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REVIEW ARTICLE

# Plasticity of dendritic spines: Molecular function and dysfunction in neurodevelopmental disorders

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Dendritic spines are tiny postsynaptic protrusions from a dendrite that receive most of the excitatory synaptic input in the brain. Functional and structural changes in dendritic spines are critical for synaptic plasticity, a cellular model of learning and memory. Conversely, altered spine morphology and plasticity are common hallmarks of human neurodevelopmental disorders, such as intellectual disability and autism. The advances in molecular and optical techniques have allowed for exploration of dynamic changes in structure and signal transduction at single-spine resolution, providing significant insights into the molecular regulation underlying spine structural plasticity. Here, I review recent findings on: how synaptic stimulation leads to diverse forms of spine structural plasticity; how the associated biochemical signals are initiated and transmitted into neuronal compartments; and how disruption of single genes associated with neurodevelopmental disorders can lead to abnormal spine structure in human and mouse brains. In particular, I discuss the functions of the Ras superfamily of small GTPases in spatiotemporal regulation of the actin cytoskeleton and protein synthesis in dendritic spines. Multiple lines of evidence implicate disrupted Ras signaling pathways in the spine structural abnormalities observed in neurodevelopmental disorders. Both deficient and excessive Ras activities lead to disrupted spine structure and deficits in learning and memory. Dysregulation of spine Ras signaling, therefore, may play a key role in the pathogenesis of multiple neurodevelopmental disorders with distinct etiologies.

**Keywords:** *actin, brain, dendritic spines, neurodevelopmental disorder, synaptic plasticity.*

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The dendritic spine, a small membranous protrusion from the dendrite, was first described by the Spanish histologist Santiago Ramón y Cajal in 1888.[1] He successfully visualized numerous tiny structures on dendrites under light microscope using Golgi silver impregnation staining, which labels a sparse subset of neurons in fixed brain tissue. Subsequent electron microscopy studies confirmed his findings that dendritic spines are postsynaptic compartments that receive the majority of excitatory input in the brain.[2–4] Live-imaging studies have demonstrated that spines can compartmentalize calcium ($Ca^{2+}$) and, therefore, can serve as biochemical signaling compartments that isolate synaptic inputs from each other.[5,6] Indeed, dendritic spines are shown to be important substrates for $Ca^{2+}$-dependent signaling pathways essential for synaptic plasticity, a possible cellular mechanism of learning and memory. At the molecular level, each spine contains a postsynaptic density (PSD), a large macromolecular structure attached to the postsynaptic membrane. The PSD serves as a general organizer of the postsynaptic signaling machinery consisting of cytoskeletal proteins, cell adhesion molecules, and receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) and N-methyl-d-aspartate receptors (NMDAR).[7–9] Importantly, the spine volume is proportional to the area of PSD, the number of synaptic AMPAR, the amplitude of the AMPAR-mediated currents, and the presynaptic area of its synaptic partner.[10–12] Thus, the spine morphology is tightly associated with synaptic function. Indeed, many studies have revealed that long-term potentiation (LTP) and depression (LTD), two major forms of synaptic plasticity, are correlated with spine enlargement and shrinkage, respectively.[13–20] Conversely, alterations in dendritic spine shape, size, and density have been implicated in many neurodevelopmental disorders, such as autism and intellectual disability (ID), suggesting that dendritic spine dysfunction is a common pathomechanism. However, due to the extremely small size and complexity of dendritic spines, it has been challenging to study individual spine function and dysfunction with high enough spatiotemporal resolution. Here, I summarize recent advances in molecular and optical techniques that have revealed the physiological functions of dendritic spines, neuropathological studies on spine abnormalities in diseased human brain tissue, and animal studies exploring the molecular, cellular, and behavioral mechanisms underlying the pathogenesis of neurodevelopmental disorders.

## Structural plasticity of dendritic spines

The dendritic spine consists of a small bulbous head (~0.1 femtoliter volume) connected to the parent dendrite through a thin neck measuring ~0.1 μm in diameter and ~0.5 μm in length. The spine neck acts as a barrier to the diffusion of biochemical signals and electrical spread of excitation; hence, an individual spine can act as an isolated biochemical and electrical compartment in the neuron. The morphology of spines is highly variable, even within the same dendritic arbor, suggesting that individual spines can be independently regulated. Moreover, spines are highly dynamic structures that are continuously formed and eliminated throughout life.[21–24] The turnover of spines is thought to be essential for the proper development and refinement of neuronal circuits. Furthermore, it has been shown that LTP and LTD are associated with spine enlargement and shrinkage, respectively.[13–20] Collectively, these studies strongly suggest that the structural remodeling of dendritic spines serves to shape and refine neuronal connectivity. Therefore, mechanistic understanding of

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signaling pathways that regulate spine morphology may provide fundamental insights into the molecular regulation of neural processing and ultimately cognitive function in the brain.

