

Transient Photoactivation of Rac1 Induces Persistent Structural LTP Independent of CaMKII in Hippocampal Dendritic Spines

Takeo Saneyoshi,  Chisato Suematsu, and  Yasunori Hayashi

Department of Pharmacology, Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan

Abstract

Structural changes in dendritic spines underlie long-term potentiation (LTP). While CaMKII has been considered as the primary driver of these changes, we show that transient, localized activation of Rac1 alone is sufficient to induce structural LTP in hippocampal slices prepared from rat pups of either sex. Using photoactivatable Rac1 (PA-Rac1), we demonstrated that Rac1 activation triggers spine enlargement and actin polymerization. This PA-Rac1-induced plasticity was blocked by Rac1 and Pak1 inhibitors but not by a CaMKII inhibitor. Our results identify Rac1 as an upstream of persistent signaling that stabilizes actin-based spine structural changes critical for synaptic memory encoding.

Key words: actin cytoskeleton; CaMKII; dendritic spine; hippocampus; LTP; photoactivation; Rac1; structural plasticity; two-photon microscopy

Significance Statement

The molecular mechanisms that trigger persistent structural long-term potentiation (sLTP) at synapses remain incompletely understood. This study demonstrated that localized activation of Rac1, a small GTPase regulating actin dynamics, is sufficient to induce and maintain sLTP in hippocampal neurons independently of CaMKII. Using two-photon photoactivation and fluorescence lifetime imaging microscopy (FLIM), we show that Rac1 induces persistent spine growth and actin polymerization. These findings identify Rac1 as a self-sustaining signaling module in synaptic plasticity and provide mechanistic insight into the biochemical encoding of long-lasting synaptic changes that underlie memory.

Introduction

Long-term potentiation (LTP) is a fundamental synaptic mechanism underlying learning and memory. While CaMKII has been widely studied as a key kinase capable of the sustained activity required for LTP maintenance (Yasuda et al., 2022), how its signaling is translated into enduring structural changes in dendritic spines remains incompletely understood.

A CaMKII-Tiam1 complex, termed reciprocal activation of kinase-effector complex (RAKEC), was previously shown to generate persistent activation of Rac1, a Rho family small GTPase, and promote spine enlargement during structural LTP (sLTP; Saneyoshi et al., 2019; Saneyoshi, 2021). Through activation of a kinase cascade eventually leading to the inactivation of the actin severing protein cofilin1, Rac1 regulates dendritic spine morphology (Saneyoshi and Hayashi, 2012), synaptic plasticity (Haditsch et al., 2009), motor learning (Hayashi-Takagi et al., 2015), and forgetting (Liu et al., 2016). In non-neuronal cells, Rac1 activity can be stabilized by positive feedback through the coronin 1A-based multiprotein complex (Castro-Castro et al., 2011), raising the possibility that Rac1 may serve as a persistent signaling module downstream of CaMKII.

Received July 14, 2025; revised Oct. 17, 2025; accepted Nov. 3, 2025.

The authors declare no competing financial interests.

Author contributions: T.S. and Y.H. designed research; T.S. and C.S. performed research; T.S. and C.S. analyzed data; T.S. and Y.H. wrote the paper.

We thank Dr. Steven Middleton on his comments on the manuscript. This research was supported by JST CREST JPMJCR20E4; AMED under JP24zf0127010; JSPS under 18H04733, 18H05434, and 16H02455 to Y.H. and 18K19377, 21H02595, and 21H05692 to T.S.; and AMED 25wm0625123h0002 to Y.H. and T.S. Takeda Science Foundation Research Grants (2020), Research Grant from the Naito Foundation (2024), and Brain Science Foundation (2024) to T.S.

Continued on next page.

To directly test this, we used photoactivatable Rac1 (PA-Rac1; Wu et al., 2009) in hippocampal slice cultures to precisely manipulate Rac1 activity in single dendritic spines and assess whether Rac1 alone can induce and maintain sLTP. We found that a transient activation of Rac1 can induce persistent increases in F-actin and enlargement of dendritic spines. This indicates that the mechanism that maintains the enlarged dendritic structure of dendritic spines lies downstream to Rac signaling, thereby opening new future direction of LTP research.

Materials and Methods

Plasmids

Photoactivatable Rac1 (PA-Rac1; Addgene plasmid #22035) was a gift from Dr. Klaus Hahn (Wu et al., 2009) and was subcloned into the pCAGGS expression vector (Niwa et al., 1991). Lifeact was obtained from Dr. Wedlich-Söldner (Riedl et al., 2008). Constructs encoding monomeric GFP, photoactivatable GFP (PAGFP), DsRed2, human Rac1, and β -actin have been described previously (Saneyoshi et al., 2008; Bosch et al., 2014).

Reagents

KN-93, EHT1864, IPA3, and MNI-caged L-glutamate were purchased from Tocris Bioscience. Picrotoxin was obtained from Nacalai Tesque, and tetrodotoxin (TTX) was from Latoxan. Latrunculin and Jasplakinolide were from FUJI-FILM.

