Structural plasticity of dendritic spines

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Key points

- Spines are structurally plastic protrusions on the dendrites.
- Spines harbor an excitatory synapse.
- Structural plasticity of spines is a basis for learning memory.
- Actin cytoskeleton plays an essential role in this process

1 Introduction

Dendritic spines, the small mushroom-shaped protrusions from dendrites, have long attracted neuroscientists due to their distinctive shape and implied potential role in synaptic transmission and plasticity, the cellular substrate underlying learning and memory (Yuste, 2023). Our understanding of the dynamics and function of dendritic spines has advanced with the development of microscopy techniques over decades that have uncovered the intricate dynamics of these structures.

The spine structure was originally discovered by Santiago Ramón y Cajal, a Spanish neuroscientist, who dedicated his life to the analysis of the morphology of the neurons with light microscopic observation of Golgi-stained tissue sections. He found that neurons have a variety of shapes and sizes depending on brain region and subtypes (Ramón y Cajal, 1899; Yuste, 2023). He noted that some populations of neurons are covered by thorny protrusions, *espinas* in Spanish or spines. He proposed that the spine is a contact site between neurons. Not only that, he also had the foresight to propose that the dendritic spines are the site of information input from another neuron.

Years later after the initial discovery of dendritic spines, the synapses in the mammalian brain tissue were visualized by electron microscopy (EM). This revealed that the spine couples with a presynaptic structure across the synaptic cleft, and there is no anastomosis of the plasma membrane between the pre- and post-synaptic neurons (Gray, 1959). As the technologies for observing the structure of the spines and synapses have advanced, neuroscientists have begun to exploit how their structural changes are associated with development, neuronal activity, and learning.

2 Diversity of dendritic spines

Spines can take different shapes, which have been traditionally categorized into four forms: filopodia, thin, mushroom, and stubby (Harris and Stevens, 1989; Pchitskaya and Bezprozvanny, 2020). Filopodia are devoid of clear head and neck and often found in immature animals and may not have synapse. The rest are typically found in mature animals. They have a head and a thin neck, which connects the head to the dendritic shaft (Fig. 1). This unique structure is considered to enable spines to function as independent regulatory units of both electrical and chemical signaling (Fifková and Van Harreveld, 1977).

At the tip of the mature spines, there is an electron-dense disk-like proteinaceous complex beneath the synaptic contact, called the postsynaptic density (PSD). The PSD is composed of membrane proteins including ion channels, cell adhesion molecules, and

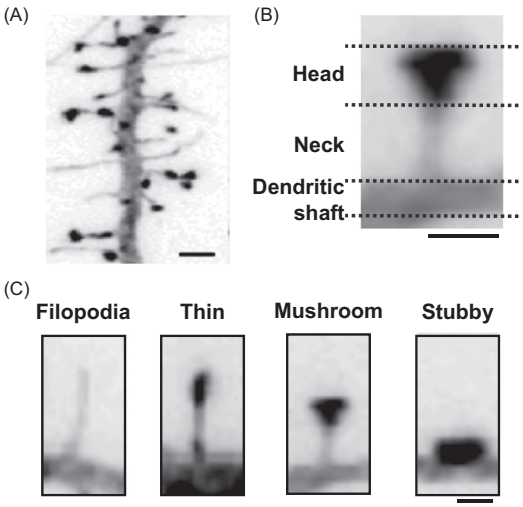

Fig. 1 Morphological diversity of dendritic spines. (A) Dendrite from a rat hippocampal neuron expressing mRFP-actin. Spines showed structural diversity. (B) Structure of a dendritic spine: head, neck and dendritic shaft. (C) Classification of dendritic spine morphology. Filopodia, a protrusion without a head; Thin, a spine with a small head; Mushroom, a spine with a clear head and neck; Stubby, a spine without a noticeable neck. Scale bars: 5 μm for (A) and 1 μm for (B and C).

neurotransmitter receptors as well as intracellular proteins such as signaling molecules, cytoskeletal proteins, and scaffolding proteins that support those functions of the membrane proteins. The PSD has a laminar structure where multiple proteins bind to each other by multifaced interaction domains mediated by liquid-liquid phase separation (LLPS) (Hayashi et al., 2021).