The study of dendritic spines has been greatly facilitated by advances in optical techniques. In particular, two-photon microscopy can reveal the morphological changes of dendritic spines within light-scattering brain tissue at a high spatial and temporal resolution *in vitro* as well as *in vivo*. Furthermore, two-photon uncaging of the 4-methoxy-7-nitroindolinyl (MNI)-caged-glutamate can mimic rapid glutamate release from single synapses and trigger responses on single dendritic spines.[12] The combined use of two-photon imaging and uncaging enables researchers to selectively stimulate a single spine with glutamate while simultaneously imaging the morphological changes. Using these techniques, it has been demonstrated that LTP and LTD can be induced at a single synapse level.[16–18,25] Studies have shown that repetitive two-photon glutamate uncaging under nominally zero $Mg^{2+}$ conditions can induce a rapid and robust increase of spine head volume. This is followed by a moderate spine volume increase sustained for hours, termed 'structural plasticity.[26] This spine enlargement is associated with an increase in postsynaptic glutamate sensitivity at single synapses, indicating a tight correlation between structural and functional plasticity. Using the same methodology with a weak stimulation, LTD was also shown to be induced at single synapses but was accompanied by a decrease in spine volume.[16,18] The low-frequency glutamate uncaging in low extracellular $Ca^{2+}$ and nominally zero $Mg^{2+}$ conditions can induce spine-specific LTD and spine shrinkage.[16] Interestingly, spine shrinkage and elimination were shown to be promoted by the activation of γ-aminobutyric acid (GABA) receptors. When both glutamate and GABA receptors are activated by two-color uncaging of glutamate and GABA in synchrony with back-propagating action potentials, marked spine shrinkage and elimination can be induced.[18] In this protocol, spine shrinkage and elimination were not restricted to the stimulated spines but spread into nearby spines. Moreover, unlike LTP and LTD, homeostatic plasticity refers to a change in the strength of synapses that occurs in response to chronic changes in neuronal activity.[27] For example, overall synaptic activity is increased and decreased when neuronal activity is chronically suppressed and enhanced, respectively. Homeostatic plasticity provides an effective compensatory mechanism to adjust synaptic strengths in the correct direction to promote stability. It has been shown that chronic blockade of neuronal activity leads to the reversible growth of dendritic spines in CA1 pyramidal neurons in the hippocampus *in vitro*.[28,29] In addition, sensory deprivation results in a homeostatic increase in spine volume in a subset of dendritic branches of pyramidal neurons in the mouse visual cortex *in vivo*.[30,31] These results suggest that homeostatic spine structural changes occur *in vitro* and *in vivo* in parallel with synaptic scaling. In addition to morphological changes of pre-existing spines, LTP induction by electrophysiological stimulation has been shown to trigger spinogenesis – the formation of new spines.[19,32,33] Similarly, two-photon glutamate uncaging onto the dendritic shafts of young neurons induced rapid spine formation within a few micrometers of the stimulated spot.[34] These studies collectively suggest that individual spines can serve as independent functional units expressing diverse forms of synaptic plasticity.

## Molecular structure of dendritic spines

The cytoskeleton provides a structural framework for the cell, serving as a scaffold that determines cell shape and the general organization of the cytoplasm.[35] In neurons, the cytoskeleton of dendrites is composed of microtubules, neurofilaments, and actin filaments. In contrast, the cytoskeleton of the dendritic spine is predominately composed of actin filaments.[36] Actin monomers (globular actin or G-actin) polymerize to form actin filaments (filamentous actin or F-actin) with the aid of a variety of actin-binding proteins (ABP). F-actin provides the force necessary for changes in cell shape, such as the formation of cellular protrusions. Actin polymerization and depolymerization are believed to be the major determinants of dendritic spine structure. Recent developments in super-resolution microscopy have led to the discovery of additional F-actin-based structures in neurons. For example, actin rings, originally discovered in axons, are periodic F-actin structures also found in the dendrites and necks of dendritic spines.[37–39] These structures are thought to support neurite shape, help in the organization of proteins along the plasma membrane, and possibly influence spine neck elasticity during synaptic plasticity.[40] Furthermore, spine actin networks are associated with other specialized substructures, such as the PSD, which consists of hundreds of proteins to organize and stabilize synaptic receptors, cytoskeletal proteins, and signaling proteins.[7–9] Therefore, the spine actin cytoskeleton forms the structural and functional networks required for synaptic transmission and plasticity. In addition, recent genetic studies have shown that many mutations associated with neurodevelopmental disorders involve genes encoding regulators of the spine actin cytoskeleton,[41] suggesting that mechanisms regulating the actin cytoskeleton may contribute to spine pathology in neurodevelopmental disorders.

## Biochemical signaling during spine structural plasticity

Free $Ca^{2+}$ is a ubiquitous second messenger that accumulates in the cytoplasm in response to diverse stimuli. The activation of NMDAR in the PSD by synaptic glutamate leads to $Ca^{2+}$ accumulation in the spine head, which can be directly visualized using fluorescent $Ca^{2+}$ indicators under high-resolution microscopy, such as two-photon microscopy.[42] Synaptic stimulation produces a short (~0.1 s) $Ca^{2+}$ transient largely restricted to the stimulated spines,[5,43–45] indicating that dendritic spines can act as independent signaling compartments. Indeed, repeated synaptic stimulation is sufficient to activate signaling cascades crucial for structural plasticity of single dendritic spines.[17,25]

Until recently, it was not possible to study dynamic signaling responses in individual spines. Förster resonance energy transfer (FRET; also referred to as 'fluorescence resonance energy transfer') is a powerful technique that can visualize the spatiotemporal dynamics of signaling pathways within living cells. FRET exploits the distance-dependence of transfer of energy from an excited donor fluorophore to an acceptor to map molecular interactions and movements,[46] such as protein–protein interactions and protein conformational changes. Furthermore, the development of two-photon fluorescence lifetime imaging microscopy (2pFLIM) has enabled us to detect FRET signals from small subcellular compartments, such as single dendritic spines deep in the light-scattering brain tissue.[25,47,48] Therefore, 2pFLIM has been used to study the signaling pathways regulating spine actin polymerization and structural changes associated with LTP.