Organotypic slice culture and transfection. Organotypic hippocampal slices cultures were prepared from postnatal day 6–9 Sprague Dawley rat pups of both sexes (Stoppini et al., 1991). At 7–8 d in vitro, cultured slices were biolistically transfected with plasmid DNA using a Gene-Gun (Bio-Rad) at 180 psi, as described previously (Saneyoshi et al., 2019). For each transfection, 10 μ g of plasmid DNA was coated onto 12.5 mg of 1.6 μ m gold particles.

Two-photon imaging and structural LTP induction. Imaging was performed using a two-photon laser scanning microscope (FV1000-MPE, Olympus) equipped with Ti-sapphire pulsed lasers (Spectra-Physics). Slices were continuously perfused at room temperature (25–27°C) in artificial cerebrospinal fluid (ACSF) containing the following (in mM): 119 NaCl, 2.5 KCl, 4 CaCl₂, 26.2 NaHCO₃, 1 NaH₂PO₄, and 11 glucose, equilibrated with 95% O₂/5% CO₂. For glutamate uncaging experiments, ACSF was supplemented with 1 μ M TTX, 50 μ M picrotoxin, and 2.5 mM MNI-L-glutamate. For PA-Rac1 experiments, ACSF contained 1 mM MgCl₂, 2.5 mM CaCl₂, and 1 μ M TTX.

Pharmacological inhibitors were bath-applied at least 30 min before stimulation. Structural LTP was induced by two-photon uncaging of MNI-glutamate at 720 nm (30 pulses, 2 ms duration, 1 Hz) with 5 mW power at the specimen. PA-Rac1 was photoactivated using a 720 nm laser (20 pulses, 0.5 ms duration, 1 Hz). Imaging was performed at 910 nm for uncaging experiments and 1,030 nm for PA-Rac1 imaging to avoid nonspecific Rac1 activation. Spine volume was quantified using the total integrated fluorescent intensity of the GFP or DsRed2 in z-stacked images of spines with background subtraction by FIJI (Schindelin et al., 2012).

Fluorescence lifetime imaging microscopy. Fluorescence lifetime was measured using time-correlated photon-counting (SPC-150 module, Becker-Hick; H7422P-40 detector, Hamamatsu Photonics), as previously described (Saneyoshi et al., 2019). Data were analyzed using a custom written macro in Igor-Pro (WaveMetrics). Lifetime values were averaged within spine heads and normalized as changes from baseline. For actin polymerization, FRET-FLIM imaging was performed using mVenus- or mGFP-Actin and Lifeact-mRFP.

Statistical analysis

All data are presented as mean \pm SEM. For comparison between two groups, unpaired two-tailed *t* tests were used. For multiple groups comparison, one-way ANOVA with Tukey's post hoc test was applied. A significance threshold was set at $p < 0.05$. Sample size (*n*) refers to individual neurons as specified in figure legends.

Results

To directly test whether Rac1 activation is sufficient for sLTP, we used a photoactivatable Rac1 (PA-Rac1; Wu et al., 2009) in CA1 pyramidal neurons of organotypic

T.S.'s present address: Department of Life Science and Technology, School of Life Science and Technology, Institute of Science Tokyo, Yokohama 226-8501, Japan

Correspondence should be addressed to Takeo Saneyoshi at saneyoshi.t.cfc3@m.isct.ac.jp or Yasunori Hayashi at yhayashi-tky@umin.ac.jp.

Copyright © 2025 Saneyoshi et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license, which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

hippocampal slice cultures. This system enabled precise spatial and temporal control of Rac1 activation through light-induced conformational change of a phototropin protein, LOV2, from *Avena sativa*. We expressed mCherry-PA-Rac1 with GFP as a volume filler using a biolistic method and imaged the expressing neurons using two-photon laser scanning microscopy (Saneyoshi et al., 2019). Our initial attempt of imaging using 910 nm light induced ectopic lamellipodia during repeated imaging, likely due to nonspecific PA-Rac1 activation by the imaging laser. We therefore performed imaging at 1,030 nm to prevent such activation (data not shown). Spine-specific Rac1 activation was achieved by two-photon activation at 720 nm (0.5 ms pulses 20 repetitions at 1 Hz), which resulted in rapid and sustained spine enlargement persisting up to 30 min (Fig. 1A–C). The volume of unstimulated adjacent spines nearby the stimulated spine did not change, indicating specificity of photoactivation of PA-Rac1 (Fig. 1B). A mutant mimicking dark state (LOV2 C450A) failed to induce enlargement, confirming the specificity of PA-Rac1-mediated effects (Fig. 1D).

Next, we tested whether Rac1-induced structural enlargement depends on the canonical Rac signaling pathway. Inhibition of Rac1 with EHT1864 (Shutes et al., 2007), or its downstream effector Pak1 with IPA3 (Deacon et al., 2008) blocked both the induction and maintenance phases of spine enlargement following PA-Rac1 activation (Fig. 1A–C). These results confirm that the Rac1-Pak1 signaling pathway is both necessary and sufficient for sustained structural spine plasticity, consistent with previous chemical-genetic studies of Pak1 and its effector LIMK1 (Dagliyan et al., 2017; Ripoli et al., 2023). In contrast, inhibition of CaMKII with KN93 (Sumi et al., 1991) has no effect on PA-Rac1-induced sLTP (Fig. 1E–G), indicating that once Rac1 is activated, structural LTP can proceed independently of ongoing CaMKII activity. The KN93 inhibited sLTP induced by glutamate uncaging, confirming the effect of the inhibitor (Fig. 1H–J).

