fig. S2) (8, 13). Thus, nuclear ERK is activated by sequential activation of a few spines.

Next, we determined the source of intracellular Ca$^{2+}$ elevation that leads to nuclear ERK activation. Uncaging-induced Ca$^{2+}$ elevation was largely restricted to the stimulated spines, and spreading along the dendrite was limited to 2 to 3 $\mu$m (figs. S3 and S4) (15, 16). This local Ca$^{2+}$ elevation was dependent mainly on N-methyl-D-aspartate-type glutamate receptors (NMDARs); there was negligible contribution from voltage-sensitive Ca$^{2+}$ channels (VSChCs), metabotropic glutamate receptor (mGLuR)-mediated internal Ca$^{2+}$ release, or Ca$^{2+}$-permeable a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (sAmpA). Inhibition of NMDARs with 2-amino-5-methyl-4-isoxazolepropionic acid (APV) (50 $\mu$m) completely prevented nuclear ERK activation (Fig. 1J and fig. S5A), as well as sLTP (Fig. 1, K and L) (13). In contrast, blockade of VSChCs with CdCl$_2$ (200 $\mu$m) did not prevent nuclear ERK activation.
ERK activation (Fig. 1J and fig. S5B) was partially inhibited (Fig. 1, K and L). However, inhibition of mGluRs did not affect sLTP (13) (Fig. 1, K and L). Thus, the requirement for nuclear ERK activation is different from that for Ca$^{2+}$ elevation or sLTP. Because group I mGluR activation increases the production of diacylglycerol, which leads to activation of protein kinase C (PKC) (17), we further tested if PKC is required for the sustained nuclear ERK activation. Because PKC inhibitor blocks sLTP (3), we applied PKC inhibitor bisindolylmaleimide I (BIM) (0.2 μM) −60 min after induction of nuclear ERK activation. Surprisingly, this delayed application of BIM caused nuclear ERK activity to gradually return to the basal level (fig. S6), suggesting that sustained PKC activity is required for maintaining nuclear ERK activation.

What is the spatial pattern of synaptic activation required for nuclear ERK activation? We first tested how many synapses are required for nuclear ERK activation. We stimulated different numbers (i.e., one, three, seven) of spines at a fixed density (i.e., one to three spines per dendritic branch, separated by more than 10 μm) in fluorescence lifetime of EKAR$_{nuc}$ after seven-spine stimulation in the absence (Ctrl) or presence of ERK inhibitor FR180204. (G) Quantification of change in fluorescence lifetime averaged over 40 to 70 min in (F). (H to I) Stimulation of seven spines leads to increased nuclear phospho-ERK level (pERK) (H) and ERK2 nuclear translocation (I). (J to L) Effects of pharmacological agents and genetic manipulation on nuclear ERK activation (J) and the transient (1 to 2 min) (K) and sustained (~70 min) (L) phases of sLTP. Fluorescence lifetime change averaged over 40 to 70 min was quantified for experiments shown in fig. S5 and (F). *P < 0.05; n.s., not significant. N = 14 for Ctrl and 7 for APV and CdCl$_2$, 6 for Ctrl and MCPG, 6 for vehicle and NPS 2390, 7 for pCI and 8 for dnRas, and 7 for U0124 and U0126. Error bars denote SEM.