The $Ca^{2+}$/calmodulin-dependent protein kinase IIα (CaMKIIα) is one of the most abundant proteins in the PSD and is required for LTP, learning, and memory. Calcium flowing into the spine through NMDAR binds to the $Ca^{2+}$-binding protein calmodulin (CaM) and $Ca^{2+}$-CaM complex activates CaMKIIα for LTP induction (Fig. 1a).[49] CaMKIIα activity during spine structural plasticity has been successfully imaged using 2pFLIM combined with a FRET-based biosensor for CaMKIIα.[15,25] Induction of LTP by glutamate uncaging triggers rapid CaMKIIα activation in hippocampal CA1 pyramidal neurons that is restricted to the stimulated spine. This activity decays with a time constant of ~10 s, two orders of magnitude longer than the $Ca^{2+}$ transient, suggesting that CaMKII serves to extend $Ca^{2+}$ signal in the spine. However, the relatively brief duration of CamKIIα activity suggests that additional downstream mechanisms are required to more directly regulate actin polymerization during spine structural plasticity over longer timescales.

The Ras superfamily of small GTPases consists of more than 150 members involved in a variety of signal transduction processes, including actin polymerization. In particular, Ras/Rho GTPases, such as Ras, RhoA, Cdc42, and Rac1, are strongly implicated in

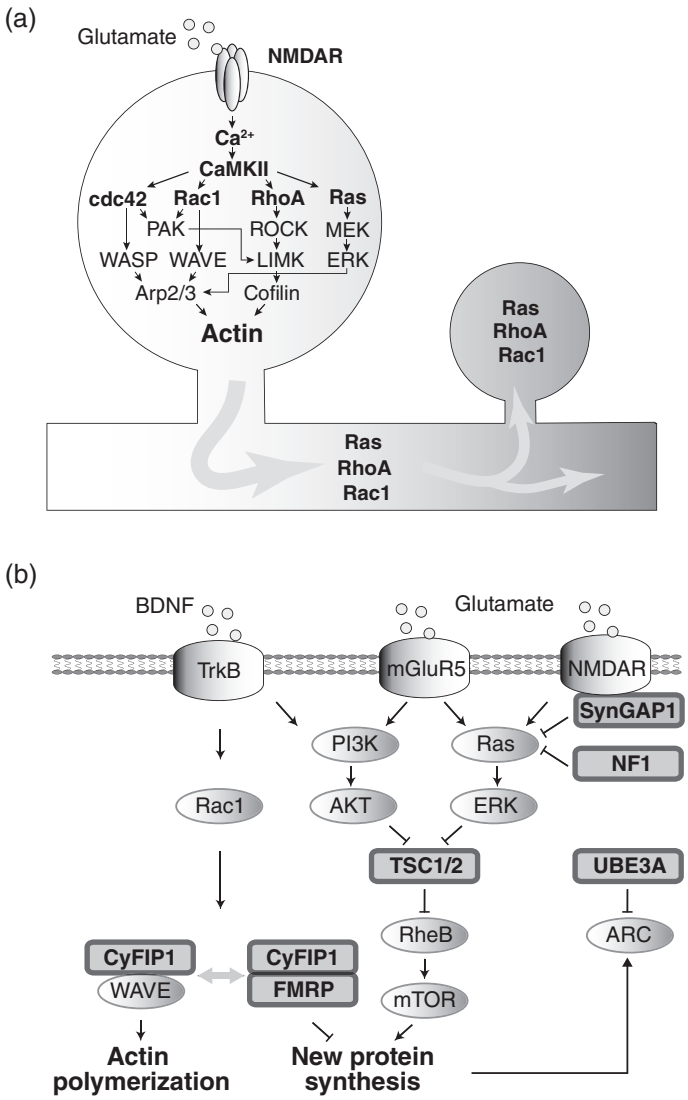


**Fig.1** Signal transduction in health and neurodevelopmental disorders. (a) Schematic diagram of hypothetical signaling pathways associated with spine structural plasticity. (b) Schematic diagram of hypothetical signaling pathways connecting neurodevelopmental disorders linked to Ras small GTPases to actin polymerization and new protein synthesis in dendrites and spines. Bold, proteins associated with single genes linked to intellectual disability and autism. BDNF, brain-derived neurotrophic factor; FMRP, fragile X mental retardation protein; NMDAR, NMDA receptor.

