Molecular mechanisms of dendrite stability

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Abstract | In the developing brain, dendrite branches and dendritic spines form and turn over dynamically. By contrast, most dendrite arbors and dendritic spines in the adult brain are stable for months, years and possibly even decades. Emerging evidence reveals that dendritic spine and dendrite arbor stability have crucial roles in the correct functioning of the adult brain and that loss of stability is associated with psychiatric disorders and neurodegenerative diseases. Recent findings have provided insights into the molecular mechanisms that underlie long-term dendrite stabilization, how these mechanisms differ from those used to mediate structural plasticity and how they are disrupted in disease.

The proper formation and long-term maintenance of neuronal connectivity is crucial for correct functioning of the brain. The size and shape of a neuron's dendrite arbor determine the number and distribution of receptive synaptic contacts it can make with afferents. During development, dendrites undergo continual dynamic changes in shape to facilitate proper wiring, synapse formation and establishment of neural circuits. Dendrite arbors are highly dynamic during development, extending and retracting branches as they mature, and only a subset of nascent dendrite branches become stabilized[1–4] (FIG. 1). During this early wiring period, synapse and dendrite arbor stabilization are intimately connected. For example, synapse formation on a nascent dendrite branch promotes its stabilization, whereas the loss or reduction of synaptic inputs destabilizes target dendrites[4–13].

The structural plasticity of dendrites decreases greatly as circuits mature (FIG. 1). Most dendrite branches become stabilized first while individual dendritic spines continue to form, change shape and turn over as circuits refine[14–18]. During this period, the formation and pruning of spines is particularly sensitive to experience and activity patterns[16,18–20]. This is followed by a period of extensive synapse and dendritic spine pruning, which can can last throughout adolescence and early adulthood in some human brain regions[17,20–26]. In stark contrast to early development, in which stabilization of dendrite branches depends critically on synapse formation, dendritic spine and dendrite branch stability become mechanistically uncoupled during this late refinement period. Such uncoupling is crucial for long-term circuit stability, as it affords mature neurons the ability to fine-tune spine-based synaptic connections, while retaining overall long-term dendrite arbor field integrity and integration within networks. Furthermore, cytoskeletal stability is crucial for maintaining long-lasting synaptic changes such as long-term potentiation (LTP). Examining the distinct mechanisms that mediate spine and dendrite stability is the major focus of this Review.

By adulthood, the dynamic behaviour of spines is greatly reduced. Transcranial two-photon imaging indicates that a large fraction of dendritic spines in the adult rodent cortex are stable for extended time periods of several months and possibly years[15–18,27] (FIG. 1). Together, these findings suggest a scenario in which most dendritic spines and dendrite arbors become stabilized for long periods of an organism's lifetime, perhaps even for decades in humans.

Losses of dendritic spine and dendrite arbor stability in humans are major contributing factors to the pathology of psychiatric illnesses such as schizophrenia and major depressive disorder (MDD), neurodegenerative diseases, such as Alzheimer's disease, and damage from stroke. Importantly, different patterns of dendritic spine and dendrite branch loss are observed in different psychiatric and neurodegenerative disorders (reviewed in REF. 28), suggesting that spine and branch stabilization mechanisms are differentially disrupted in different disease pathologies. The altered synaptic connectivity resulting from dendrite arbor and dendritic spine destabilization is thought to contribute to the impaired perception, cognition, memory, mood and decision-making that characterize these pathological conditions.

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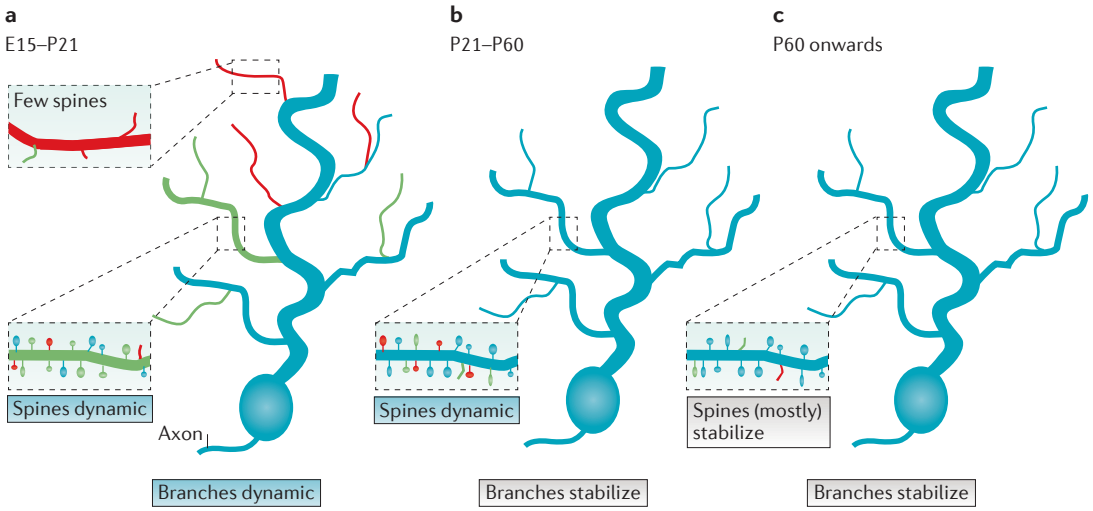

Figure 1 | **Dendrite branch and dendritic spine dynamics change during development.** **a** | During early development in mice (embryonic day 15 (E15) to postnatal day 21 (P21)), dendritic branches are highly dynamic, extending new branches (green) and retracting some existing branches (red). Failure to form productive synaptic contacts (inset, red dendrite segment) results in fewer spines and dendrite branch retraction; more stable branches (inset, green dendrite segment) contain a mix of stable spines, new spines and destabilizing spines. **b** | As animals enter and transit adolescence (P21–P60), some dendrite branches stabilize, while a fraction of dendritic spines remain dynamic, with a net loss of spines. **c** | As animals enter adulthood, dendritic spine dynamics slow and most of the spines remain stable.

A growing number of recent studies have begun to dissect the mechanisms that mediate long-term dendritic spine and dendrite branch stability. Here, I provide an up-to-date review of the molecules (TABLE 1) and cellular and molecular mechanisms that differentially regulate dendritic spine versus dendrite branch stability and highlight how these mechanisms are targeted by pathology.

Determinants of dendrite and spine stability

Dendritic spines are supported by an F-actin framework. At most excitatory synapses, presynaptic boutons synapse onto small dendritic spines that emerge from the dendritic shaft. Spines have diverse shapes, including thin, short and stubby, and mushroom-shaped, with a club-like head and thinner neck[15,29–32]. Dendritic spines are enriched in filamentous (F) actin, which provides the spine shape, organizes the postsynaptic signalling machinery, drives changes in spine structure and maintains spine stability[33–37]. The spine cytoskeleton (referred to here as the 'spinoskeleton')[38] is composed of a mix of linear and branched actin networks, which extend from the base of the spine to the postsynaptic density (PSD)[31,39]. This actin network fills the spine — it is closely apposed to the spine membrane, and it retains this association during activity-driven remodelling of spine structure[31] (FIG. 2). Changes in the amount and structure of F-actin also mediate long-lasting alterations in spine size and synaptic efficacy. For example, repetitive firing of synapses, such as that occurring during high-frequency synaptic stimulation to induce LTP, promotes actin polymerization within the spine, causing the spine to enlarge[40,41]. Conversely, treatment that weakens synaptic efficacy, such as low-frequency stimulation that results in long-term depression, causes actin loss and dendritic spine shrinkage[40,42].