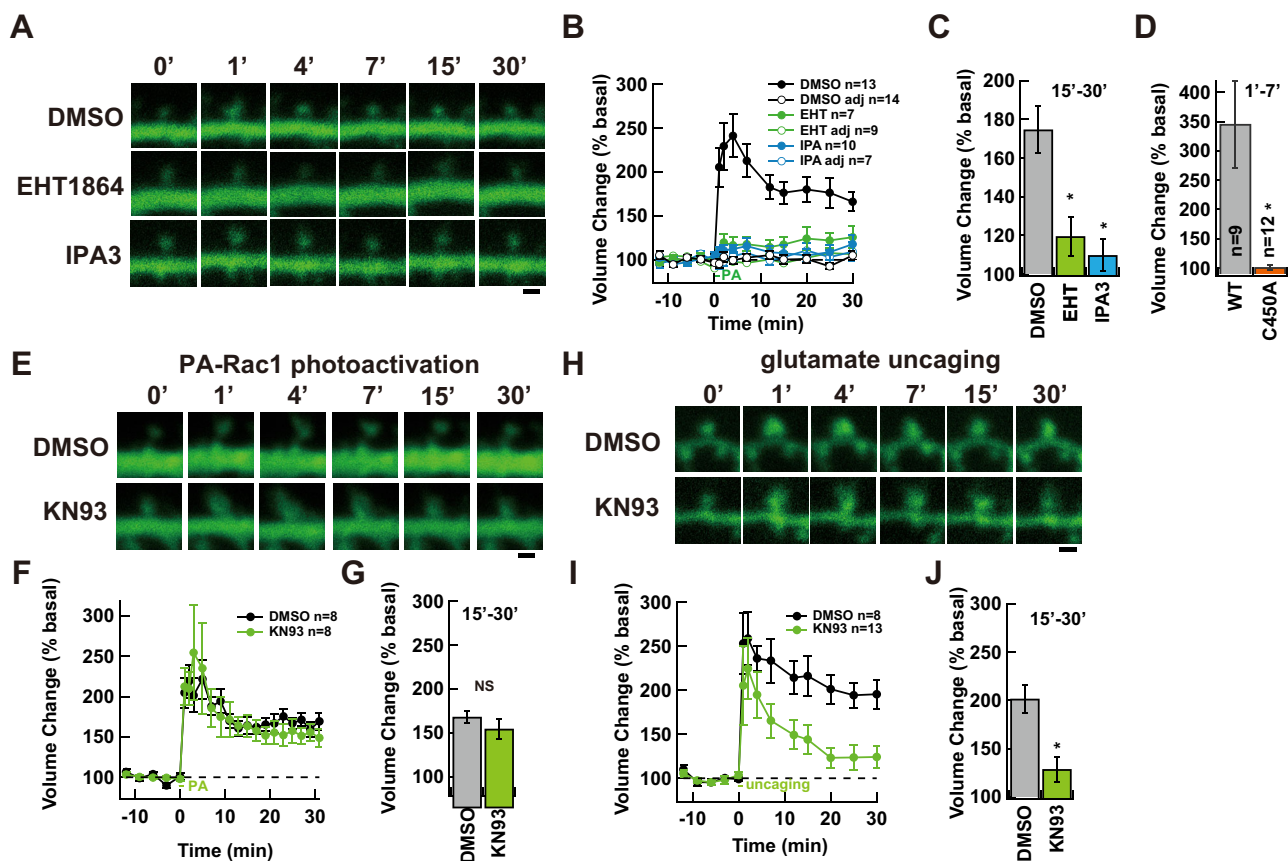


Figure 1. Rac1-dependent induction of structural LTP in organotypic hippocampal slice culture expressing GFP and PA-Rac1, following photoactivation under control (DMSO, 0.1%), Rac inhibitor (EHT1864, 50 μ M), or Pak inhibitor (IPA3, 30 μ M) conditions. **B**, Time course of spine volume changes during PA-Rac1 activation (DMSO, black; EHT1864, green; IPA3, blue). **C**, Quantification of spine volume in the persistent phase (15–30 min) under each condition (mean \pm SEM; $n = 13$ neurons for DMSO, $n = 7$ neurons for EHT1864, $n = 10$ for IPA3). **D**, Quantification of spine volume in initial phase (1–7 min) using PA-Rac1 WT or LOV2 dark mutant, C450A (mean \pm SEM; $n = 9$ neurons for WT, $n = 12$ neurons for C450A). **E**, Representative images showing PA-Rac1-induced sLTP in the presence of DMSO or the CaMKII inhibitor KN93 (10 μ M). **F**, Time course of spine volume changes (DMSO, black; KN93, green). **G**, Comparison of spine volume during the persistent phase (15–31 min) between DMSO and KN93 (mean \pm SEM; $n = 8$ neurons per group). **H**, Representative images showing glutamate uncaging-induced sLTP in the presence of DMSO (0.1%) or the CaMKII inhibitor KN93 (10 μ M). **I**, Time course of spine volume changes (DMSO, black; KN93, green). **J**, Comparison of spine volume during the persistent phase (15–30 min) between DMSO and KN93 (mean \pm SEM; $n = 8$ neurons for DMSO; $n = 13$ neurons for KN93). Statistical analysis: two-way ANOVA with post hoc Tukey's test for **C**; unpaired two-tailed t tests for **D**, **G**, and **J**; * $p < 0.05$; NS, not significant; Scale bars, 1 μ m.