actin reorganization, spine morphogenesis, and LTP (Fig. 1a). The small GTPases are signaling proteins that cycle between the active GTP-bound state and the inactive GDP-bound state. The GDP–GTP cycle is regulated by guanine nucleotide exchange factors (GEF) and GTPase-activating proteins (GAP). GEF turn on GTPase signaling by catalyzing the exchange of G-protein-bound GDP for GTP, whereas GAP terminate signaling by inducing GTP hydrolysis to GDP.[50] A number of studies have identified Ras GTPases and associated GEF and GAP at synapses.[7–9] In particular, H-Ras is the most abundant Ras isoform expressed in the adult brain.[51] The activity of H-Ras during LTP has been successfully imaged by 2pFLIM using a specific FRET-FLIM sensor.[52] When structural plasticity was induced in single spines, H-Ras activity was rapidly increased at the stimulated spine.[52] Importantly, in contrast to the restricted activation of CaMKII, H-Ras activation spread along the parent dendritic shaft for over ~10 μm and decayed at a much slower rate (over ~20 min vs ~10 s for CaMKII). Inhibition of CaMKII suppressed H-Ras activation in spines, indicating that H-Ras is downstream of CaMKII. Similarly, the spatiotemporal activities of RhoA, Cdc42, and Rac1 have been resolved with 2pFLIM-FRET. These studies show that RhoA and Rac1 activities also spread into the parent dendrite and nearby spines, whereas Cdc42 activity remains highly compartmentalized in the stimulated spine.[53,54] Although the activation of these small GTPases in stimulated spines appears essential for spine structural plasticity (reviewed below), the functions conferred by signal spread remain elusive. One possibility is that spread of activated H-Ras during induction of structural plasticity at the stimulated spine may tune the threshold for plasticity at nearby synapses.[55] In this way, plasticity can modulate subsequent plasticity.

Activated Ras GTPases, in turn, regulate the actin affinity of ABP, such as cofilin and Arp2/3 (Fig. 1a). Imaging by 2pFLIM showed sustained increases in cofilin–actin and cofilin–cofilin interactions in stimulated spines, leading to the formation of a stable cofilin-decorated F-actin complex on LTP induction.[56] In addition to activating this Ras–ABP pathway, CaMKII may also regulate F-actin dynamics more directly. In contrast to CaMKIIα, CaMKIIβ possesses an F-actin-binding domain, enabling the dodecameric CaMKII complex to stabilize actin networks in spines. It has been shown that CaMKIIβ can dissociate and reassociate rapidly with actin networks during structural plasticity, limiting the time window for actin remodeling during structural plasticity.[57] These results suggest that CaMKII has both kinase signaling and structural functions in spine plasticity. Activation of various Ras/Rho GTPases and associated ABP cooperate to link CaMKII activity to actin polymerization, leading to structural changes at dendritic spines.

Genetic studies have likened neurodevelopmental disorders to various synaptic GEF and GAP for Rho GTPases, such as Kalirin (Rho-GEF), TRIO (Rho-GEF), OPHN1 (Rho-GAP), ARHGEF9 (Cdc42-GEF), and ORCL1 (Rac-GAP).[58–63] Further studies will be required to understand how specific GEF and GAP shape the spatiotemporal activation patterns of Rho GTPases, and how disruption of these signaling processes alters synaptic plasticity in neurodevelopmental disorders.

## Dendritic spines in neurodevelopmental disorders

Given that dendritic spines are essential for synaptic transmission, plasticity, and ultimately for learning and memory, it is not surprising that disruption of dendritic spine plasticity leads to cognitive dysfunction in the human brain. Indeed, accumulating evidence from post-mortem human brain studies as well as animal models supports this idea. These studies, together with findings from human genetics, suggest that aberrant dendritic spine plasticity is shared among various neurodevelopmental disorders with distinct etiologies. While many key questions remain, these studies have provided significant insights into how dendritic spines regulate cognitive function and how altered spine plasticity contributes to neurodevelopment disorders.

### Human brain pathology

The post-mortem human brain is an invaluable resource to directly study pathological changes in patients' brains and often provides promising cellular and molecular markers translatable to animal studies. Indeed, post-mortem human brain studies have revealed several structural and histological changes associated with psychiatric disorders. These studies have led to the recognition in the 1960s and 1970s that major psychiatric disorders are diseases of the brain, and that at least some are associated with altered brain anatomy.[64] Based on this notion, brain pathology in psychiatric disorders has been intensively studied in subsequent decades, together with neuroimaging studies of human subjects. However, neuropathological studies of post-mortem tissue are often complicated by confounding factors, such as variable time from death to fixation, traumatic damage, and

the differential effects of the cause of death and medications. Further, such studies may have low statistical power and reproducibility due to limited sample availability. In addition, neuropathological findings cannot distinguish between cause and consequence. Despite these limitations, neuropathological studies have consistently reported synaptic dysfunction, including aberrant spine morphology, in human neurodevelopmental disorders.[65–73]

The neurodevelopmental disorders are a group of conditions that result in early-onset deficits in cognitive function.[74] ID, previously called 'mental retardation,' is a major neurodevelopmental disorder characterized by deficits in both intellectual functioning and adaptive behavior. It affects approximately 1%–3% of the population and can be caused by a variety of genetic and non-genetic factors. Importantly, an association between dendritic spine abnormalities and neuropsychiatric disorders was first suggested by Golgi staining of post-mortem brain tissue from ID patients.[65–67] These studies reported two types of dendritic spine abnormalities in cortical pyramidal neurons of ID patients, dendritic spine loss (paucity of spins) and an overabundance of long thin 'immature' spines. Although these early studies do not offer any quantitative information and Golgi staining suffers from limited resolution for imaging dendritic spines, these findings provide the first evidence of the association between dendritic spine dysgenesis and ID.[68]