Direct measurements using sophisticated live microscopy techniques reveal that actin exists in distinct pools that differ in their movement and stability within the spine[36,43,44]. Photoactivation studies indicate that actin at the spine tip and periphery, also known as the spinoskeleton 'shell', is dynamic, cycling between monomeric globular and polymeric (filamentous) forms that turn over with half-lives of tens of seconds. By contrast, actin in the centre and base of the spine, also known as the spinoskeleton 'core', turns over in tens of minutes[43]. Consistent with this model, when single photoactivated actin molecules were tracked in spines, half remained largely stationary, as would be expected of actin associated with a more stable actin core, whereas one-third underwent slow retrograde movement, which is consistent with a more dynamic pool of actin associated with the shell[44]. It has recently been noted that distinct actin-binding proteins (ABPs) localize to discrete regions within the spinoskeleton[38]. For example, cortactin, which both stimulates actin polymerization and stabilizes branched actin networks, localizes to the spinoskeleton core[45], whereas cofilin, an F-actin-severing protein that is associated with dynamic F-actin turnover, localizes to the shell[46] (FIG. 2). Together, these studies suggest that the periphery of spine structure — near the outer membrane — is likely to be capable of greater morphological change, whereas the central core remains more stable. This is consistent with the observation that spines can be highly motile and could reflect a need, during synaptogenesis, to keep the overall position of new spines constant while the more dynamic outer portion searches for suitable synaptic contacts or adjusts in size in response to new activity patterns.

Table 1 | **Molecules influencing dendritic spine and dendrite arbor stability**

	Key molecules	Refs
Stabilizes spines	RAC1	66
	WAVE1	69,70
	Cortactin	76,84,89
	β-adducin	96,97
	EPHB–FAK	98
	FYN	100
	p130CAS	101
	MARCKS	78,203
	p140CAP	85
Stabilizes dendrites	MAP1A	60,61
	MAP2	62,63
	p190RHOGAP	77,114
	NDR1 and NDR2	138
	Cypin	142,143
Stabilizes both spines and dendrites	BDNF–TRKB	108,140,141
	Integrin α3β1	101,114,115
	ARG	26,77,114
	EB3	85,204
Destabilizes both spines and dendrites	RHOA–ROCK	68,71,72,147–151
	Amyloid-β-derived diffusible ligands	175–178,181
	Tau and tau kinases	179,181
	GATA1	185
	Corticosteroids	205–208
	Increased NMDAR activation	84,89,198–202

BDNF, brain-derived neurotrophic factor; EB3, end-binding protein 3; FAK, focal adhesion kinase; GATA1, GATA-binding factor 1; MAP, microtubule-associated protein; MARCKS, myristoylated alanine-rich C-kinase substrate; NDR, nuclear Dbf2-related kinase; NMDAR, NMDA receptor; p130CAS, p130 Crk-associated substrate; p140CAP, p130 Cas-associated protein; p190RHOGAP, 190 kD RHO GTPase-activating protein A; ROCK, RHO-associated protein kinase.

Microtubule arrays in dendrite branches support morphological structural and membrane trafficking. In the mature nervous system, the cytoskeleton within dendrite branches consists of a packed network of microtubules, which provides structural stability, anchors organelles and serves as a highway for the transport of cargoes, including all manner of dendritic building materials and organelles[47]. In contrast to microtubules in axons, which are polarized (with the plus ends facing the end of the axon), microtubules within dendrites are of mixed polarity, which may facilitate bidirectional trafficking of cargoes by microtubule-based motors[48,49]. That said, polarized microtubule arrays have been observed in large-diameter apical dendrites[50], possibly reflecting the bias of transport of cargoes away from soma out to their elaborate dendrite arbors. Serial electron micrographic reconstructions reveal that ordered dendritic microtubules lie regularly spaced, parallel to each other and to the dendritic shaft[51] (FIG. 2).

A dynamic cytoskeleton, but stable branches and spines. Although the gross structure of dendritic spines and branches is stable for months or years, the individual actin filaments and microtubules that make up their structures turn over in matters of minutes to hours; this is the case in both the developing and mature nervous systems. Over 80% of F-actin in spines turns over every minute[36,43,44], whereas 75% of the microtubules in dendrites turn over within tens of minutes[52,53]. It is important to note that the remaining proportion of F-actin and microtubules undergo depolymerization-mediated turnover in dendrites but at a slower rate — on the order of tens of minutes for spine F-actin and a probably a few hours for dendrite shaft microtubules, as has been measured for axon microtubules[52,53].

Given the high turnover of molecules that confer structural integrity, how is structural stability and functional integrity achieved? The secret appears to lie in the precise regulation of the molecules that control dendritic cytoskeletal dynamics. Dendritic spines and shafts are replete with proteins that promote the formation of actin filaments and microtubules, respectively, and organize and stabilize these cytoskeletal structures (FIG. 2). In addition to shaping the cytoskeleton, these proteins ensure that only a fraction of the cytoskeleton undergoes remodelling at any given time and that existing actin filaments and microtubule networks can both maintain dendritic structure and serve as scaffolds for their own replenishment. For example, the actin-related protein 2/3 (ARP2/3) complex, which localizes to dendritic spines[54], has been shown to nucleate new actin branches from the side of an existing actin filament *in vitro*[55]. Selective inactivation of the ARP2/3 complex in excitatory neurons leads to significant spine and synapse loss in cortical and hippocampal neurons, further supporting a crucial role for actin replenishment in the maintenance of spine and synapse structural integrity[56].

Dendritic microtubules also contain high concentrations of microtubule-associated proteins (MAPs), including MAP1A and MAP2 (FIG. 2), both of which can promote microtubule polymerization and stabilize existing microtubules[57–59]. Upregulation of MAP1A and MAP2 expression strongly correlates with dendrite stabilization in cultured neurons, and both proteins are required for activity-dependent enlargement and stabilization of developing dendrite arbors[60,61]. Importantly, knockout of these proteins in mice disrupts microtubule spacing within dendrites and leads to significant reductions in dendrite arbor size[62,63]. In addition to their effects on microtubule spacing and stability, MAPs may also protect dendritic microtubules from the activities of endogenous microtubule-severing enzymes, such as katanin, which could otherwise destabilize dendrite arbors by triggering microtubule instability[64]. Covalent modifications of dendritic microtubules that are associated with microtubule stability include detyrosination of the α-tubulin subunit, which stabilizes the microtubules by making them poor substrates for the kinesin-13 family of microtubule depolymerases[65]. In summary, structural integrity of spines and dendrites is achieved by a careful balance between stabilization and destabilization mechanisms.