Two factors determine the activity profile of PA-Rac1 after photoactivation: (1) intrinsic properties of the LOV2 domain conformational changes and (2) the diffusion of the PA-Rac1 protein out of dendritic spine. The LOV2 domain undergoes conformational changes within seconds of light illumination and returns to the dark state within ~ 1 min (Wu et al., 2009; Zoltowski et al., 2009). To assess the contribution of protein diffusion, we analyzed the fluorescence decay of PAGFP-tagged PA-Rac1 and Rac1 within dendritic spines following local photoactivation. All conditions—PAGFP-Rac1 under basal condition, PAGFP-Rac1 in glutamate uncaging stimulated spines, and PAGFP-PA-Rac1 following photoactivation—showed comparable fluorescence decay kinetics ($\tau \approx 1.35$ – 1.80 min), suggesting similar mobility regardless of activation state (Fig. 2A–C). Although not statistically significant, there was a trend toward slower decay for activated PA-Rac1, consistent with previous findings that the active Rac1 mutant shows reduced diffusion in spines (Chazeau et al., 2014). These data suggest that photoactivated Rac1 is quickly lost from dendritic spines due to both intrinsic inactivation of the LOV2 domain and protein diffusion and thus imply that transient Rac1 activity triggers a persistent change specifically in the photoactivated dendritic spine that leads to an enlargement of the structure.

To evaluate actin cytoskeletal remodeling, we developed a novel approach to detect actin polymerization using two-photon fluorescence lifetime imaging microscopy with mGFP- or mVenus-Actin and Lifeact-mRFP, a protein specifically interact with F-actin (Riedl et al., 2008) as FRET pairs. To test if it can indeed detect actin polymerization, we treated neurons with latrunculin, an inhibitor of actin polymerization, or jasplakinolide, an inducer of actin polymerization to observe the actin polymerization and compared the lifetimes changes before and after these treatments. Latrunculin-induced actin depolymerization increased the fluorescence lifetime while jasplakinolide-induced actin polymerization decreased it, indicating that this approach can detect actin polymerization/depolymerization status bidirectionally within dendritic spines (Fig. 3A–C).

We then compared actin polymerization/depolymerization status between glutamate uncaging-induced sLTP and PA-Rac1 activation (Fig. 3D–H). Both stimuli produced comparable spine enlargement (Fig. 3E) and actin polymerization