Additionally the spines also contain the molecular machinery for endocytosis and exocytosis to regulate the dynamics of surface receptors. Interestingly, a specialized endocytotic zone is located lateral to PSD. The cytoskeleton in the spine is exclusively filamentous actin (F-actin) (Hotulainen and Hoogenraad, 2010), which serves as structural scaffold, as well as direct and indirect binding site for various proteins including receptors, thereby determining the capacity of synaptic proteins (Hayashi et al., 2024).

3 Electron microscopy unveils structural plasticity of dendritic spines

In the 1970s, the long-term potentiation (LTP) was discovered by Bliss and Lømo, who described that in the hippocampus of the mammalian brain, synaptic transmission was persistently enhanced after a transient high-frequency stimulation (Bliss and Lomo, 1973). Since then, LTP has been considered as a cellular model for learning and memory (Nicoll, 2017). Two years later, Fifková and coworkers looked for evidence of structural changes in dendritic spines during LTP using electron microscopy. They found that in dendrites which had been electrically stimulated at high frequency, the size of dendritic spines was larger than that of unstimulated control. This enlargement was observed in 2–6 min after the stimulation and persisted for 23 h (Van Harreveld and Fifkova, 1975). They reported that the neck structure of a stimulated spines were wider and shorter than controls, which may increase the electrical coupling between the dendritic spine and the shaft (Fifková and Van Harreveld, 1977). Using a similar approach, Desmond and Levy observed an increase in PSD area associated with the concave spine profile after LTP induction (Desmond and Levy, 1986).

In addition to the shape changes induced by LTP, the constituents of the spine were also altered. Ostroff et al. found that polyribosomes, involved in local protein synthesis, were increased in dendritic spines 2 h after the tetanic stimulation (Ostroff et al., 2002). The polyribosome-containing spines also had a significantly larger PSD area, suggesting a positive correlation between LTP and new protein synthesis. A method to purify PSDs by density gradient centrifugation was developed in the 1970s (Cotman et al., 1974). Using this fraction, the components of the PSDs were analyzed, leading to the insightful hypothesis that the changes in the concentration and conformation of the PSD proteins could be associated with long-lasting effects on synaptic structure in these circuits (Siekevitz, 1985).

While the snapshots provided by these techniques contributed to the basic idea of the mechanism of plasticity and changes in dendritic spine morphology, they had limitations. These analyses relied on statistics of multiple spines to determine whether there was a difference between naïve and potentiated tissue, but were limited in their sensitivity since not every spine in the tissue underwent synaptic plasticity. Also, electron microscopy does not allow us to follow how a single dendritic spine changes shape and size over time. Likewise, with biochemical analysis of the PSD, it is not possible to measure the change in protein concentrations or conformation in real time.

4 Dynamic imaging with fluorescence microscopy: Capturing spine dynamics in real time

These limitations of the early snapshot studies were finally overcome with the use of time-lapse imaging of the neurons during LTP. Hosokawa et al. were the first to attempt time-lapse imaging of dendritic spines from acute hippocampal slices subjected to LTP induction using confocal microscopy (Hosokawa et al., 1995). They used DiI-labeled neurons to monitor the spine structure and found that a subset of spines was elongated 3 h after chemical LTP induction.

The discovery of the green fluorescent protein (GFP) and development of two-photon microscopy further facilitated the studies of dendritic spine by allowing a time-lapse imaging in deep living tissue (Shimomura, 2005; Denk et al., 1990). The striking feature of the dendritic spines revealed by early time-lapse studies is that they were not static, but constantly changed shape (Fischer et al., 1998). Maletic-Savatic et al. expressed GFP in cultured hippocampal slices to visualize neurons and induced LTP by locally stimulating the presynaptic fibers through a glass electrode. The formation of new filopodial structures was specifically observed in the LTP-induced segment of dendrites (Maletic-Savatic et al., 1999). In the same year, Engert and Bonhoeffer performed a similar experiment to induce local LTP by perfusing the entire slice with Cd^{2+}-containing extracellular solution, while locally applying a Ca^{2+}-containing solution to restrict the stimulation to an area of ~30 μm diameter. They observed that new spines appeared on the dendrite with LTP induction, but not in the surrounding area (Engert and Bonhoeffer, 1999). This is also replicated by Okamoto et al. who used an electrode to locally stimulate presynaptic fibers (Okamoto et al., 2004). They also observed a bidirectional volume change depending on the stimulation paradigm. While tetanic stimulation, which induces LTP, increased spine volume, low frequency stimulation (1 Hz for 15 min), which induces long-term depression (LTD), promoted spine volume shrinkage.