Similar to ID, autism spectrum disorder (ASD) is a broad group of neurodevelopmental conditions characterized by deficits in social communication and social interaction. ASD, in contrast to ID, is associated with significantly increased spine density on various neuronal types, such as layer (L) 2/3 cortical pyramidal neurons in the frontal, temporal, and parietal lobes and neurons in the lateral nucleus of the amygdala.[69,70] Furthermore, high spine density in cortical pyramidal neurons was correlated with lower cognitive function, supporting the idea that hyperconnectivity of cortical networks may underlie the cognitive dysfunction in ASD.[69] Indeed, human functional MRI studies have shown functional hyperconnectivity across multiple brain regions, including cortical and subcortical areas in children with ASD.[75] As clinical characteristics of ID and ASD often overlap, spine disruption might be a common underlying mechanism for neurodevelopmental disorders.

Further, several genetic disorders share the dysfunctional spine structures of ID or ASD, providing clues to the molecular regulation of spine function. For example, Fragile X syndrome (FXS) is one of the most common inherited causes of ID and autism. FXS patients often exhibit hypersensitivity to sensory stimuli and epileptic seizures, indicative of hyperexcitable brain circuitry. Golgi staining of post-mortem brain tissue from FXS patients revealed increased numbers of long, irregularly thin, immature spines on cortical pyramidal neurons, similar to that in ASD.[71–73]

## Animal models

The identification of single gene alterations associated with neurodevelopmental disorders permits the establishment of experimental animal models to explore the detailed molecular, cellular, and behavioral mechanisms underlying disease pathogenesis.

FXS is caused by loss-of-function of the X-linked gene *Fmr1* encoding fragile X mental retardation protein (FMRP).[76] Inspired by findings from post-mortem brains of FXS patients, spine abnormalities have been intensively studied in *Fmr1* knockout mice. Consistent with findings from post-mortem human brain, spine density and length were increased in L5 pyramidal neurons of occipital cortex and primary somatosensory cortex compared to wild-type (WT) mice.[77–79] However, several studies reported no change in spine length or density in primary somatosensory cortex L4 spiny stellate neurons, cortical L5 pyramidal neurons, or hippocampal CA1 pyramidal neurons of *Fmr1* knockout mice.[80–82] These differences may be attributed to age at experimentation, studied brain area, genetic background, and/or methodology. Alternatively, histological studies of fixed tissue provide only a snapshot of highly dynamic processes and so may not capture abnormalities in spine turnover associated with ID. Recent advances in *in vivo* two-photon microscopy have permitted the imaging of such dynamic changes on living neuronal dendrites in the living mouse brain. *In vivo* imaging studies confirm that dendritic spines are highly motile structures that appear and disappear over days even without gross changes in spine density in L5 and L2/3 pyramidal neurons in various cortical areas and CA1 hippocampal pyramidal neurons.[24,83–85] Therefore, loss of *Fmr1* function may alter spine turnover and motility without causing a detectable change in spine density within fixed mouse brain tissue. Consistent with this idea, *in vivo* two-photon imaging revealed high spine turnover ratios on pyramidal neurons in various brain regions of *Fmr1* knockout mice, including L5 and L2/3 of the barrel cortex and L5 of the motor and visual cortex, while overall spine density appeared to be largely unchanged compared to WT mice.[86–89] Furthermore, *Fmr1* knockout mice exhibited reduced sensitivity of spine turnover ratio in L5 pyramidal neurons to sensory experience and motor learning.[86,89] These results suggest that *Fmr1* regulates spine dynamics and experience-dependent plasticity during development. The molecular mechanisms of synaptic dysfunction through loss of FMRP have been extensively studied using *Fmr1* knockout mice. FMRP is an RNA-binding protein that associates with ~4% of all brain mRNA,[76] and negatively regulates the translation of specific mRNA at the synapse. This negative regulation appears essential for normal spine plasticity. For example, activity-regulated cytoskeleton-associated protein (ARC), a synaptic protein critical for the internalization of glutamate receptor trafficking at synapses, is one mRNA target of FMRP at the synapses. Indeed, the rapid translation of ARC mRNA is required for metabotropic glutamate receptor (mGluR)-dependent LTD.[90] *Fmr1* knockout mice exhibit abnormally high levels of ARC mRNA translation and enhanced LTD in response to mGluR stimulation, suggesting that under normal conditions FMRP suppresses mGluR-dependent protein synthesis and LTD.[91] Moreover, both genetic and pharmacological inhibition of mGluR5 signaling can rescue many phenotypes of *Fmr1* knockout mice, such as elevated protein synthesis, spine density, and LTD as well as audiogenic seizures.[92–94] These results provide a compelling rationale for the use of mGluR5 antagonists in FXS treatment.[95] However, despite promising results in FXS animal models, clinical trials using mGluR5 antagonists failed to improve behavioral symptoms in patients.[96] These disappointing results may be explained by the involvement of pathways other than mGluR signaling in FXS pathogenesis, as FMRP binds to numerous mRNA to regulate synaptic structure and function. In addition to regulation of mGluR signaling, FMRP has been shown to regulate actin remodeling in spines (Fig. 1b). Loss of FMRP results in elevated Rac1-cofilin signaling and aberrant actin dynamics, leading to abnormal spine structures. Pharmacological inhibition of Rac1-cofilin signaling rescued impaired synaptic transmission, sensory processing, and fear conditioning in FMRP knockout mice.[97,98] Furthermore, cytoplasmic FMRP interacting protein 1 (CyFIP1), a key functional binding partner of FMRP, is linked to autism and ID.[99] CYFIP1 is present in two protein complexes important for synapse development and plasticity, one of which inhibits local protein synthesis together with FMRP and the other, termed the 'WAVE regulatory complex,' regulates actin polymerization. Brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin-related receptor kinase B (TrkB) are critical for many forms of synaptic plasticity and memory formation. Recently, it has been shown that BDNF is released from single dendritic spines, leading to the activation of postsynaptic TrkB during spine enlargement induced by two-photon glutamate uncaging.[100] In addition, BDNF–TrkB signaling activates Rac1, which drives the dissociation of CyFIP1 from FMRP and the association with the WAVE complex, leading to spine structural remodeling through actin polymerization. Furthermore, CyFIP1 can physically interact with proteins associated with many psychiatric disorders, including ID, autism, and schizophrenia.[99] These results highlight the roles of CyFIP1 in local protein translation and actin remodeling within spines that may underlie neurodevelopmental disorders.