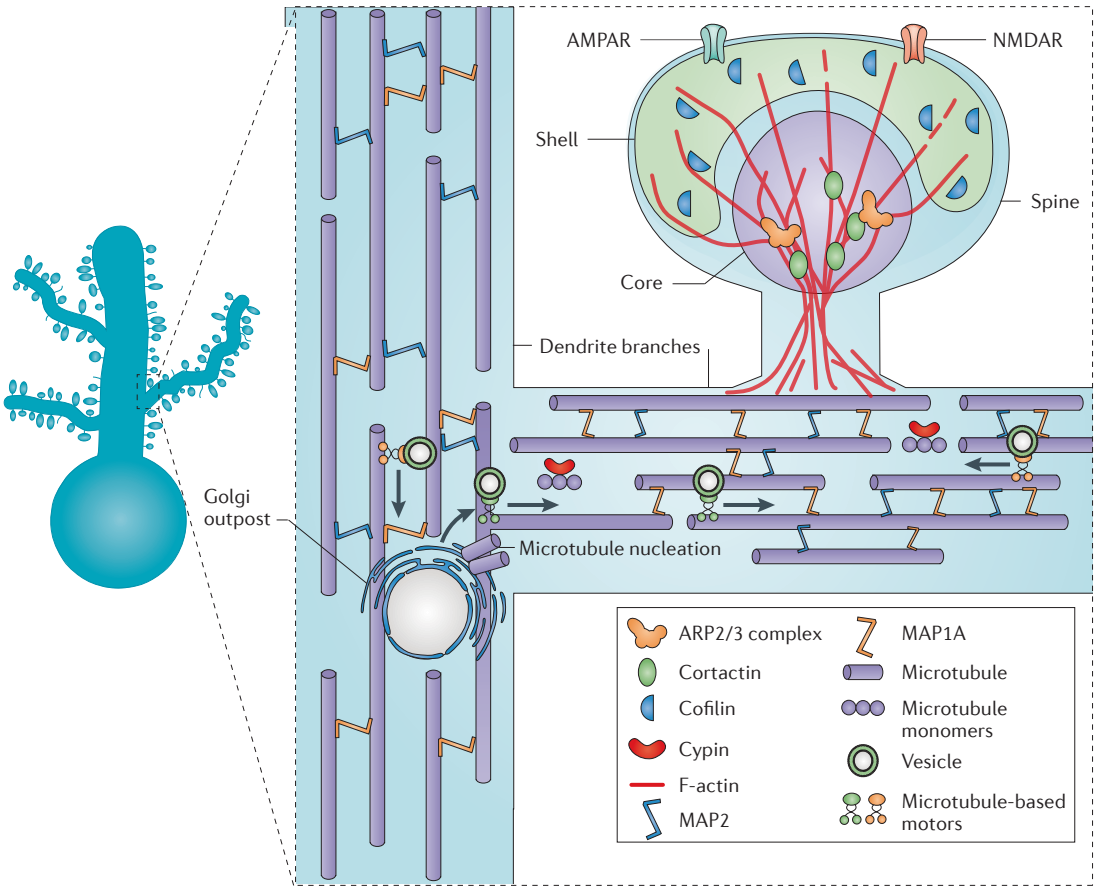

Figure 2 | **Cytoskeletal structure in dendrites.** The cytoskeletal ultrastructure in a small region of a dendrite (boxed area on the neuron). Dendritic shafts contain large parallel bundles of microtubules bridged by microtubule-associated protein 1A (MAP1A) and MAP2. The microtubule array near a dendritic branch site serves as a docking site for a Golgi outpost, which services the trafficking of vesicles (cargo) to and from the more terminal regions of dendritic membrane and serves as a site for microtubule nucleation. Cypin helps to promote the assembly and stability of new microtubules. The dendritic spine is supported by network of actin filaments. The more stable central core contains the actin-related protein 2/3 (ARP2/3) complex and the actin nucleation-promoting factor and actin branch stabilizer cortactin. F-actin filaments are less densely packed and more dynamic in the spine shell, which is enriched in the actin-severing protein cofilin. AMPAR, AMPA receptor; NMDAR, NMDA receptor.

Molecular mechanisms of spine maintenance

The long-term stability of dendrite and spine structure in the mature nervous system thus intimately depends on the actin control machinery, which is regulated by a wide range of extracellular molecules that act on cell surface adhesion receptors, including EPH receptors, immunoglobulin superfamily receptors, cadherins and integrins (FIG. 3). Upon engagement with their extracellular binding partners, these receptors influence a wide range of downstream signalling molecules. These include cytoplasmic non-receptor tyrosine kinases of the focal adhesion kinase (FAK), SRC and ABL families that modulate the activities of RHO-family GTPases and other downstream cytoskeletal stabilizing proteins, and serve to control spinoskeletal structure and ensure long-term spine stability (FIG. 3). Some of these molecules operate throughout neuronal development and into adulthood to control actin dynamics in spines. In many cases, changes in the expression and/or activity of these proteins closely follow changes in synaptic activity. Other spine signalling components become engaged only late in development as network stability emerges, possibly to help stabilize spines for the long-term. Importantly, all of these pathways converge on a single target, the spine actin cytoskeleton, and thus there is significant crosstalk between these mechanisms. The roles of these molecules in regulating actin cytoskeletal dynamics in the spine are discussed in more detail below.

RHO-family GTPases exert differential effects on spine stability. The RHO-family GTPases RHOA, RAC1 and cell division control protein 42 homologue (CDC42) are central regulators of cytoskeletal dynamics in neurons[66]. All of these GTPases are active in the spine, where they have been shown to mediate spine formation in developing neurons and activity-based changes in spine size[67]. Beyond this initial period of spinogenesis, these GTPases continue to influence spine shape and stability, even into adulthood. For example, the introduction of dominant-negative RAC1 into hippocampal neurons leads to progressive spine loss, reflecting a requirement for RAC1 in long-term spine stability[68]. Among its downstream

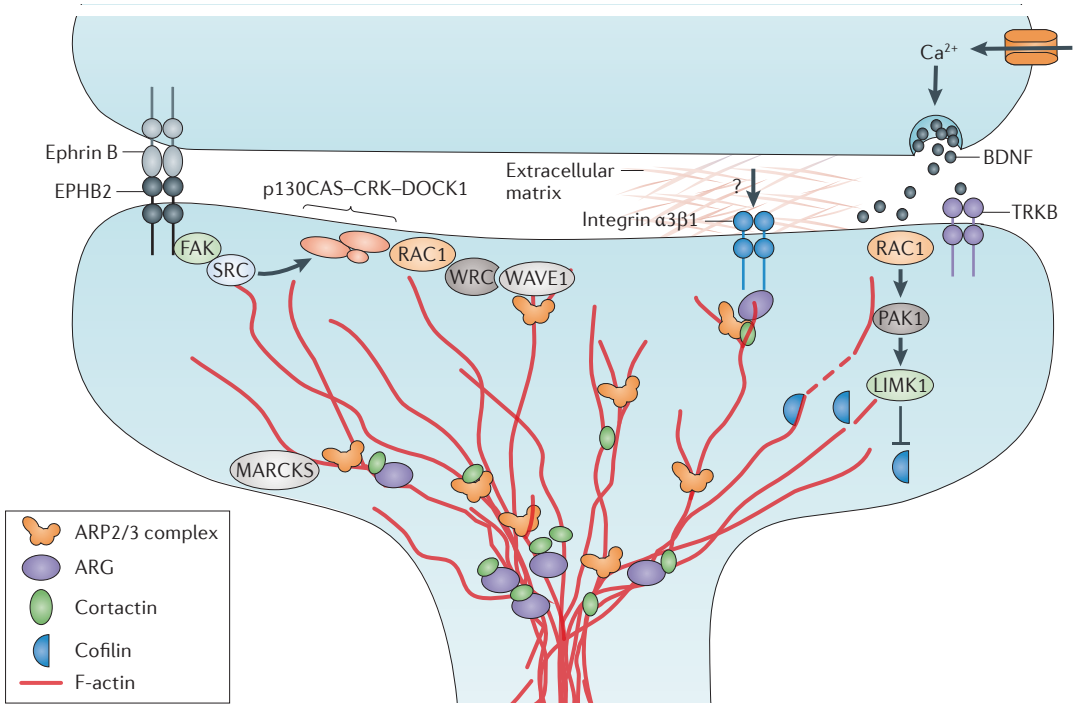

Figure 3 | **Cytoskeletal signalling pathways that stabilize spines.** Several cytoskeletal signalling pathways mediate dendritic spine stabilization. On the left, presynaptic ephrin B binding to its receptor EPHB2 triggers a focal adhesion kinase (FAK)–SRC kinase relay that activates the p130 Crk-associated substrate (p130CAS)–adaptor molecule CRK–dedicator of cytokinesis protein 1 (DOCK1) complex, which in turn activates the GTPase RAC1. RAC1 releases the WAVE regulatory complex (WRC) from WAVE1, allowing it to activate the actin-related protein 2/3 (ARP2/3) complex to nucleate a new actin branch. In response to an unknown signal, integrin α3β1 activates tyrosine protein kinase ARG and promotes its phosphorylation of and binding to cortactin. Cortactin can activate actin branch nucleation through the ARP2/3 complex and can stabilize the branches that are formed. ARG binding can also stabilize actin filaments and promote cortactin recruitment to actin filaments. On the right, presynaptic depolarization triggers Ca^{2+}-dependent release of brain-derived neurotrophic factor (BDNF), which binds to the receptor tyrosine kinase TRKB to activate RAC1. RAC1 acts through a serine/threonine-protein kinase PAK1–LIM domain kinase 1 (LIMK1) cascade to phosphorylate and inhibit cofilin and block its severing of actin filaments. Myristoylated alanine-rich C kinase substrate (MARCKS) tethers actin filaments to the membrane to stabilize F-actin in spines.