These studies provided evidence that LTP-inducing stimuli can induce the enlargement of existing spines or growth of new dendritic spines and filopodia. However, while there is a correlation between LTP induction and spine growth, it was still not clear whether LTP induction increased dendritic spine size at the single spine level. Therefore, a method to reliably stimulate single spine LTP was sought.

4.1 Two-photon uncaging of glutamate allows visualization of structural modifications: Spine enlargement, shrinkage and de novo spinogenesis

Kasai and co-workers developed a method to achieve the single spine induction of LTP (Matsuzaki et al., 2001), using a glutamate uncaging technique to release glutamate in a very small volume of tissue just adjacent to the tip of a dendritic spine using a two-photon laser. They used caged glutamate, a compound where glutamate is protected by a photosensitive group, that can be photolysed with UV light to release functional glutamate. By combining this method with electrophysiological recordings, they showed that the spine volume was increased by LTP induction using glutamate uncaging on to a given spine under observation (Matsuzaki et al., 2004). Consistent results were also observed by several other groups (Fig. 2) (Bosch et al., 2014; Lee et al., 2009; Oh et al., 2013). The changes in synaptic strength and those of spine volumes correlated well with each other (Nägerl et al., 2004; Zhou et al., 2004; Oh et al., 2013). Similarly, it has been demonstrated that uncaging of caged-γ-aminobutyric acid (GABA) results in long-lasting spine shrinkage (Fig. 2) (Hayama et al., 2013).

In developing neurons, de novo spine formation is also activity-dependent (Saneyoshi et al., 2010), however, it was unclear whether these newly formed spines were functional. Kwon and Sabatini investigated the mechanisms of spine formation in young cortical slices (Kwon and Sabatini, 2011). Using glutamate uncaging and two-photon microscopy, they found that glutamate stimulation was sufficient to induce de novo spine growth from the dendritic shaft. Moreover, the new spines were functional and able to transduce the glutamate signal, suggesting that LTP-induced spinogenesis also rapidly induces functional synapses to contribute to the potentiation of synaptic strength. Using a similar experimental approach with a caged GABA, Kwon and co-workers found that GABA has the potency to induce de novo synapse formation, both inhibitory and excitatory, in developing cortical neurons, suggesting that the developing brain also uses GABA as a determinant to establish neuronal circuits (Oh et al., 2016).

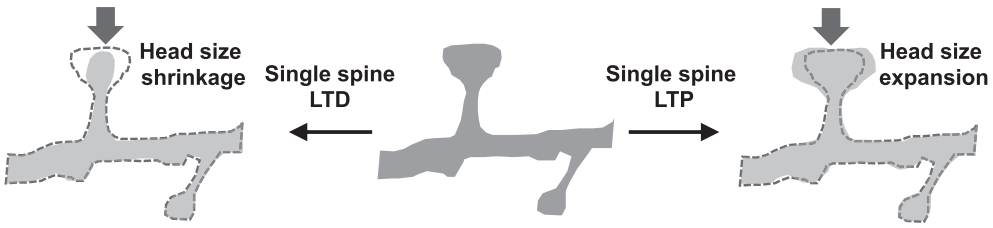

Fig. 2 Structural plasticity of dendritic spines. Neuronal activity induces changes in spine morphology. LTP induction of a single spine (indicated by the arrow on the right) results in an increase in spine head size, whereas LTD-stimulated spine (indicated by the arrow on the left) leads to a decrease in spine head size. Dashed lines represent the morphology before stimulation.