Tuberous sclerosis complex (TSC) is another single-gene cause of ID and autism associated with dysregulation of mRNA translation

as well as actin remodeling. TSC is characterized by multiple benign tumors in many parts of the body resulting from mutations in the *TSC1* or *TSC2* gene. Hamartin and tuberin, the products of *TSC1* and *TSC2*, respectively, form a protein complex that acts as a tumor suppressor. TSC1 and TSC2 regulate mRNA translation by inhibiting the mammalian target of rapamycin complex 1 (mTORC1), a key protein kinase stimulator of protein synthesis (Fig. 1b). The TSC1-TSC2 complex has GAP activity for Ras homolog enriched in the brain (Rheb), a Ras-like GTP-binding protein required for mTORC1 activation. Therefore, loss of TSC1-TSC2 complex formation and ensuing disinhibition of Rheb can lead to mTOR hyperactivity and excessive protein synthesis. Since TSC and FXS patients share clinical symptoms, it has been hypothesized that excessive protein synthesis may be a core pathophysiological mechanism of both ID and autism.[101] Consistent with this idea, mice engineered to carry heterozygous loss-of-function mutations in *TSC1* or *TSC2* have hippocampus-dependent learning and memory deficits.[102,103] However, in contrast to *Fmr1* knockout mice, heterozygous *TSC2* mice show a decreased translation of ARC mRNA and mGluR-LTD in the hippocampus.[101,104] Furthermore, impairments in protein synthesis, mGluR5-LTD, and hippocampus-dependent behavior in heterozygous *TSC2* mice were restored by positive modulation of mGluR5.[101] How the loss of FMRP and TSC leads to differential dysregulation of protein synthesis and synaptic plasticity is not yet understood. However, these results suggest that balanced regulation of new protein synthesis could be a key mechanism for proper synaptic function and plasticity and that disruption of this mechanism may underlie synaptic dysfunction of neurodevelopmental disorders.

Angelman syndrome (AS) is a rare genetic disorder characterized by severe developmental and intellectual disability and a high prevalence of autism, with prevalence estimates ranging from 1 in 20 000 to 1 in 12 000.[91,105] Most cases are caused by the loss of expression of the maternally inherited copy of the imprinted gene ubiquitin–protein ligase E3A (*Ube3a*) on chromosome 15q11q13. Importantly, duplications and triplications of the maternally inherited 15q11-13 chromosomal region are among the most common genomic copy number variations identified in patients with autism.[106] Mice that carry a deletion in the maternal copy of *Ube3a* (AS mice) show impairments in LTP, mGluR-dependent LTD, and learning in the hippocampus and experience-dependent maturation of excitatory circuits in the visual cortex.[107–109] The number of dendritic spines was significantly reduced in hippocampal and cortical pyramidal neurons in AS mice and in cortical pyramidal neurons in AS patients.[108,110,111] *Ube3a* encodes a homologous to E6-Associated Protein C-Terminus (HECT) domain ubiquitin E3 ligase, which promotes the degradation of ubiquitinated substrate by proteasome. Strikingly, it has been shown that ARC is one of the targets for Ube3A and the expression of ARC is regulated by synaptic activity through ubiquitination.[112] Ube3A knockout mice showed the increased ARC expression and a decreased number of AMPAR at excitatory synapses. These results suggest that the neuronal activity-dependent expression of ARC may be a possible convergence point for UBE3A, FMRP, and TSC mutations in the etiology of ASD.[91]