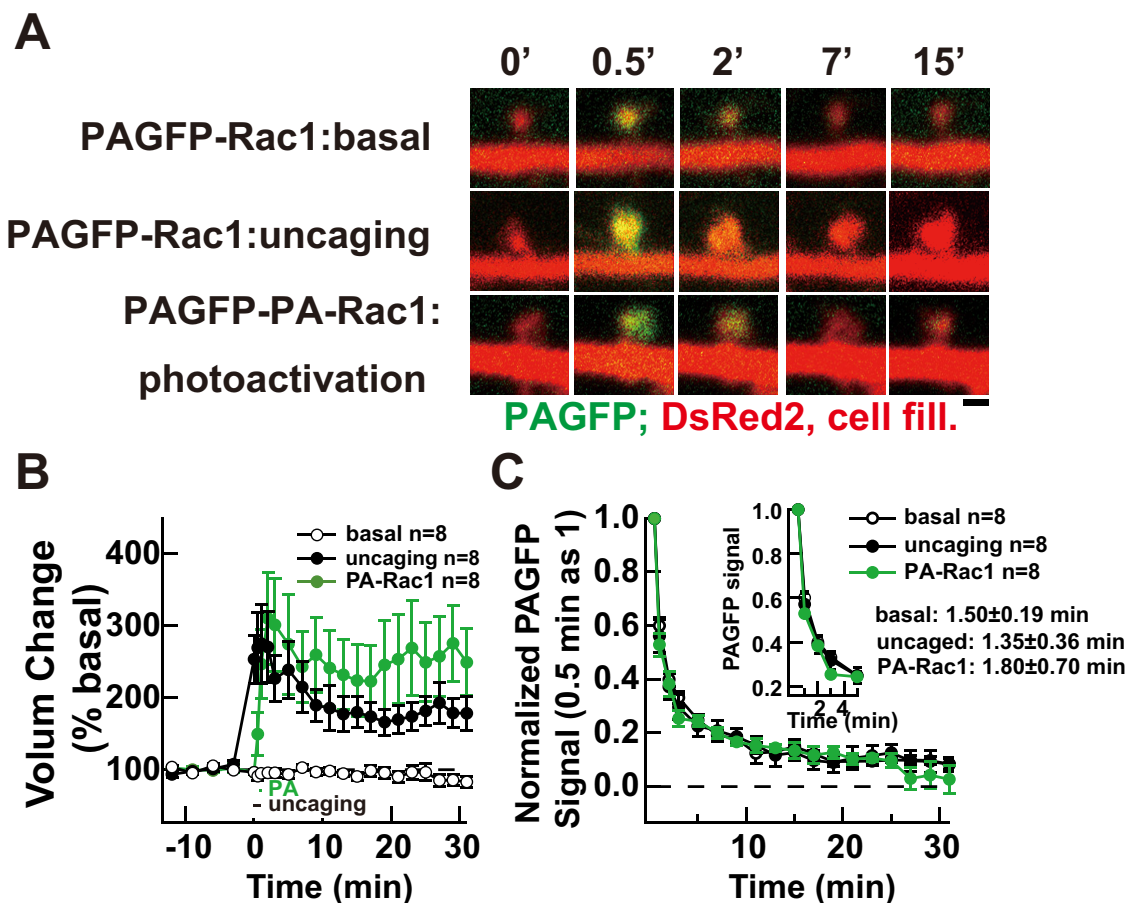


Figure 2. Molecular dynamics of Rac1 and PA-Rac1 in dendritic spines during sLTP. **A**, Representative merged images showing spine morphology (DsRed2) and PAGFP fluorescence for Rac1 or PA-Rac1 under basal conditions (top), uncaging-induced sLTP (middle), or PA-Rac1 photoactivation (bottom). **B**, Quantification of normalized spine volume changes across conditions (PA-Rac1, green; basal, white; uncaging-induced sLTP, black; $n = 8$ neurons per group). **C**, Normalized PAGFP decay curves showing fluorescence intensity divided by spine volume at 0.5 min poststimulation: Rac1 in basal (white, $\tau = 1.50 \pm 0.19$ min), Rac1 in glutamate uncaging-induced sLTP (black, $\tau = 1.35 \pm 0.36$ min), photoactivated PA-Rac1 (green, $\tau = 1.80 \pm 0.70$ min). Scale bars, 1 μ m.