5 FRET imaging reveals dynamic regulation of actin during LTP

Another advancement of microscopic technique was Förster resonance energy transfer (FRET) (Miyawaki, 2005; Ueda et al., 2013). It is typically accomplished by labeling protein(s) of interest with two fluorescent proteins with an emission spectrum of one overlapping with an excitation of the other. Changes in protein conformation or interaction can be detected as a change in the efficiency in the energy transfer. Additionally, fluorescent life-time Imaging microscopy (FLIM) imaging allows for the measurement of FRET efficiency in small volumes within dendritic spine (Ueda et al., 2013; Yasuda, 2006).

Actin is one of the most abundant proteins in the dendritic spine, which is nearly exclusive cytoskeletal protein, with the exception of a minimal presence of others such as microtubules or intermediate filaments (Hotulainen and Hoogenraad, 2010). The importance of the actin cytoskeleton in synaptic plasticity has been demonstrated using agents that can modify actin function. Treatment of neurons with actin polymerization inhibitors (latrunculin B or cytochalasins) or the actin filament stabilizer (phalloidin) before or a few minutes after LTP induction, inhibited LTP (Krucker et al., 2000; Kim and Lisman, 1999). A treatment of hippocampal neurons with the latrunculin A also reduced surface AMPA-R, suggesting that F-actin maintains AMPA-R-mediated synaptic transmission underlying LTP (Gu et al., 2010). However, application of latrunculin 30–60 min after LTP induction showed no effect on LTP maintenance (Kelly et al., 2007; Ramachandran and Frey, 2009). This indicates that the role of actin during the LTP has time-window.

Okamoto et al. were the first to visualize the actin dynamics with FRET between actin molecules in a dendritic spine during synaptic plasticity. They found that the ratio of filamentous (F)- and globular (G)-actin was shifted toward F-actin in the LTP-stimulated expanding spines, but toward G-actin in LTD-stimulated shrinking spines (Okamoto et al., 2004), both lasting for at least for 30 min. Bosch et al. revealed that actin in the dendritic spine is regulated by an actin regulatory factor, cofilin, which is enriched in spines after the induction of LTP (Bosch et al., 2014). By using FRET imaging, they revealed that cofilin forms a persistent stable complex with F-actin, thereby stabilizing it (Bosch et al., 2014).

Goto et al. took a different approach to test the involvement of actin in LTP (Goto et al., 2021). They developed a method to photoinactivate cofilin by fusing it with a photosensitizing fluorescent protein, SuperNova. Illumination of cofilin-SuperNova specifically erased both functional and structural LTP within a 30 min time window but not at 50 min. These results are consistent with the idea that actin is required after the early (<30 min) phase of LTP but not in the later phase.

5.1 Observation of intracellular dynamics of actin regulatory mechanism

The actin cytoskeleton is regulated by Rho-GTPase, downstream of calmodulin-dependent protein kinase II (CaMKII) in synaptic plasticity (Murakoshi et al., 2011; Saneyoshi and Hayashi, 2012; Saneyoshi et al., 2019) (Fig. 3). During the induction of LTP, the duration of calcium influx via the NMDA-type glutamate receptor (NMDA-R) and CaMKII activation in the spine are transient (<1 min), whereas Rho-GTPase activity, increased actin polymerization, and spine volume changes are both persistent (Lee et al., 2009; Murakoshi et al., 2011). Therefore, the conversion from transient to persistent signaling during LTP should occur between CaMKII and Rho-GTPase. Elucidation of the signal conversion is then critical to understanding the molecular mechanisms of long-term memory.

Among the NMDA-R-regulated Rho-GTPase signaling molecules, Tiam1, a calcium-dependent Rac guanine nucleotide exchange factor, stably interacts with CaMKII (Saneyoshi et al., 2019). Interestingly, this interaction locks CaMKII into an active conformation. In turn, Tiam1 can be activated by CaMKII phosphorylation. Thus, the binding of CaMKII and Tiam1 forms a reciprocally-activating kinase-effector complex (RAKEC) (Saneyoshi et al., 2019; Saneyoshi, 2021), and this activation loop converts transient signals into sustained Rac activity. Activated Rac is required to maintain the increase in spine volume by regulating actin (Saneyoshi et al., 2019). At present, persistent CaMKII activity at the synapse has not been detected by FRET sensors (Lee et al., 2009; Saneyoshi et al., 2019). In the future, as FRET sensors for CaMKII become more sensitive and higher resolution, the persistent component of CaMKII at synaptic sites may be detected.