De novo, heterozygous, loss-of-function mutations in *SYNGAP1* cause a syndromic form of ID with varying severities of epilepsy and autism. *SYNGAP1* encodes the synaptic Ras-GAP (SynGAP), which is a major constituent of both the PSD and NMDAR complex in spines. *SYNGAP1* knockout mice showed increased Ras signaling (due to loss of GAP activity) concomitant with impaired LTP, learning, and memory.[113–115] Interestingly, SynGAP dissociates from the PSD within a few minutes following LTP induction.[116] This delocalization of SynGAP may help to transiently increase Ras activity in stimulated spines, thereby stabilizing LTP. Indeed, the active form of Ras (Ras-GTP) was increased in synapses after LTP induction, and restoring normal SynGAP protein levels in *SYNGAP1* heterozygous knockout mice rescued both LTP and Ras-related signaling impairments.[117,118] In addition, SynGAP has been shown to suppress spine formation and maturation. Reduced SynGAP expression results in increased spine volume and accelerated spine maturation in hippocampal cultured neurons and cortical neurons *in vivo*.[119,120] Overall, SynGAP appears to support normal spine maturation and plasticity through suppression of Ras signaling.

Neurofibromin is another Ras-GAP in dendritic spines (Fig. 1b). In humans, loss-of-function mutations of the neurofibromatosis type 1 gene (*Nf1*), which encodes neurofibromin, causes a complex disorder characterized by brown pigmentation on the skin (café-au-lait spots) and multiple benign tumors (neurofibromatosis) together with ID. Neurofibromin is a giant multifunctional protein that regulates Ras, adenylyl cyclase, and microtubule association.[121] Neurofibromin is expressed in synapses and required for normal development of dendritic spines.[122,123] Similar to *SYNGAP1* heterozygotes knockout mice, *Nf1* heterozygous knockout mice show impaired LTP, learning, and memory. The Ras-GAP function of neurofibromin in spines has been studied using 2pFLIM.[124] Reduced expression of neurofibromin enhanced and sustained H-Ras activation in spines, suggesting that neurofibromin is essential for Ras inactivation in spines. Suppression of Ras signaling rescued defects in spine structural plasticity, LTP, and learning in neurofibromin deficit neurons.[121,124] Collectively, these results suggest that excessive Ras signaling in spines may underlie the pathogenesis of ID in NF1 patients.

Rett syndrome (RTT) is a genetic neurodevelopmental disorder that mainly affects girls and is characterized by the loss of acquired motor and language skills by 2 years of age.[125] Major symptoms include microcephaly, loss of speech, stereotypic hand movements, seizures, and autistic-like symptoms, such as lack of social interaction and communication. RTT occurs in ~1 in 10 000 live female births and is sporadic in over 99% of cases. In most cases, RTT is caused by a loss-of-function of the X-linked gene encoding methyl CpG binding protein 2 (MeCP2).[126] MeCP2 is a nuclear protein that binds to methylated cytosine of CpG islands in the promoter regions of DNA and thereby suppresses the expression of sets of genes.[127] However, MeCP2 depletion leads to both an increase and decrease in gene expression, suggesting that MeCP2 is not a classical transcriptional repressor. Additionally, MeCP2 binds broadly throughout the genome and may play a histone-like role in the regulation of chromatin structure and gene expression. The defects in synapse structure and plasticity in RTT have been suggested in both humans and mice. Neuropathological studies in post-mortem human brain tissue have suggested altered dendritic complexity and spine density in the cortex and hippocampus in RTT.[128–130] Mice heterozygous for the deletion (RTT mice) and missense mutations of *Mecp2* recapitulate many phenotypes of RTT patients.[131–136] Moreover, loss of MeCP2 leads to impairments in both LTP and LTD and a reduced number of glutamatergic synapses and spines in the hippocampus.[137–139] *In vivo* two-photon imaging has revealed reduced spine dynamics in pyramidal neurons in the somatosensory cortex in RTT mice.[140] Furthermore, RTT mice show defects in the experience-dependent pruning of retinogeniculate synapses of the thalamus and synaptic plasticity in response to visual deprivation. Remarkably, mice with conditional deletion of *Mecp2* in GABAergic cell-types exhibit the behavioral phenotypes associated with RTT, suggesting a significant role for inhibitory interneurons in the pathophysiology of RTT.[141–147] These results indicate that MeCP2 plays multiple roles in the activity-dependent epigenetic regulation for the synaptic development and plasticity in the brain.[148] Molecular mechanisms underlying RTT have been intensively studied in the past decades. As there are many functional targets of MeCP2, no single pathway or gene is expected to be involved in the pathogenesis of RTT. However, postnatal restoration of *MeCP2* function partially or completely rescues the phenotype in RTT mice, highlighting the reversibility of this disease and holding promise for treatment.[149–151] Indeed, several potential therapeutic targets have been identified. For example, BDNF–TrkB signaling activates phosphatidylinositide three kinase (PI3K)/Akt (also known as protein kinase B) kinase /mitogen-activated protein kinase (MAPK) pathways, which are important for synapse functions and are downregulated in RTT mice. MeCP2 selectively binds to the