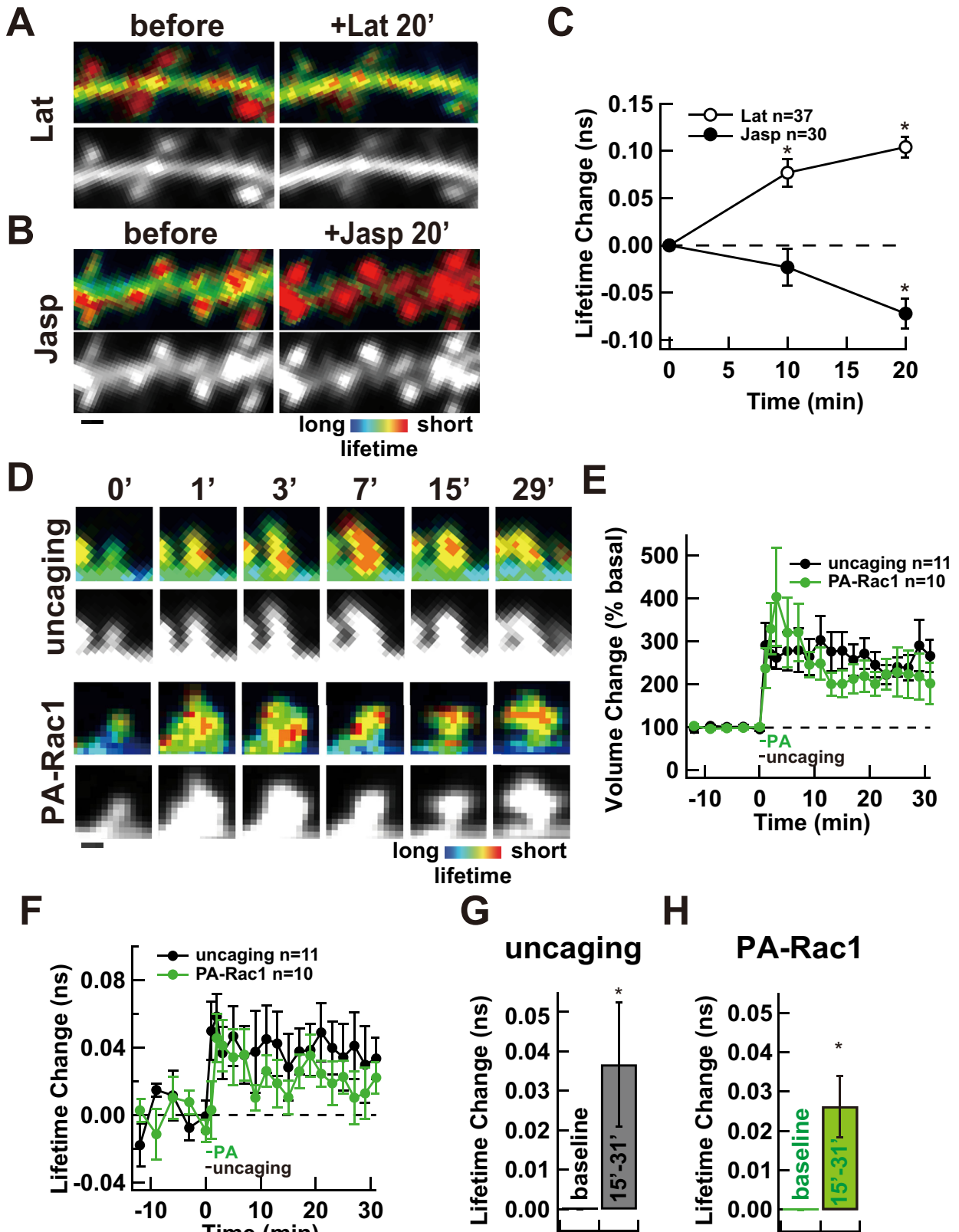


Figure 3. Actin polymerization during uncaging- or PA-Rac1-induced structural LTP. **A, B**, Representative FRET-FLIM images (top) and spine morphology images (bottom, using the GFP-Actin signal as a proxy) showing actin polymerization before and 20 min after pharmacological treatment with an inhibitor of actin polymerization (latrunculin, 10 μ M; **A**) or an inducer of actin polymerization (jasplakinolide, 1 μ M; **B**). Warmer colors indicate shorter lifetimes, which are indicative of increased actin polymerization. **C**, Time course of lifetime changes in dendritic spines by pharmacological treatment (open dot: latrunculin,

during the maintenance phase of LTP (Fig. 3F–H), suggesting that Rac1 activation alone can mimic the structural and actin cytoskeletal remodeling observed with synaptic stimulation.

Discussion

Our study establishes that localized and transient Rac1 activation is sufficient to induce and maintain structural LTP independently of upstream CaMKII signaling, positioning Rac1 as a trigger of the self-sustaining signal which is required for inducing long-lasting synaptic structural plasticity. While CaMKII is essential for LTP induction, our findings suggest that once Rac1 is activated, it can autonomously maintain spine structure via downstream effectors such as Pak1 and actin remodeling. These findings extend the proposed reciprocal activation of the kinase-effector complex (RAKEC) model, in which CaMKII and Tiam1 form a signaling complex that reciprocally activates both CaMKII and Tiam1-mediated Rac1 activity during LTP. Our results indicate that Rac1, once activated—whether downstream of RAKEC or via PA-Rac1 activation—can maintain plasticity independently. This supports a modular view of synaptic signaling in which RAKEC initiates plasticity through coordinated dual activation, while Rac1 ensures its structural persistence (Hedrick et al., 2016; Saneyoshi et al., 2019).

Recent studies have highlighted that CaMKII contributes to LTP not only through its kinase activity but also via kinase-independent mechanisms (Tullis et al., 2023; Chen et al., 2024; Claiborne et al., 2024). Specifically, CaMKII kinase activity is essential for LTP induction, where autophosphorylation at T286 is the only action to be required (Chen et al., 2024). However, maintenance of LTP appears to depend on kinase-independent roles of CaMKII, such as structural or scaffolding interactions within the postsynaptic density. Although the precise molecular mechanisms remain under debate, this emerging model aligns with our finding that Rac1 activation alone is sufficient to induce sLTP in a CaMKII-independent manner (Fig. 1).

Persistent Rac1 activation during the late phase of LTP may arise through at least two upstream signaling routes: one mediated by the CaMKII-Tiam1 RAKEC mechanism, which sustains Rac1 activity downstream of CaMKII (Saneyoshi et al., 2019), and another involving autocrine BDNF/TrkB signaling (Tep et al., 2012; Harward et al., 2016) and PKC (Tu et al., 2020). Because the RAKEC complex mechanism requires CaMKII kinase activity but not necessarily T286 autophosphorylation, inhibition of CaMKII would be expected to disrupt this pathway—consistent with our observation that direct Rac1 activation can bypass this requirement. Nonetheless, how persistent actin polymerization is maintained during the late phase of LTP remains unresolved. Further studies are needed to clarify how Rac1 signaling integrates with kinase-independent functions of CaMKII to stabilize spine structural plasticity.

A major remaining question is how transient Rac1 activity is converted into persistent signaling that maintains actin remodeling over time. In non-neuronal systems, Rac1 participates in positive feedback loops that maintain its GTP-bound state via the coronin 1A multiprotein complex (Castro-Castro et al., 2011). In neurons, similar mechanisms may exist—such as Rac1-mediated recruitment of Rac-specific GEFs (Penzes et al., 2008), inhibition of Rac-GAPs, or local assembly of a postsynaptic protein complex through mechanisms such as liquid-liquid phase separation (Chen et al., 2017). Such feedback could promote persistent, spatially restricted Rac1 signaling in spines during memory encoding.

The present study did not directly assess whether PA-Rac1-induced sLTP is accompanied by potentiation of AMPAR-mediated synaptic current. However, several lines of evidence suggest that Rac1 activation may be sufficient to promote AMPAR surface expression. For instance, Rac1 activation has been shown to drive AMPAR insertion in developing neurons (Wiens et al., 2005), and phosphorylation of its downstream effector PAK3 enhances AMPAR surface trafficking (Hussain et al., 2015). These findings imply that Rac1-dependent actin polymerization may facilitate the structural remodeling necessary for AMPAR stabilization at the postsynaptic membrane. Thus, combining photoactivatable signaling tools such as PA-Rac1 with electrophysiological or super-resolution imaging approach could provide a powerful strategy to dissect the molecular coupling between actin dynamics and AMPAR recruitment during LTP expression and maintenance.