6 Presynaptic mechanical pressure-induced plasticity: A novel perspective

Induction of LTP in a spine triggers enlargement of the dendritic spine head (Matsuzaki et al., 2001; Lee et al., 2009; Bosch et al., 2014; Saneyoshi et al., 2019), which can be ~4 times larger than the original in the first few min then settles at 1.5–2.0 times larger than the original. Kasai et al. probed the functional significance of this initial increase in spine head size (Ucar et al., 2021). Using the cutting-edge technology of imaging and manipulation of neuronal circuits, it was shown that the mechanical force onto the presynaptic side by the enlarged spine head during LTP, enhanced the release of neurotransmitter from the presynaptic side, providing a new role of the spine head expansion during plasticity, called PREssure Sensation and Transduction, PREST (Ucar et al., 2021). The expansion of the head size during LTP induction is not only for expanding the area for AMPA-type glutamate receptors, but also enhances the release probability of neurotransmitter from the presynaptic side.

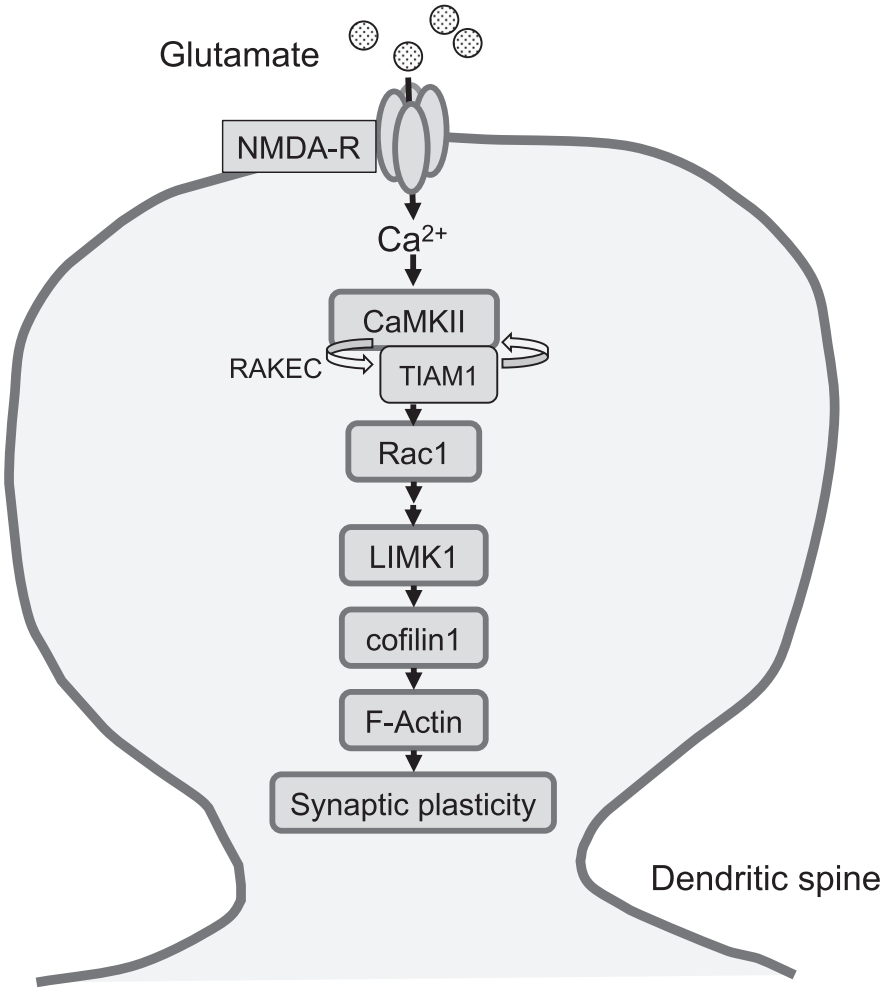

Fig. 3 The signaling molecules mediating the structural plasticity of dendritic spines. Upon release from the presynaptic terminal, glutamate binding to the NMDA-type glutamate receptor (NMDA-R) induces calcium influx into the dendritic spine cytosol. Subsequently, the calcium ion binds to calmodulin and activates CaMKII. Upon activation, CaMKII forms a complex with the Rac-guanine nucleotide exchange factor (GEF), TIAM1. A reciprocal activation within kinase-effector complex (RAKEC) is formed, which creates a feedback loop to maintain the activity of CaMKII and TIAM1. The RAKEC persistently activates Rac1 and its downstream effectors, including LIMK1, which then phosphorylate and inactivate cofilin1. Inactivation of cofilin1 results in the stabilization of F-actin, which in turn induces structural plasticity.

6.1 In vivo observation of spine dynamics in live animals

Most studies of synaptic plasticity use brain slices or isolated neurons either in acute preparation or in culture. It is of great interest to test the same molecular and cellular mechanisms take place during learning in the intact brain. With the development of two-photon microscopy, researchers have been able to perform time-lapse imaging of the morphological changes of the same spine over time in vivo (Grutzendler et al., 2002). In the neocortical region of a mouse expressing fluorescent protein, the stability of dendritic spine increased from young to adult mice (Holtmaat et al., 2005; Zuo et al., 2005). In 4–6-month-old mice, more than 74 % of the spines were observed to be stable. Moreover, the stability of the spines correlated well with their shape, with thin filopodia being short-lived, while mushroom-shaped spines had a longer lifetime.