targets, RAC1 activates WAVE (also known as WASF) family proteins to stimulate the ARP2/3 complex, which (as explained above) is associated with F-actin in the spinoskeleton core and acts to nucleate new actin filament branches from existing actin filaments[41,66,67] (FIG. 3). WAVE1 also localizes to dendritic spines and knockdown or knockout of *Wave1* causes reductions in dendritic spine density[69,70]. These observations strongly suggest that RAC1 acts through WAVE1 and the ARP2/3 complex to refresh the spinoskeleton core and therefore supports long-term spine stability.

In contrast to RAC1, activated RHO mutants or increased RHOA levels cause reductions in dendritic spine density[71,72], whereas RHOA inhibition or knockdown of the RHO activator guanine nucleotide exchange factor 1 (GEF1) increases spine density[71,73]. Inhibition of the major RHO target RHO-associated protein kinase (ROCK) can block spine loss resulting from increased RHOA protein levels[72]. These studies suggest that RHO–ROCK signalling antagonizes the stability of previously formed dendritic spines, although the target of RHO signalling in regulating spine stability is not clear. RHO signalling through ROCK stimulates LIM kinase to phosphorylate cofilin and inhibit its actin severing activity[74,75], but this might be expected to promote spine stability rather than reduce it (see below).

Studies of the effects of synaptic activity on cytoskeletal stability have revealed a more complex relationship between RHOA signalling and spine stability. Local uncaging of glutamate to stimulate individual spines caused increased RHOA activity in the spine, which mediated activity-based spine enlargement[67]. By contrast, blocking AMPA receptors in cultured hippocampal neurons with NBQX for several days caused increased RHOA activity, which led to spine loss[73]. Moreover, several additional studies have shown that although RHOA activation or loss of a RHOA inhibitory cascade in mature neurons leads to a dramatic shrinkage of dendrite arbors, it does not affect dendritic spine stability[68,76,77]. Overall, it appears that specific activity patterns, developmental timing or other biological factors greatly influence the impact of RHOA–ROCK signalling on dendritic spine stability. Ongoing work in the field should identify how these factors influence RHOA–ROCK activation and its effect on spine stability.

ABPs have key roles in long-term spine stabilization. Dendritic spines are enriched in several ABPs that mediate activity-dependent effects on dendritic spine shape, size and stability, including cortactin, drebrin, ABP1, caldesmon, myristoylated alanine-rich C-kinase substrate (MARCKS) and β-adducin[78–82]. Cortactin is an F-actin-binding protein that stimulates new actin branch nucleation through the ARP2/3 complex and stabilizes the association of the new branch with the mother filament[83]. This protein is enriched in the spinoskeleton core, where about 50% is stable (as measured by fluorescence recovery after photobleaching; see FIG. 3)[45,84]. Cortactin has also been reported to interact with several proteins with known or suspected roles in long-term spine stabilization, including cortactin-binding protein 2, the postsynaptic density (PSD) scaffolding protein SHANK3, p130 Cas-associated protein (p140CAP; also known as SRCIN1) and tyrosine protein kinase ARG (also known as ABL2) (see below)[76,85–88].

Because of its interactions with SHANK3 and F-actin within spines, it is perhaps not surprising that cortactin localization and function in spine stability are tightly regulated by synaptic activity. Indeed, chronic stimulation of cultured hippocampal neurons by bath application of NMDA, which is known to destabilize dendritic spines, leads to cortactin redistribution away from postsynaptic sites to dendritic shafts[76,84,89,90]. Interestingly, activation of the receptor tyrosine kinase TRKB (also known as NTRK2) by its ligand brain-derived neurotrophic factor (BDNF) leads to the opposite effect, increasing cortactin localization to spines. These findings indicate that TRKB and the NMDA receptor (NMDAR) exert opposite effects on spine stability via their different effects on cortactin levels in spines. Together, these findings reveal cortactin as a key effector of spine stability downstream of both stabilizing and destabilizing cues.

Drebrin, MARCKS and ABP1 also have key roles in dendritic spine morphogenesis, particularly in the transition from thin immature dendritic spines to larger, more stable mushroom spines[79,91,92]. Knockdown of MARCKS in mature hippocampal neuron cultures reduces F-actin content in spines, leading to fewer spines that are smaller in size[78]. Phosphorylation of MARCKS by protein kinase C (PKC) inhibits its association with the membrane and its ability to crosslink F-actin, and has been implicated in activity-dependent control of spine size and stability[93–95]. Indeed, direct activation of PKC in mature hippocampal neuron cultures causes spine shrinkage and loss. Importantly, PKC-induced spine destabilization can be blocked using a non-phosphorylatable MARCKS mutant[78], indicating that PKC triggers spine destabilization through inhibition of MARCKS.

β-adducin proteins cap actin filaments, and removal of these caps allows the polymerization process to be coordinated by inputs from different signalling pathways into changes in actin structure. Dendritic spines of mice lacking the gene that encodes β-adducin (*Add2*$^{-/-}$ mice) are relatively normal in appearance but exhibit greatly increased rates of turnover[96]. Increasing sensory stimulation by housing these mice in enriched environments can induce new spine formation, but a significant fraction of these new spines lack PSD95-positive PSDs, indicating that they are unable to form functional synapses[96,97]. This increased spine lability and defective synapse formation probably contributes to the observed reductions in spine density in adult *Add2*$^{-/-}$ mice[97]. Together, these studies illustrate how the organization of spinoskeletal structure and spine stability, and the influence of synaptic activity on this stability, is coordinated by multiple ABPs.

EPHB signalling through FAK stabilizes mature dendritic spines. Although originally identified as a kinase associated with integrin signalling, FAK can be activated by a large variety of cell surface receptors that regulate spine stability, including EPHB receptors (FIG. 3). Disruption of EPHB2 signalling or knockdown of FAK in established hippocampal neuron cultures leads to a significant loss of mature mushroom-shaped dendritic spines and an increase in immature thin 'filopodia-like' spines[98]. FAK can activate RHOA through 190 kDa GEF (p190RHOGEF; also known as ARHGEF28), and subsequent downstream inactivation of the actin-severing protein cofilin via phosphorylation by LIM kinase may mediate spine stabilization. Consistent with this model, an inactive phosphomimetic cofilin mutant can block the spine destabilization induced by loss of EPHB2–FAK signalling[98]. In addition, activated FAK also recruits and activates SRC family kinases to phosphorylate p130 Crk-associated substrate (p130CAS; also known as BCAR1), a scaffolding molecule that promotes RAC1 activation[99]. Interestingly, knockout of the SRC family kinase FYN leads to a progressive age-dependent loss of dendritic spines in mice[100], whereas knockdown of p130CAS leads to spine loss in established hippocampal neuron cultures[101]. These manipulations suggest a mechanism by which spine shrinkage and/or destabilization results from disruption of FAK-mediated RAC1 activation.