BDNF promoter and represses the expression of the *bdnf* gene, which is dysregulated in RTT mice. Importantly, studies have shown that increasing BDNF signaling improved symptoms in RTT mice.[152–154] A clinical trial for fingolimod, which increases BDNF levels, has recently been completed although the results have not yet been published.[155] Additionally, insulin-like growth factor 1 (IGF-1) is another trophic factor critical for synaptic maturation and plasticity. Similar to BDNF, IGF-1 activates PI3K/Akt/MAPK pathways. Importantly, IGF-1 levels are reduced in RTT mice and systemic treatment with recombinant IGF-1 partly improves phenotypes in RTT mice.[156] A synthetic analog of IGF-1 improved several RTT symptoms in phase 2 clinical trials and the advance to a phase 3 trial has been announced.[157]

## Conclusions and future perspective

I have described the mechanisms underlying the plasticity of dendritic spines and their association with neurodevelopmental disorders. During the past couple of decades, significant progress has been made in optical techniques to image the dynamics of spine plasticity. The two-photon microscope allows for stimulation and imaging of single dendritic spines in living neurons *in vitro* as well as *in vivo.* Studies using the two-photon microscope have revealed that structural plasticity of dendritic spines is tightly correlated with several forms of functional plasticity, learning, and memory.[17,83,158] Furthermore, the development of 2pFLIM has enabled us to explore the spatiotemporal patterns of biochemical signaling initiated by short $Ca^{2+}$ pulse in dendritic spines. These studies have revealed that CaMKII is transiently activated in the stimulated spines and the local CaMKII activity is relayed to multiple small GTPases, leading to actin remodeling critical for spine enlargement.[25,53,54] Regarding the pathological roles of dendritic spines, studies from humans and animal models have suggested that spine pathology is a convergent point of many neurodevelopmental disorders. Perhaps one of the most significant findings over the past decade is the identification of possible signaling pathways relevant to spine pathology, which can be studied in experimental conditions. For example, as we discussed above, dysregulation of Ras signaling pathways, which is associated with activity-dependent expression of ARC and BDNF, may underlie the common pathogenesis of dendritic spine abnormalities in neurodevelopmental disorders. Therefore, it is essential to study the details of how these molecular pathways that are possibly relevant to neurodevelopmental disorders are regulated in a physiological condition and how they are disrupted in a diseased state using appropriate animal models.

Despite remarkable recent progress, many challenges remain to be addressed to understand spine plasticity and pathology. First, previous electrophysiological and biochemical studies have identified many molecules essential for the induction of LTP and LTD. However, in many cases, it is still not known how these molecules regulate structural plasticity in dendritic spines. Currently, signaling activities of ~10 molecules have been successfully imaged during structural plasticity using 2pFLIM–FRET. However, for the understanding of the basic principle of complex signaling networks in spines, it is essential to image signaling activities of many other signaling molecules. One current limitation of this strategy is that these experiments require the expression of FRET-based biosensors. The artificially overexpressed FRET sensors often disrupt native cell signaling, limiting the applicability of this strategy. In this regard, the recent development of techniques that enable fluorescent tagging of endogenous proteins may open a new avenue to precisely image endogenous signal transduction without the effects of overexpressed FRET sensors.[159,160] The second challenge is to image the process of signal transduction in behaving animals *in vivo.* To link the *in vitro* findings in brain slices with *in vivo* brain function, such as learning and memory, it is critical to image signaling activities in neurons and spines during behavior. However, those experiments require the identification of activated neurons and spines associated with learning and memory during imaging and thereby a high-resolution imaging technique of simultaneous detection of multiple signaling activities with distinct detection channels. Recently, the dual color FRET imaging in spines has been successfully achieved by using fluorophores with different spectral properties.[161,162] By further extending this strategy, it might be possible to image the dynamics of signaling activities at a single spine resolution in behaving animals *in vivo.* This will provide previously unachievable insights into molecular regulation of spine plasticity. Third, these advanced techniques and insights are also expected to promote the understanding of spine pathology underlying neurodevelopmental disorders. This can be achieved by using animal and cellular models with defined genetic alterations that cause neurodevelopmental disorders. Although neurodevelopmental disorders constitute highly heterogeneous groups of diseases, recent studies have identified multiple rare mutations with strong effects in genes encoding synaptic proteins in patients with neurodevelopmental disorders.[161,162] The rapid progress in human genetics will continue to further provide valuable information to dissect the genetic complexity of neurodevelopmental disorders. Newly discovered, rare functional genetic variations are expected to provide critical information to generate animal and cellular models of neurodevelopmental disorders, enabling molecular analysis of signaling pathways and potential therapeutic intervention underlying spine pathology. Alternatively, the advent of the induced pluripotent stem cell (iPSC) to reprogram cells from human patients provides a powerful opportunity to model and directly study human diseases with complex genetic background, such as many neurodevelopmental disorders, in an experimental laboratory. Human iPSC-neurons and organoids can recapitulate key cellular processes of neuronal and brain development, enabling us to study the molecular process underlying neurodevelopmental disorders. Indeed, studies have shown that synaptic defects were observed in iPSC-derived human neurons from patients with several neurodevelopmental disorders.[163–165] Human iPSC-derived models hold great promise for rapidly and efficiently translating the findings in rodent models into human neurons and screening for new treatment and prevention strategies to address synaptic defects in neurodevelopmental disorders. Thus, a better understanding of molecular regulation of spine plasticity based on technical advances will improve our understanding and treatment for neurodevelopmental disorders.

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## Disclosure statement

The author declares no conflicts of interest.

## Author contributions

J.N. wrote the manuscript.

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