Studies have also been conducted to directly link between spine formation and learning in vivo. Hayashi-Takagi et al. developed a novel tool to express photoactivatable Rac (PA-Rac) in spines where activity-dependent protein synthesis resulting from LTP took place. The photoactivation of PA-Rac then allowed for specific elimination of those spines (Hayashi-Takagi et al., 2015). Using this tool, they observed that elimination of learning-associated spines inhibited memory recall in a rotor rod learning task (Hayashi-Takagi et al., 2015). Goto et al. expressed cofilin-SuperNova in the mouse hippocampus and illuminated the structure after a learning task. They found that the illumination and the resulting inactivation of cofilin after the learning erased memory retrospectively (Goto et al., 2021). Therefore, LTP of the spine is associated with functional plasticity leading to memory storage in brain tissue.

7 Conclusion

With the advance of microscopic techniques, dendritic spine research has evolved from static morphological analyses to dynamic investigations of synaptic plasticity. From the electron microscopic discovery of structural changes, to in vivo imaging of real-time changes in spine dynamics during learning, each innovation has advanced our understanding of the dynamic nature of synaptic connections. By integrating diverse techniques, including molecular imaging, in vivo observations, and biomechanical studies, we continue to unravel the intricate mechanisms underlying dendritic spine plasticity, shedding new light on the fundamental processes of learning, memory, and brain function.

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References

Bliss TV and Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *The Journal of Physiology* 232: 331–356.

Bosch M, Castro J, Saneyoshi T, Matsuno H, Sur M, and Hayashi Y (2014) Structural and molecular remodeling of dendritic spine substructures during long-term potentiation. *Neuron* 82: 444–459.

Cotman CW, Banker G, Churchill L, and Taylor D (1974) Isolation of postsynaptic densities from rat brain. *The Journal of Cell Biology* 63: 441–455.

Denk W, Strickler JH, and Webb WW (1990) Two-photon laser scanning fluorescence microscopy. *Science* 248: 73–76.

Desmond NL and Levy WB (1986) Changes in the postsynaptic density with long-term potentiation in the dentate gyrus. *Journal of Comparative Neurology* 253: 476–482.

Engert F and Bonhoeffer T (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* 399: 66–70.

Fifková E and Van Harreveld A (1977) Long-lasting morphological changes in dendritic spines of dentate granular cells following stimulation of the entorhinal area. *Journal of Neurocytology* 6: 211–230.