BDNF signalling through TRKB influences spine size and stability. BDNF is released from both pre- and postsynaptic compartments during neuronal depolarization or direct stimulation with glutamate, where it has crucial roles in synapse development and stability[102–106]. During development, BDNF signalling through presynaptic TRKB receptors has been shown to stabilize neurotransmitter release sites and reduce synapse turnover[105,106]. Interestingly, blockage of NMDARs also disrupts clustering of the presynaptic release sites, but this can be rescued by application of BDNF, suggesting that NMDA-mediated BDNF release is critical for stabilization of the presynaptic compartment[105]. Although the mechanism by which BDNF–TRKB signalling regulates presynaptic stabilization is not completely clear, it is possible that TRKB signalling to cortactin or other ABPs leads to stabilization of the presynaptic actin network, which in turn stabilizes the synaptic vesicle release machinery.

In the postsynaptic neuron, many actin regulatory pathways central to spine enlargement and stabilization are modulated by BDNF–TRKB signalling. BDNF stimulates activity of serine/threonine-protein kinase PAK (a major target of RAC) and also induces increased inhibitory phosphorylation of cofilin, which is associated with

increased spine size and stability (see above). TRKB can also activate the GTPase RAS, which promotes spine enlargement and increased stability[107]. Interestingly, BDNF signalling through the TRKB receptor can interact with activity-induced molecular pathways to stabilize dendritic spines. For example, exposure of hippocampal slices to modest electrophysiological stimulation and BDNF leads to greater increases in spine F-actin content than either treatment alone, suggesting a synergistic effect. Furthermore, high-frequency stimulation of presynaptic afferents in hippocampal slices also increases spine F-actin content, which is attenuated by blocking TRKB signalling[108].

Integrin β1 signalling through ARG and cortactin mediates dendritic spine stability. Integrins are heterodimeric receptors that are composed of α and β subunits and are widely expressed in the nervous system, with numerous subunits expressed throughout the brain[109]. They localize to synapses, where they link pre- and postsynaptic membranes to the extracellular matrix, and are involved in signalling complexes that regulate the cytoskeletal structure. The integrin β1 subunit is localized particularly to the centre of the PSD[110]. Importantly, integrins appear to be involved in the stabilization of LTP, which is associated with a rapid, long-lasting increase in polymerized F-actin and an increase in spine stability[111–113]. Indeed, inhibition or loss of integrin α3β1 destabilizes spines and reduces synapse density during adolescence and early adulthood (postnatal day 21 (P21)–P42) in mice[101,114,115]. The extracellular molecules that activate integrins at synapses are not known but may include both traditional extracellular matrix molecules, such as laminins and thrombospondins, and guidance cues, such as netrins and reelin, which can interact with integrins[116–119].

Integrins signal through the ABL family kinases, ABL and ARG in vertebrates, to coordinate changes in cytoskeletal structure[120]. Importantly, biochemical and genetic experiments indicate that the integrin β1 cytoplasmic tail binds directly to ARG and that this interaction is crucial for dendritic spine and dendrite stability (FIG. 3). ARG is particularly abundant in the nervous system[121], where it localizes to dendritic spines[76,114,122], and *Arg*$^{-/-}$ mice exhibit widespread losses of spine and synapse density throughout the cortex and hippocampus (20–35% depending on the brain region) as they mature to adulthood[77,123]. Interestingly, these phenotypes closely resemble those observed in conditional knockout mice that have neurons lacking the integrin subunit α3 or β1 (REFS 101,114,115), which is consistent with a role for ARG as a major downstream mediator of spine stabilization by integrin α3β1.

ARG acts by binding cooperatively to actin filaments and preventing their depolymerization[124,125]. ARG binding to actin filaments also changes their helical pitch[126], promoting increased cortactin binding, which may further stabilize filaments[125]. Knockdown of ARG in cultured hippocampal neurons leads to cortactin loss from spines, a parallel decrease in spine F-actin content and a 50% loss of spines[76]. These data suggest that binding of an ARG–cortactin complex to F-actin stabilizes actin, which promotes spine stability. Indeed, fusion of just the ARG F-actin-binding domains to cortactin results in its accumulation in spines and rescues spine instability in ARG knockdown neurons[76]. Interestingly, the synapses that remain on ARG knockdown neurons are enlarged and exhibit increased mini-excitatory postsynaptic current amplitudes, consistent with increased signalling by postsynaptic AMPA receptors and NMDARs. NMDAR antagonists prevent cortactin depletion from spines and rescue spine loss resulting from ARG knockdown in cultured neurons. These observations suggest that ARG may also attenuate NMDAR activity to counter its antagonism of cortactin localization to spines. One possibility is that ARG may regulate NMDAR subunit phosphorylation[127,128], which is known to regulate localization and trafficking of NMDARs[129]. Thus, ARG appears to serve as a general brake on the spine destabilizing influence of excessive NMDAR signalling.

Control of dendrite arbor stabilization

The size and shape of a dendrite arbor determine where it can receive inputs and how it can integrate into neural networks. Dendrite branches are especially stable in mature neurons, and this stability is essential for the durability of neural networks. Dendrite branches are supported and maintained through the combined actions of an extensive network of microtubules and associated proteins.

Role of microtubule-based trafficking in stabilizing dendrite arbors. *Drosophila melanogaster* dendrite-arborizing sensory neurons undergo complete dendrite arbor loss during metamorphosis, and this model system has been particularly useful for studying the mechanisms that control microtubule networks in dendrite branch stability. Severing of individual dendritic branches is initiated by a localized depletion of microtubules and thinning of the dendrite branch, followed by severing and fragmentation of the branch[130]. Genetic studies indicate that the microtubule-severing protein Katanin 60 is crucial for this process[131]. These observations underscore the central role of microtubule integrity for maintaining overall dendrite arbor stability.

Dendrites are composed of large amounts of proteins and lipids, which must be continually replenished. Although this is partially accomplished by local protein synthesis, other components must be synthesized and processed elsewhere and trafficked to dendrites. Microtubules play crucial parts in the protein and lipid trafficking events that are required to sustain dendrite structure, and perturbations that disrupt trafficking of microtubule-based cargoes have devastating effects on dendrite formation during development and stability. For example, disruptions in microtubule orientation and spacing correlate with disruptions of local dendrite architecture and have been noted in neurons from individuals with neurodevelopmental disorders[132,133]. Moreover, the Golgi apparatus in the soma is highly polarized towards dendrites, and Golgi outposts, which serve as local sites for post-Golgi trafficking, and recycling of membrane-based cargoes as well as new microtubule nucleation,

Golgi outposts
Clusters of Golgi-like cisternae that reside in neuronal processes and act as local trafficking centres for membrane-bound vesicles.

are localized at many dendrite arbor branch points[134,135] (FIG. 2). Mutations in the microtubule-based motors dynein and kinesin also significantly disrupt trafficking within the dendrites as well as proper localization of Golgi outposts, and lead to deficits in dendrite arbor formation and stabilization[136,137]. Nuclear Dbf2-related kinase 1 (NDR1; also known as STK38) and NDR2 (also known as STK38L), which are key regulators of dendrite arbor development in vertebrates, have recently been shown to target several proteins with known or suspected roles in protein trafficking[138].

Activity and BDNF signalling through the TRKB receptor regulate microtubule-binding proteins to stabilize dendrites. Neuronal activity is a potent stimulator of dendritic remodelling and stabilization in developing neurons, and the neurotrophic factor BDNF, signalling through TRKB, appears to be an important mediator of this effect[60,61,139] (FIG. 4). Experimental approaches such as membrane depolarization, repetitive electrophysiological stimulation and direct application of NMDA have all been shown to promote BDNF release at synapses in cultured hippocampal neurons[102–104]. Moreover, brain-specific ablation of BDNF or its receptor TRKB leads to a striking shrinkage of cortical excitatory neuron dendrites from early adolescence to adulthood[140,141]. The molecular mechanisms by which synaptic activity stimulates neurotrophic factor signalling that leads to dendrite microtubule stability are coming into focus. Direct application of BDNF or nerve growth factor to cultured neurons is sufficient to induce expression of the microtubule-stabilizing proteins MAP1A and MAP2 (REFS 58–61). In addition, BDNF also induces expression of cypin, a guanine deaminase that promotes microtubule assembly, and dendrite formation and stability. These data suggest that BDNF signalling through TRKB converges on microtubule-binding proteins to mediate dendrite stability[142,143].