Our findings also intersect with previous studies implicating Rac1 in both memory formation and forgetting. For example, a prolonged photoactivation of Rac1 (150 ms light pulse at 1 Hz for 1 h) specifically in potentiated synapses after a motor learning task can erase acquired memories (Hayashi-Takagi et al., 2015), suggesting that widespread and prolonged Rac1 activity may promote synapse weakening or elimination. In contrast, our approach—brief, localized photoactivation at single spines—induces long-lasting spine enlargement. This is consistent with *in vivo* observations in the nucleus accumbens medium spiny neurons, where Rac1 photoactivation using 473 nm light (0.5 ms light pulse at 10 Hz for 10 min) produced a small but significant increase in spine head diameter and mushroom spine density (Wright et al., 2020). These divergences likely reflect differences in the spatial and temporal pattern of Rac1 activation as well as in brain regions. Broad activation may

←
n = 37 spines from six neurons; closed dot: jasplakinolide, *n* = 30 spines from five neurons). Statistical analysis: unpaired two-tailed *t* tests; **p* < 0.05. Scale bars: 2 μm. **D**, Representative FRET-FLIM images (top) and spine morphology (bottom, using the GFP-Actin signal as a proxy) showing actin polymerization before and after sLTP induction. Warmer colors indicate shorter lifetimes, indicative of increased actin polymerization. **E**, Time course spine volume changes during sLTP induced by glutamate uncaging (black, *n* = 11 neurons) or PA-Rac1 activation (green, *n* = 10 neurons). **F**, Time course of fluorescence lifetime changes during uncaging (black) or PA-Rac1 activation (green). **G**, **H**, Comparison of lifetime values at baseline and during the persistent phase (15–31 min) for uncaging-induced sLTP (**G**) or PA-Rac1-induced sLTP (**H**). Statistical analysis: unpaired two-tailed *t* tests; **p* < 0.05. Scale bars, 1 μm.

engage signaling crosstalk with RhoA pathways (Wu et al., 2009) or trigger homeostatic plasticity across dendritic segments, shifting the role of Rac1 from potentiation to facilitating synapse pruning.

Finally, our application of a FLIM-FRET biosensor (mGFP-Actin and Lifeact-mRFP; Fig. 3) enabled quantitative, spine-specific monitoring of actin remodeling in live brain tissue. This actin probe system provides a powerful tool to dissect the biochemical dynamics downstream of Rac1 and can be applied to future studies to understand feedback regulation and synaptic remodeling in memory.