Fischer M, Kaech S, Knutti D, and Matus A (1998) Rapid actin-based plasticity in dendritic spines. *Neuron* 20: 847–854.

Goto A, Bota A, Miya K, Wang J, Tsukamoto S, Jiang X, Hirai D, Murayama M, Matsuda T, Mchugh TJ, Nagai T, and Hayashi Y (2021) Stepwise synaptic plasticity events drive the early phase of memory consolidation. *Science* 374: 857–863.

Gray EG (1959) Electron microscopy of synaptic contacts on dendrite spines of the cerebral cortex. *Nature* 183: 1592–1593.

Grutzendler J, Kasthuri N, and Gan W-B (2002) Long-term dendritic spine stability in the adult cortex. *Nature* 420: 812–816.

Gu J, Lee CW, Fan Y, Komlos D, Tang X, Sun C, Yu K, Hartzell HC, Chen G, Bamburg JR, and Zheng JQ (2010) ADF/cofilin-mediated actin dynamics regulate AMPA receptor trafficking during synaptic plasticity. *Nature Neuroscience* 13: 1208–1215.

Harris KM and Stevens JK (1989) Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: Serial electron microscopy with reference to their biophysical characteristics. *The Journal of Neuroscience* 9: 2982–2997.

Hayama T, Noguchi J, Watanabe S, Takahashi N, Hayashi-Takagi A, Ellis-Davies GC, Matsuzaki M, and Kasai H (2013) GABA promotes the competitive selection of dendritic spines by controlling local Ca2+ signaling. *Nature Neuroscience* 16: 1409–1416.

Hayashi Y, Ford LK, Fioriti L, McGurk L, and Zhang M (2021) Liquid-liquid phase separation in physiology and pathophysiology of the nervous system. *The Journal of Neuroscience* 41: 834–844.

Hayashi Y, Bosch M, Liu P-W, Hosokawa T, and Saneyoshi T (2024) Molecular mechanisms underlying synaptic tagging and consolidation. In: Sajikumar S and Abel T (eds.) *Synaptic Tagging and Capture: From Synapses to Behavior*. Cham: Springer International Publishing.

Hayashi-Takagi A, Yagishita S, Nakamura M, Shirai F, Wu YI, Loshbaugh AL, Kuhlman B, Hahn KM, and Kasai H (2015) Labelling and optical erasure of synaptic memory traces in the motor cortex. *Nature* 525: 333–338.

Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, and Svoboda K (2005) Transient and persistent dendritic spines in the neocortex in vivo. *Neuron* 45: 279–291.

Hosokawa T, Rusakov DA, Bliss TV, and Fine A (1995) Repeated confocal imaging of individual dendritic spines in the living hippocampal slice: Evidence for changes in length and orientation associated with chemically induced LTP. *The Journal of Neuroscience* 15: 5560–5573.

Hotulainen P and Hoogenraad CC (2010) Actin in dendritic spines: Connecting dynamics to function. *The Journal of Cell Biology* 189: 619–629.

Kelly MT, Yao Y, Sondhi R, and Sacktor TC (2007) Actin polymerization regulates the synthesis of PKMzeta in LTP. *Neuropharmacology* 52: 41–45.

Kim CH and Lisman JE (1999) A role of actin filament in synaptic transmission and long-term potentiation. *The Journal of Neuroscience* 19: 4314–4324.

Krucker T, Siggins GR, and Halpain S (2000) Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* 97: 6856–6861.

Kwon HB and Sabatini BL (2011) Glutamate induces de novo growth of functional spines in developing cortex. *Nature* 474: 100–104.

Lee SJ, Escobedo-Lozoya Y, Szatmari EM, and Yasuda R (2009) Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* 458: 299–304.

Maletic-Savatic M, Malinow R, and Svoboda K (1999) Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* 283: 1923–1927.

Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, and Kasai H (2001) Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nature Neuroscience* 4: 1086–1092.

Matsuzaki M, Honkura N, Ellis-Davies GC, and Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature* 429: 761–766.

Miyawaki A (2005) Innovations in the imaging of brain functions using fluorescent proteins. *Neuron* 48: 189–199.