Integrin signalling to p190RHOGAP attenuates RHO activity to stabilize dendrite branches. In addition to regulating dendritic spine stability, as discussed above, integrin α3β1 signalling through the ABL and ARG kinases is crucial for dendrite branch stabilization (FIG. 4). Dendrite arbors of cortical or hippocampal excitatory neurons lacking either ARG or both kinases develop normally until P21, approaching their fully mature size. As the mice reach adulthood, however, dendrite arbor size reduces significantly, leading to an overall thinning or shrinkage of the cortex and hippocampus[77,144]. Similar phenotypes are observed in hippocampal CA1 neurons deficient in the integrin subunit α3 or β1, both of which are key upstream regulators of ABL and ARG. However, no defects in dendrite branch stability are observed in animals with disruption of integrin subunit α5 or β3, suggesting that integrin α3β1 interacts selectively with ARG to control dendrite stability.

The 190 kD RHO GTPase-activating protein (GAP) A (p190RHOGAP) is a major ARG substrate in the postnatal brain[145]. ARG-mediated phosphorylation of p190RHOGAP promotes its binding to membrane-associated p120RASGAP, which allows p190RHOGAP to inhibit membrane-bound active RHO[145,146]. p190RHOGAP phosphorylation and p190RHOGAP–p120RASGAP complex formation are decreased in the brains of *Arg*$^{-/-}$ mice , leading to net increases in RHO activity. Importantly, dominant-negative RHOA or reducing the gene-dosage of *Rock2* suppresses the dendrite branch loss observed in *Arg*$^{-/-}$ mice[76,77]. Together, these studies indicate that the integrin α3β1–ARG–p190RHOGAP axis preserves dendrite structure in early adulthood by attenuating RHO activity. Identification and characterization of the integrin ligand that activates this pathway should reveal how and why this pathway is activated to stabilize branches and spines.

Pathological dendritic destabilization by RHO–ROCK signalling. Although most dendrite arbors exhibit long-term stability, there are circumstances under which dendrite stability is compromised, such as in neurodegenerative diseases and after an excitotoxic insult during stroke (see below). The GTPase RHO appears to be a central mediator of this dendrite destabilization. For example, expression of constitutively active RHO, or reduced activation of RHO inhibitors, such as p190RHOGAP (see above), destabilizes growing[147–150] and mature[68,151] dendrite arbors (FIG. 4). RHO-mediated activation of ROCK probably acts through several mechanisms to disrupt dendrite architecture. Inhibition of ROCK can promote microtubule assembly and the stabilization of dendrite-like neuronal processes in cells, suggesting that ROCK antagonizes dendrite stability by disrupting microtubules[152]. Indeed, ROCK phosphorylates MAP2 on a site in its microtubule-binding region. ROCK-mediated phosphorylation of a similar conserved site in tau disrupts its interactions with microtubules[153]. MAP2 phosphorylation in other contexts has been shown to reduce its ability to bind microtubules and promote their assembly[154,155]. Activated RHO also suppresses translation of the microtubule stabilizer cypin (see above), but cypin overexpression in cultured hippocampal neurons can overcome the reduction in dendrite arbors induced by increased RHO activity[151]. These studies indicate that the loss of MAP- and cypin-mediated microtubule stabilization contributes to RHO-mediated dendrite arbor destabilization.

Coordination of spine and dendrite stability

Although the mechanisms that regulate dendritic spine and dendrite branch stability have been discussed separately so far, there are several instances in which signalling between the compartments influences stability. This crosstalk is particularly evident under conditions in which activity-driven circuit remodelling requires a coordinated shaping and stabilizing of spine and dendrite structure.

Activity-driven spine enlargement and stabilization. Activity and activity-mediated neurotrophic factor signalling affects dendritic spine size and stability throughout a neuron's lifetime, including mature cortical and hippocampal neurons. Growing microtubule ends are enriched in 'plus end tip-binding proteins', notably