Fig. 1. Stimulation of seven spines in a sequential fashion triggers activation of ERK in the nucleus. (A) Schematic for seven-spine stimulation. (B) A neuron transfected with EKAR$_{nuc}$. Arrowheads indicate the locations of seven stimulated spines. (Inset) Low-brightness view of the same neuron shows nuclear localization of EKAR$_{nuc}$. (C) Representative images of spine sLTP induced by glutamate uncaging in the neuron shown in (B). (D) Fluorescence lifetime images of EKAR$_{nuc}$ before and after seven-spine stimulation in the same neuron as in (B) and (C). (E) Quantification of the transient (1 to 2 min) and sustained (~70 min) phases of sLTP after seven-spine stimulation in EKAR$_{nuc}$-expressing neurons and in mEGFP-expressing neurons. The number of neurons (n) is indicated at the bottom of each bar. (F) Time course of change in fluorescence lifetime of EKAR$_{nuc}$ after seven-spine stimulation in the absence (Ctrl) or presence of ERK inhibitor FR180204. (G) Quantification of change in fluorescence lifetime averaged over 40 to 70 min in (F). (H to I) Stimulation of seven spines leads to increased nuclear phospho-ERK level (pERK) (H) and ERK2 nuclear translocation (I). (J to L) Effects of pharmacological agents and genetic manipulation on nuclear ERK activation (J) and the transient (1 to 2 min) (K) and sustained (~70 min) (L) phases of sLTP. Fluorescence lifetime change averaged over 40 to 70 min was quantified for experiments shown in fig. S5 and (F). *P < 0.05; n.s., not significant. N = 14 for Ctrl and 7 for APV and CdCl$_2$, 6 for Ctrl and MCPG, 6 for vehicle and NPS 2390, 7 for pCI and 8 for dnRas, and 7 for U0124 and U0126. Error bars denote SEM.
Glutamate uncaging at a single spine failed to cause any detectable signal in the nucleus, whereas glutamate uncaging at three spines led to significantly elevated nuclear ERK activity (Fig. 1, A and B). For ERK$_{\text{nuc}}$ signal at a near-physiological temperature (34°C), we found a similar correlation with the number of stimulated spines (Fig. 2B and fig. S7), as well as for the levels of nuclear phospho-ERK and ERK$_2$ translocation into the nucleus (Fig. 2C and figs. S1 and S2).

In most of the experiments, we stimulated proximal branches within 200 µm from the soma. However, when we stimulated distal branches at more than 200 µm away from the soma, ERK activation showed a long delay (~40 min) before it started to increase to the level similar to that caused by proximal stimulation (Fig. 2, A and B). This slow process suggests that fast biochemical processes such as Ca$^{2+}$ waves (18) and electrochemical signaling (2, 19) are unlikely to underlie the nuclear ERK activation induced by activating a few spines.

Next, we varied the number of dendritic branches on which the stimulated spines reside to find out which input pattern—clustered or dispersed—produces nuclear ERK activation more efficiently. Clustered stimulation of all seven spines on a single branch failed to induce any nuclear ERK activity increase (Fig. 2, D and E). In contrast, stimulating three or seven spines distributed over two to seven branches resulted in marked activation of nuclear ERK (Fig. 2, D and E; all $P < 0.05$). Thus, signal integration over multiple dendritic branches is required to induce nuclear activation of ERK. We further investigated why dispersed stimulation is more efficient by imaging ERK activation at the branching point in the primary dendritic trunk after stimulating within a dendritic branch (fig. S8). ERK activity at the branching point was saturated when we stimulated two spines within a dendritic branch. Thus, additional stimulation to a branch should not cause additional ERK activation in the primary dendrite or in the nucleus.