Murakoshi H, Wang H, and Yasuda R (2011) Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature* 472: 100–104.

Nägerl UV, Eberhorn N, Cambridge SB, and Bonhoeffer T (2004) Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron* 44: 759–767.

Nicoll RA (2017) A brief history of long-term potentiation. *Neuron* 93: 281–290.
Oh WC, Hill TC, and Zito K (2013) Synapse-specific and size-dependent mechanisms of spine structural plasticity accompanying synaptic weakening. *Proceedings of the National Academy of Sciences of the United States of America* 110: E305–E312.
Oh WC, Lutzu S, Castillo PE, and Kwon H-B (2016) De novo synaptogenesis induced by GABA in the developing mouse cortex. *Science* 353: 1037–1040.
Okamoto K, Nagai T, Miyawaki A, and Hayashi Y (2004) Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. *Nature Neuroscience* 7: 1104–1112.
Ostroff LE, Fiala JC, Allwardt B, and Harris KM (2002) Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* 35: 535–545.
Pchitskaya E and Bezprozvanny I (2020) Dendritic spines shape analysis—Classification or clusterization? Perspective. *Frontiers in Synaptic Neuroscience* 12: 31.
Ramachandran B and Frey JU (2009) Interfering with the actin network and its effect on long-term potentiation and synaptic tagging in hippocampal CA1 neurons in slices in vitro. *The Journal of Neuroscience* 29: 12167–12173.
Ramón y Cajal S (1899) *Textura del sistema nervioso del hombre y de los vertebrados: estudios sobre el plan estructural y composición histológica de los centros nerviosos adicionados de consideraciones fisiológicas fundadas en los nuevos descubrimientos.* Volumen I. Nicolas Moya.
Saneyoshi T (2021) Reciprocal activation within a kinase effector complex: A mechanism for the persistence of molecular memory. *Brain Research Bulletin* 170: 58–64.
Saneyoshi T and Hayashi Y (2012) The Ca2+ and Rho GTPase signaling pathways underlying activity-dependent actin remodeling at dendritic spines. *Cytoskeleton (Hoboken)* 69: 545–554.
Saneyoshi T, Fortin DA, and Soderling TR (2010) Regulation of spine and synapse formation by activity-dependent intracellular signaling pathways. *Current Opinion in Neurobiology* 20: 108–115.
Saneyoshi T, Matsuno H, Suzuki A, Murakoshi H, Hedrick NG, Agnello E, O'Connell R, Stratton MM, Yasuda R, and Hayashi Y (2019) Reciprocal activation within a kinase-effector complex underlying persistence of structural LTP. *Neuron* 102: 1199–1210.e6.
Shimomura O (2005) The discovery of aequorin and green fluorescent protein. *Journal of Microscopy* 217: 3–15.
Siekevitz P (1985) The postsynaptic density: A possible role in long-lasting effects in the central nervous system. *Proceedings of the National Academy of Sciences of the United States of America* 82: 3494–3498.
Ucar H, Watanabe S, Noguchi J, Morimoto Y, Iino Y, Yagishita S, Takahashi N, and Kasai H (2021) Mechanical actions of dendritic-spine enlargement on presynaptic exocytosis. *Nature* 600: 686–689.
Ueda Y, Kwok S, and Hayashi Y (2013) Application of FRET probes in the analysis of neuronal plasticity. *Frontiers in Neural Circuits* 7: 163.
Van Harreveld A and Fifkova E (1975) Swelling of dendritic spines in the fascia dentata after stimulation of the perforant fibers as a mechanism of post-tetanic potentiation. *Experimental Neurology* 49: 736–749.
Yasuda R (2006) Imaging spatiotemporal dynamics of neuronal signaling using fluorescence resonance energy transfer and fluorescence lifetime imaging microscopy. *Current Opinion in Neurobiology* 16: 551–561.
Yuste R (2023) *Dendritic Spines.* MIT Press.
Zhou Q, Homma KJ, and Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron* 44: 749–757.
Zuo Y, Lin A, Chang P, and Gan WB (2005) Development of long-term dendritic spine stability in diverse regions of cerebral cortex. *Neuron* 46: 181–189.