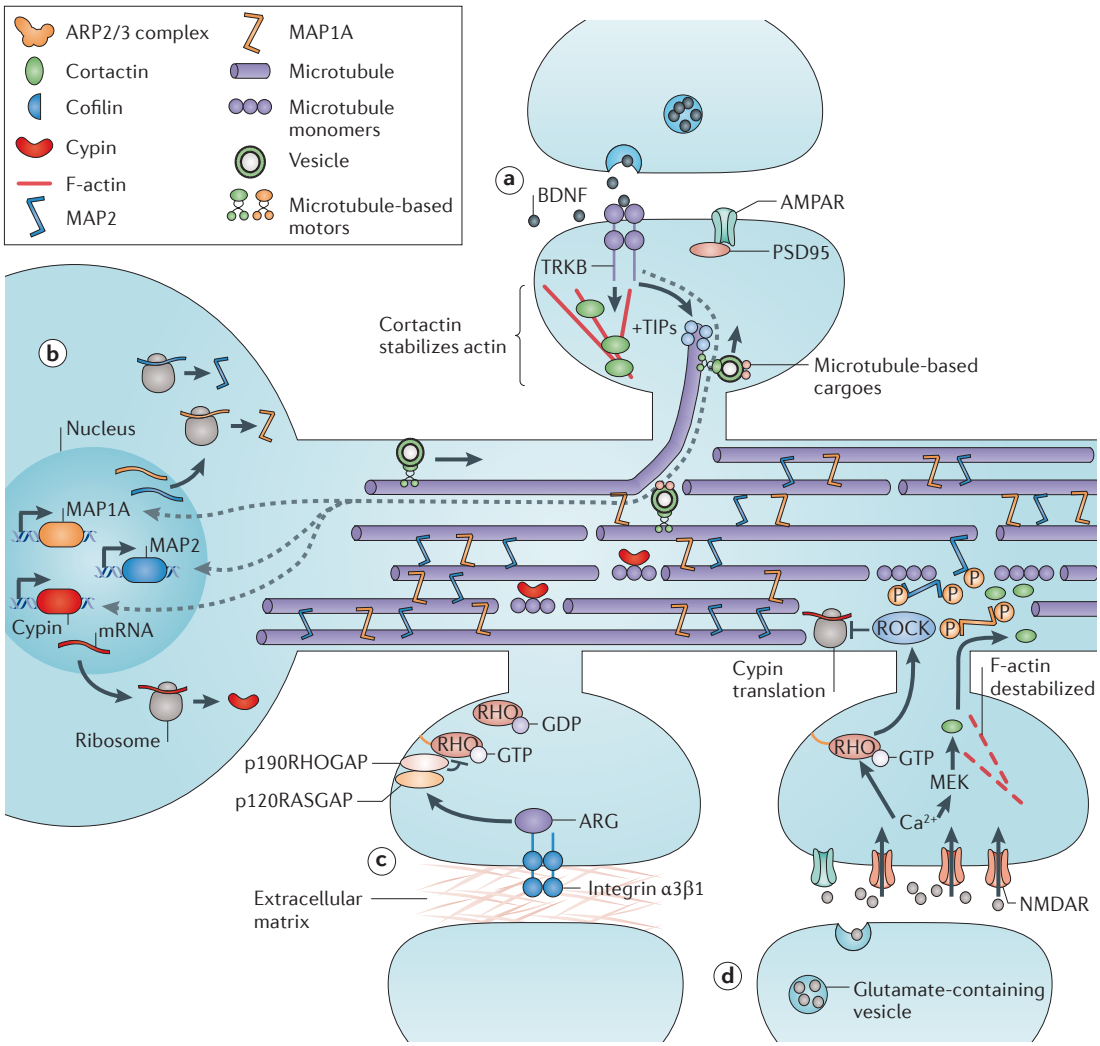

Figure 4 | **Pathways that mediate crosstalk between dendritic spines and dendrite arbors.** Several pathways mediate crosstalk between the spine and shaft cytoskeletons. **a** | Brain-derived neurotrophic factor (BDNF) signalling through the receptor tyrosine kinase TRKB triggers assembly of microtubules containing plus end tip-binding proteins (+TIPs), such as end-binding protein 3 (EB3), to target to spines and allow delivery of cargoes that stabilize spine structure. BDNF also promotes accumulation of cortactin in spines to stabilize F-actin structure. **b** | BDNF triggers increased expression of microtubule-associated protein 1A (MAP1A), MAP2 and cypin, all of which promote microtubule stabilization in the dendrite shaft. **c** | Integrin α3β1 signalling through the receptor tyrosine kinase ARG and 190 kD RHO GTPase-activating protein A (p190RHOGAP) attenuates RHO activity to preserve dendrite shaft structure. **d** | Increased AMPA recptor (AMPAR) and NMDA receptor (NMDAR) activity, such as that associated with stroke, results in Ca^{2+} influx that activates RHO–RHO-associated protein kinase (ROCK) signalling which phosphorylates MAPs and dissociates them from microtubules. Increased levels of ROCK also inhibit localized cypin translation, which further destabilizes spines. NMDAR hyperactivation also triggers cortactin relocalization to the dendrite shaft, which destabilizes dendritic spine F-actin. MEK, MAPK/ERK kinase.

end-binding protein 3 (EB3; also known as MAPRE3). Live-imaging studies involving tracking of the growing microtubule tips using EB3-fluorescent protein fusions have found that neuronal activity results in EB3-labelled microtubule tips making transient visits to dendritic spines[85,156–158] (FIG. 4). Microtubule invasion of individual spines is accompanied by increased accumulation of PSD95 and the EB3-binding protein p140CAP, both of which are required for spine maintenance[85,157]. These events are accompanied by increased spine F-actin content and spine enlargement, both of which are features that are generally associated with greater stability. By contrast, disruption of microtubule dynamics using drugs or by knocking down EB3 leads to reductions of PSD95 and p140CAP in spines, destabilizing some of them and decreasing the size of those remaining[85,156,158].

Intriguingly, these microtubule 'visits' to dendritic spines are greatly increased by membrane depolarization or stimulation with BDNF[85,156–158] (FIG. 4). A decrease in BDNF-induced structural reinforcement of the spine may explain the reductions in hippocampal spine density reported in mice with reduced gene-dosage of the BDNF

receptor *Trkb*[159]. Conversely, treatment of cultured neurons with a chemical long-term depression induction protocol arrests the mobility and targeting of EB3-positive microtubule tips, preventing them from entering the spine[160]. Under these conditions, EB3 relocates to the dendritic shaft, where it associates with microtubule-bound MAP2, and the loss of microtubule targeting to the spines leads to spine shrinkage and loss.

Stabilization of dendrites by synaptic activity. As mentioned above, new synapse formation is essential for dendrite stabilization in developing neurons, but maintenance of this synaptic input appears to be crucial for ongoing dendritic stability[18,19]. Accordingly, a reduction in synaptic inputs, by experimental deafferentation or removal of sensory inputs, leads to dendrite loss in a wide variety of model systems[7–9,77,161]. Both Ca^{2+}/calmodulin-dependent kinase II (CaMKII) and MEK (MAPK/ERK kinase; also known as MAP2K) signalling modules are crucial for activity-dependent dendrite stabilization in cultured sympathetic neurons[60]. Interestingly, arrest of dynamic dendrite behaviour and premature stabilization of dendrite arbors can also be achieved via expression of a constitutively activated form of the postsynaptic signalling protein CaMKII[139]. CaMKIIα has many substrates that can affect the actin cytoskeleton within the spine, including the RAC activators T lymphoma invasion and metastasis-inducing protein 1 (REF. 162) and kalirin 7 (REF. 163), and the RAS GTPase activating protein SYNGAP[164]. Although CaMKIIα and its substrates are predominantly localized to dendritic spines[165], it can relocalize to dendritic shafts upon stimulation in cultured neurons[166]. The exact mechanism by which CaMKIIα functions to stabilize dendrites awaits further study.

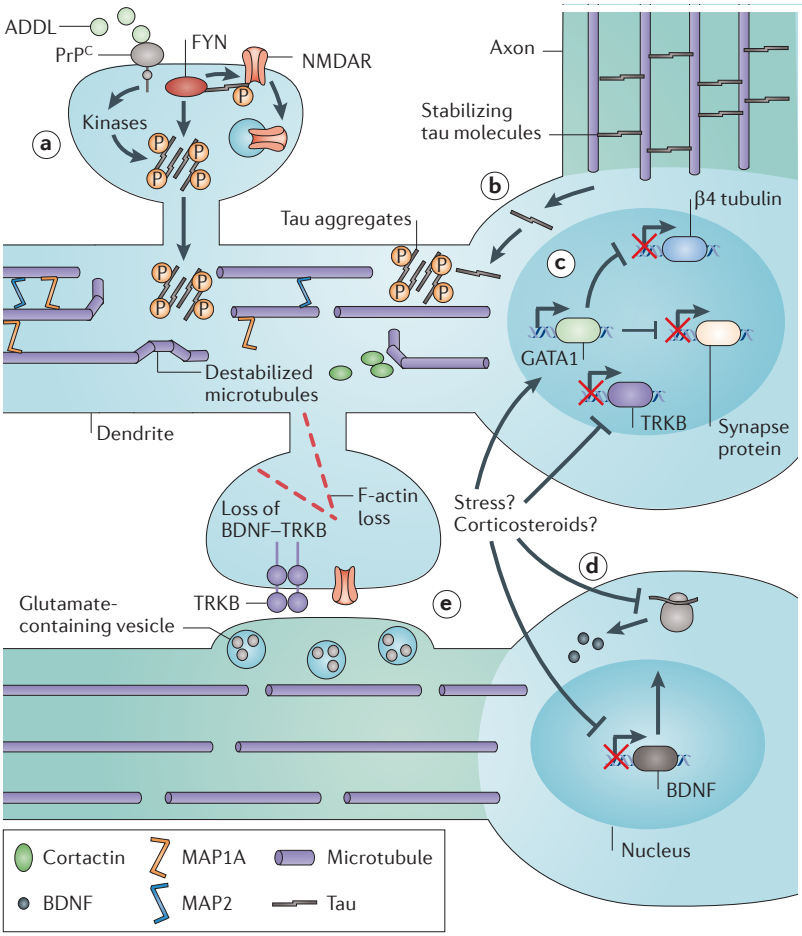

Figure 5 | **Disruption of spine and dendrite stabilization mechanisms in disease.** Disease pathology specifically disrupts dendritic spine and dendrite arbor stabilization. **a** | In Alzheimer's disease, amyloid-β-derived diffusible ligands (ADDLs) bind to the cellular prion protein (PrP^C) receptor and activate receptor tyrosine kinase FYN as well as other kinases. This triggers transient NMDA receptor (NMDAR) phosphorylation, followed by NMDAR internalization and tau hyperphosphorylation and accumulation in spines and shafts. **b** | There is also redistribution of tau from axons to dendrites. **c** | In major depressive disorder (MDD), upregulation of GATA-binding factor 1 (GATA1) inhibits expression of the dendrite shaft component β4 tubulin and also several genes required for synaptic function. **d** | Stress and increased corticosteroid levels, which are known risk factors for MDD, inhibit expression of brain-derived neurotrophic factor (BDNF) in the presynaptic neuron and its receptor the receptor tyrosine kinase TRKB in the postsynaptic neuron. **e** | This disrupts spine and dendrite stabilization by this BDNF–TRKB axis. AMPAR, AMPA receptor.