What is the range of the spatiotemporal integration of the nuclear ERK activation? To address this question, we stimulated two spines in a dendritic branch first, waited for 30 min, and then stimulated two additional spines in the same branch or another dendritic branch 5 to 80 µm away from the first branch (Fig. 3A). As expected, the first set of stimulation did not activate nuclear ERK because both stimulated spines were on the same branch (see Fig. 2D). However, the second set of stimulation, when applied to another branch separated by more than 30 µm, significantly increased nuclear ERK activity (Fig. 3). Thus, nuclear ERK signaling can integrate synaptic stimulation over more than 30 min. When the second set of stimulation was applied to the same branch or a nearby branch (within 30 µm), we did not observe a significant increase in nuclear ERK activity (Fig. 3). Thus, nuclear ERK is activated more efficiently by a spatially distributed pattern of stimulation.
Is nuclear ERK activation induced by stimulation of a few dendritic spines sufficient to regulate gene transcription? To address this question, we used immunostaining to measure the activity of two transcription factors—cyclic adenosine monophosphate response element–binding protein (CREB) and E26-like transcription factor-1 (Elk-1)—because these molecules are known to be activated by neuronal activity via ERK activation (14). After glutamate uncaging at seven spines of mEGFP-expressing CA1 neurons, the slices were immunostained for CREB phosphorylated at Ser383 (Fig. 4, A and B) or Elk-1 phosphorylated at Ser383 (Fig. 4, D and E), the phosphorylation sites required for their transcriptional activity (20, 21). The levels of phosphorylated CREB and Elk-1 were higher in uncaged neurons than in surrounding untransfected neurons at 45 and 90 min after uncaging (Fig. 4, B and E). In contrast, mEGFP-positive, unstimulated neurons in the same slices did not show any increase in phosphorylated CREB and Elk-1 (Fig. 4). Furthermore, CREB and Elk-1 phosphorylation was abolished when ERK inhibitor FR180204 was applied before stimulation (Fig. 4, C and F). Thus, stimulation of a few spines regulates activities of transcription factors CREB and Elk-1 through ERK.

Induction of structural LTP in three to seven spines led to nuclear ERK activation and subsequent regulation of downstream transcription factors CREB and Elk-1. Because each CA1 pyramidal neuron has roughly 10,000 synapses, activation of only a tiny fraction (~0.1%) of its synapses can activate nuclear signaling that regulates gene transcription. Many studies have demonstrated that somatonuclear Ca2+ transients, caused by somatic depolarization and Ca2+ wave propagation, play an important role in regulation of activity-dependent gene transcription [(19, 22), reviewed by (23)]. However, under our experimental condition, this mechanism is unlikely to play a role. Our LTP induction protocol produced Ca2+ elevation highly restricted to the vicinity of the stimulated spines and only small somatic voltage changes. In addition, activation of VSCCs was not required for nuclear ERK activation. Moreover, the slow signal transmission strongly suggests that biochemical messengers relay information from activated synapses to the nucleus (10, 11, 24–27). Because Ras activity is known to spread over ~10 μm upon single-spine stimulation (3), downstream ERK may diffuse further and invade the nucleus as a result of multiple spine stimulation. Consistent with this hypothesis, we observed that ERK2 translocates into the nucleus in response to stimulation of a few spines. Further, the onset of ERK activation in response to distal dendrite stimulation was consistent with the speed of ERK diffusion (15). Considering that the size of the nucleus is ~2000 times larger than that of an average spine (2) and that the phosphatase-rich cytosol needs to be traversed by phosphorylated ERK, additional mechanisms such as a PKC-ERK positive feedback loop and physical protection would be required to aid the long-distance, persistent synapse-to-nucleus ERK signaling (27, 28).

The long-distance spatiotemporal integration in inducing nuclear ERK activation may have important implications for the functional organization of dendritic inputs. Many studies have shown that synaptic potentiation tends to occur in a spatially clustered fashion (29–31) due to electrical integration and biochemical cross talk within a short stretch of dendrite (32–35). However, because potentiated synapses would recruit stronger local membrane depolarization and biochemical signaling in the surrounding region in a positive-feedback manner, this mechanism potentially leads to accumulation of potentiated synapses in one dendritic branch (36). The nuclear signaling efficiently induced by spatially dispersed inputs may be important for counterbalancing the tendency to accumulate potentiated spines in one branch and developing balanced spatial distribution of synaptic weights.