References

- Bosch M, Castro J, Saneyoshi T, Matsuno H, Sur M, Hayashi Y (2014) Structural and molecular remodeling of dendritic spine substructures during long-term potentiation. *Neuron* 82:444–459.
- Castro-Castro A, Ojeda V, Barreira M, Sauzeau V, Navarro-Lérida I, Muriel O, Couceiro JR, Pimentel-Muñíos FX, Del Pozo MA, Bustelo XR (2011) Coronin 1a promotes a cytoskeletal-based feedback loop that facilitates Rac1 translocation and activation. *EMBO J* 30:3913–3927.
- Chazneau A, et al. (2014) Nanoscale segregation of actin nucleation and elongation factors determines dendritic spine protrusion. *EMBO J* 33:2745–2764.
- Chen B, Chou H-T, Brautigam CA, Xing W, Yang S, Henry L, Doolittle LK, Walz T, Rosen MK (2017) Rac1 GTPase activates the wave regulatory complex through two distinct binding sites. *Elife* 6:E29795.
- Chen X, Cai Q, Zhou J, Pleasure SJ, Schulman H, Zhang M, Nicoll RA (2024) Camkii autophosphorylation is the only enzymatic event required for synaptic memory. *Proc Natl Acad Sci U S A* 121: E2402783121.
- Claiborne N, Anisimova M, Zito K (2024) Activity-dependent stabilization of nascent dendritic spines requires nonenzymatic Camkii function. *J Neurosci* 44:E1393222023.
- Dagliyan O, Karginov AV, Yagishita S, Gale ME, Wang H, Dermardirossian C, Wells CM, Dokholyan NV, Kasai H, Hahn KM (2017) Engineering Pak1 allosteric switches. *ACS Synth Biol* 6:1257–1262.
- Deacon SW, Beeser A, Fukui JA, Rennefahrt UEE, Myers C, Chernoff J, Peterson JR (2008) An isoform-selective, small-molecule inhibitor targets the autoregulatory mechanism of P21-activated kinase. *Chem Biol* 15:322–331.
- Haditsch U, Leone DP, Farinelli M, Chrostek-Grashoff A, Brakebusch C, Mansuy IM, McConnell SK, Palmer TD (2009) A central role for the small GTPase Rac1 in hippocampal plasticity and spatial learning and memory. *Mol Cell Neurosci* 41:409–419.
- Harward SC, Hedrick NG, Hall CE, Parra-Bueno P, Milner TA, Pan E, Laviv T, Hempstead BL, Yasuda R, Mcnamara JO (2016) Autocrine BDNF-TrkB signalling within a single dendritic spine. *Nature* 538:99–103.
- Hayashi-Takagi A, Yagishita S, Nakamura M, Shirai F, Wu YI, Loshbaugh AL, Kuhlman B, Hahn KM, Kasai H (2015) Labelling and optical erasure of synaptic memory traces in the motor cortex. *Nature* 525:333–338.
- Hedrick NG, Harward SC, Hall CE, Murakoshi H, Mcnamara JO, Yasuda R (2016) Rho GTPase complementation underlies BDNF-dependent homo- and heterosynaptic plasticity. *Nature* 538:104–108.
- Hussain NK, Thomas GM, Luo J, Huganir RL (2015) Regulation of AMPA receptor subunit GluA1 surface expression by Pak3 phosphorylation. *Proc Natl Acad Sci U S A* 112:E5883–E5890.
- Liu Y, Du S, Lv L, Lei B, Shi W, Tang Y, Wang L, Zhong Y (2016) Hippocampal activation of Rac1 regulates the forgetting of object recognition memory. *Curr Biol* 26:2351–2357.
- Niwa H, Yamamura K, Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 108:193–199.
- Penzes P, Cahill ME, Jones KA, Srivastava DP (2008) Convergent CaMK and RacGEF signals control dendritic structure and function. *Trends Cell Biol* 18:405–413.
- Riedl J, et al. (2008) Lifeact: a versatile marker to visualize F-actin. *Nat Methods* 5:605–607.
- Ripoli C, et al. (2023) Engineering memory with an extrinsically disordered kinase. *Sci Adv* 9:Eadh1110.
- Saneyoshi T (2021) Reciprocal activation within a kinase effector complex: a mechanism for the persistence of molecular memory. *Brain Res Bull* 170:58–64.
- Saneyoshi T, Hayashi Y (2012) The Ca²⁺ and Rho GTPase signaling pathways underlying activity-dependent actin remodeling at dendritic spines. *Cytoskeleton* 69:545–554.
- Saneyoshi T, Wayman G, Fortin D, Davare M, Hoshi N, Nozaki N, Natsume T, Soderling TR (2008) Activity-dependent synaptogenesis: regulation by a CaM-kinase kinase/CaM-kinase I/betapix signaling complex. *Neuron* 57:94–107.
- Saneyoshi T, Matsuno H, Suzuki A, Murakoshi H, Hedrick NG, Agnello E, O'connell R, Stratton MM, Yasuda R, Hayashi Y (2019) Reciprocal activation within a kinase-effector complex underlying persistence of structural LTP. *Neuron* 102:1199–1210.E6.
- Schindelin J, et al. (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9:676–682.
- Shutes A, Onesto C, Picard V, Leblond B, Schweighoffer F, Der CJ (2007) Specificity and mechanism of action of Eht 1864, a novel small molecule inhibitor of Rac family small GTPases. *J Biol Chem* 282:35666–35678.
- Stoppini L, Buchs PA, Muller D (1991) A simple method for organotypic cultures of nervous tissue. *J Neurosci Methods* 37:173–182.
- Sumi M, Kiuchi K, Ishikawa T, Ishii A, Hagiwara M, Nagatsu T, Hidaka H (1991) The newly synthesized selective Ca²⁺ calmodulin dependent protein kinase II inhibitor Kn-93 reduces dopamine contents in Pc12h cells. *Biochem Biophys Res Commun* 181:968–975.
- Tep C, Kim ML, Opincariu LI, Limpert AS, Chan JR, Appel B, Carter BD, Yoon SO (2012) Brain-derived neurotrophic factor (BDNF) induces polarized signaling of small GTPase (Rac1) protein at the onset of Schwann cell myelination through partitioning-defective 3 (Par3) protein. *J Biol Chem* 287:1600–1608.
- Tu X, Yasuda R, Colgan LA (2020) Rac1 is a downstream effector of PKC α in structural synaptic plasticity. *Sci Rep* 10:1777.
- Tullis JE, et al. (2023) LTP induction by structural rather than enzymatic functions of CaMKII. *Nature* 621:146–153.
- Wiens KM, Lin H, Liao D (2005) Rac1 induces the clustering of AMPA receptors during spinogenesis. *J Neurosci* 25:10627–10636.
- Wright WJ, et al. (2020) Silent synapses dictate cocaine memory destabilization and reconsolidation. *Nat Neurosci* 23:32–46.
- Wu YI, Frey D, Lungu OI, Jaehrig A, Schlichting I, Kuhlman B, Hahn KM (2009) A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* 461:104–108.
- Yasuda R, Hayashi Y, Hell JW (2022) CaMKII: a central molecular organizer of synaptic plasticity, learning and memory. *Nat Rev Neurosci* 23:666–682.
- Zoltowski BD, Vaccaro B, Crane BR (2009) Mechanism-based tuning of a LOV domain photoreceptor. *Nat Chem Biol* 5:827–834.