References and Notes

Fig. 4. Transcription factors are phosphorylated in response to seven-spine stimulation in an ERK-dependent fashion. (A) Immunofluorescent images of phosphorylated CREB (pCREB) (red). Seven spines of a neuron expressing mEGFP (green) were stimulated, and the slice was fixed 45 min after the stimulation (Uncaged). An unstimulated mEGFP neuron in the same slices is also shown as negative control (Unstim). The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). The relatively high basal level of pCREB in a subpopulation of neurons is probably due to spontaneous circuit activity (7). (B) Quantification of the fluorescence intensity of pCREB in the nuclei identified with DAPI. The fluorescence in the nucleus of each mEGFP-expressing neuron was normalized to the average fluorescence in the nuclei of five surrounding untransfected neurons. Uncaged neurons were always paired with unstimulated neurons in the same slices (Z5). The numbers of neurons are indicated at the bottom of the bars. *P < 0.05. (C) ERK inhibitor FR180204 blocks uncaging-induced CREB phosphorylation. Dimethyl sulfoxide (DMSO) was used as vehicle control. (D to F) Same as (A) to (C), except phosphorylated Elk-1 (pElk-1) was analyzed instead of pCREB. Arrowheads in (A) and (D) indicate unstimulated or uncaged mEGFP-expressing neurons. Data are presented as mean ± SEM (error bars).
Neural Activity in Human Hippocampal Formation Reveals the Spatial Context of Retrieved Memories

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In many species, spatial navigation is supported by a network of place cells that exhibit increased firing whenever an animal is in a certain region of an environment. Does this neural representation of location form part of the spatiotemporal context into which episodic memories are encoded? We recorded medial temporal lobe neuronal activity as epilepsy patients were asked to recall memories of the places they had visited. Neuronal firing during the retrieval of each memory was similar to the activity that represented the locations in the environment where the memory was initially encoded.

When one encounters an old friend and remembers the time they last met, often the place of meeting and surrounding circumstances come to mind. This is the hallmark of episodic memory: the capacity to remember the time, and other aspects of one’s current cognitive milieu. When the brain forms a new episodic memory, these theories predict that the content of the experience becomes associated with the current spatial and temporal context. When the memory is retrieved, this prior context is partially reinstated, focusing one’s thoughts on the time and place of the remembered episode. This reinstatement not only provides the phenomenological experience of remembering, but also helps to cue other memories experienced within the same or related contexts.

Although it is well established that the hippocampus and surrounding medial-temporal-lobe (MTL) structures play a central role in the formation and retrieval of context-mediated memories (3–5), we know far less about how these memory processes manifest in the activities of individual MTL neurons. Much of what is known about the neural coding properties of hippocampal and MTL neurons comes from studies of rodent spatial navigation, where individual neurons respond preferentially at specific locations within a given contextually defined spatial environment (6, 7). Similar neuronal responses have also been identified in the human hippocampus during virtual spatial navigation (8, 9). The context-dependent firing of these neurons (10, 11) and their dependence on the animal’s goal state or past history of experienced cues (12, 13) have led some to speculate that the neural representation of space in the hippocampus is part of a broader network of neurons that encode episodic memories more generally (14–17). This hypothesis suggests that the same neural structures and computations that enable the learning of a spatial layout via place-cell activity also facilitate the encoding of episodic memories. However, according to a prominent alternative account, the spatial coding functions of the hippocampus are part of a context module that operates independently of the computations that encode the content of a memory (18, 19).

We designed a virtual-reality memory game in which participants played the role of a delivery person, driving through a virtual town and delivering items to stores. Our participants were patients with drug-resistant epilepsy who were implanted with depth electrodes to localize the focus of their seizures and to map cognitive function in surrounding healthy tissue. In an initial phase of the game, participants explored the town using a computer controller to navigate from store to store as they attempted to learn the layout of the environment illustrated in Fig. 1A. After this initial familiarization phase, during which participants visited each store twice, a series of “delivery days” began. On each delivery day, participants were instructed to travel from store to store, visiting 13 randomly chosen stores (of the 16 total) in a randomly determined order. Upon their arrival at each store, participants were presented with an item [either visually for 2 s for participants one to five or aurally for participants six and seven (20)]. Upon arrival at the final (13th) store, no item was presented. Instead, the screen

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