Events that destabilize spines and branches

Several human brain diseases that arise later in life are associated with losses of dendritic spine density and dendrite arbor complexity, including psychiatric diseases, such as schizophrenia and depression, and neurodegenerative diseases, such as Alzheimer's disease[167]. The consequent loss of connectivity at both the local and interregional level is thought to contribute to disease symptoms. Emerging evidence suggests that the pathology associated with these diseases specifically targets dendritic spine and dendrite branch stabilization mechanisms.

Roles of ADDLs and tau in spine and dendrite instability in Alzheimer's disease. Extensive synapse loss and dendrite arbor atrophy is observed in Alzheimer's disease[168–171], and the reductions in synapse number strongly correlate with the magnitude of cognitive and memory impairment[172,173].

Work over the past several years has focused on soluble amyloid-β (Aβ)-derived diffusible ligands (ADDLs; also known as Aβ oligomers) as key triggers for spine loss and dendritic atrophy in Alzheimer's disease pathology[174] (FIG. 5). Application of ADDLs to cultured neurons or slices has been found to lead to a significant loss of dendritic spines[175–177]. Insight into the mechanism underlying this effect has been gained from the finding that binding of ADDLs to the cellular form of the prion protein (PrP^C) triggers FYN-mediated phosphorylation of the GluN2B (also known as NR2B) subunit of the NMDAR[178]. This initially leads to an increase in the number of NMDARs at the postsynaptic surface followed by a persistent loss of surface NMDAR by endocytosis[175,176,178] (FIG. 5). The initial rise in NMDAR surface activity may contribute to spine destabilization, possibly by triggering the loss of drebrin and cortactin from the spine[175]. However, NMDAR activation probably also mediates spine destabilization by signalling through calcineurin[179] to activate cofilin[180], which as mentioned above, regulates actin disassembly. Both calcineurin

and cofilin have been found to be required for ADDL-induced spine destabilization[176]. ADDL treatment also disrupts the surface localization of other receptors, such as EPHB2 (discussed above), that are also important regulators of spine stability[175].

ADDLs also trigger significant dendrite arbor shrinkage[179]. ADDLs stimulate the activities of several kinases (including MARK1, p70S6K, BRSK and FYN) that can phosphorylate the microtubule-binding protein tau[181,182]. Hyperphosphorylated tau relocalizes from a primarily axonal distribution to the soma and some dendrites (FIG. 5), an effect that is associated with a significant reduction in microtubules and localized dystrophic beading of the dendrites[179,181]. Interestingly, these tau missorting events are also accompanied by reductions in spine density[181]. Although a small portion of tau is normally found in spines, where it tethers FYN to the PSD[183], hyperphosphorylated tau accumulates in spines, where it interferes with NMDA localization to the spine and synaptic anchoring, which contributes to cytoskeletal destabilization[183,184]. Thus, ADDL-induced tau phosphorylation contributes to destabilization of both dendrite arbors and dendritic spines, albeit through distinct mechanisms.

Dendritic spine destabilization and dendrite atrophy in depression. MDD is associated with a reduction in synapse density[185] and reduced dendrite arbor size in the prefrontal cortex and hippocampus[186–189]. A recent study found that levels of GATA-binding factor 1 (GATA1), a transcriptional repressor and master regulator of several genes that has crucial roles in dendritic spine and dendrite arbor maintenance, are reduced in the brains of individuals with MDD[185] (FIG. 5). Several GATA1 target genes are significantly downregulated in the MDD brain, including β4 tubulin (the major β-tubulin subunit in neurons) and the membrane trafficking regulators RAB3A and RAB4B[185]. These reductions may cause a decrease in the size and stability of the microtubule network and perturb the trafficking of key dendritic building blocks that support dendrite structure. This model is supported by the observation that GATA1 overexpression alone is sufficient to significantly reduce dendrite arbor size in cortical neurons[185].

Chronic stress and the associated increase in circulating corticosteroid levels are major risk factors for the development of MDD. The application of restraint stress and the administration of exogenous corticosterone have both been used as models for MDD states in rodents. Both treatments reduce BDNF and TRKB levels in the hippocampus and prefrontal cortex[190–194] (FIG. 5), which probably explains the reduction in BDNF in the brains of patients with MDD[195]. Reduced BDNF signalling would be expected to compromise the BDNF-mediated support of dendritic spine and dendrite arbor stability via mechanisms detailed above, thereby contributing to the neuronal destabilization found in this disease. Recent work shows that chronic corticosterone treatment also decreases expression of caldesmon, an F-actin-stabilizing molecule, increasing F-actin turnover in dendritic spines and resulting in smaller, more unstable spines[82].

Disruption of spine and dendrite stability in stroke. Ischaemic events such as stroke cause destabilization of spines and dendrites on neurons within and adjacent to the infarct area. This destabilization may clear dendrites away from areas in which damage or death of afferents has disrupted connectivity, thus freeing up molecular components and metabolic resources to remodel the dendrite arbor during the recovery phase. In a murine ischaemia model, the reductions in dendrite arbor size in the peri-infarct region that follow stroke are closely matched by growth of distal dendrites during the recovery period[196], suggesting that the neuron may attempt to reach some homeostatic 'set point' in dendrite size and synaptic inputs as it reintegrates into networks.

Increased NMDAR activity and the resulting excitotoxicity has long been known to mediate neuronal damage and death after stroke (for a review, see REF. 197). Application of NMDA to cultured neurons or focal application of NMDA to specific brain areas *in vivo* has been widely used to model this glutamate-induced excitotoxicity.

Whereas prolonged, excessive stimulation leads to neuronal death, excitotoxic but sublethal NMDAR stimulation of neurons, either in culture or focally *in vivo*, causes spine loss, dendrite branch swelling and dendrite shrinkage[84,89,198–202]. These changes are closely associated with disruptions in actin and microtubule structure in spines and dendrites, respectively[89,127,203]. In spines, the cytoskeletal destabilization appears to be linked to disrupted functioning of actin regulatory proteins. Accordingly, bath application of NMDA to cultured hippocampal neurons causes cortactin to relocalize from dendritic spines to the dendritic shaft, and this correlates with the loss of spine stability[84,89]. In addition, inhibitors of the cathepsin B family of proteases attenuate NMDA-induced spine loss, Indeed, NMDAR stimulation leads to reduced levels of the spine stabilizing protein MARCKS (see above), but this decrease can be prevented by cathepsin B inhibition, and this suggests that the spine collapse induced by NMDAR stimulation results from proteolysis by cathepsin B of MARCKS and possibly other key actin regulatory proteins[203]. Increased NMDAR stimulation also disrupts the targeting of dynamic microtubules to spines, which prevents delivery of key proteins, including PSD95 and p140CAP[160], which are required for spine stability.

Exactly how increased NMDAR stimulation triggers dendrite branch destabilization is less clear. One potential candidate for mediating these effects is the GTPase RHOA. As noted above, focal synaptic stimulation can activate RHOA in the spine, which can then diffuse into the dendritic shaft[67]. The summed signalling from a group of spines in aggregate, such as that following excessive NMDAR stimulation, could trigger RHOA–ROCK signalling at levels sufficient to disrupt MAP function[154,155] and cypin expression[151], thereby destabilizing the dendritic cytoskeleton. In support of this, the ROCK inhibitor fasudil can reduce the severity of NMDA-induced damage to the dendrite-rich inner plexiform layer of the retina[202]. Moreover, RHO activation and NMDA stimulation have both been shown to decrease cypin levels in cultured

neurons[200]. Finally, it is possible that NMDA-mediated disruption of microtubule structure affects dendrites by disrupting cargo delivery to the dendrite.

Conclusions

Neurons are continually in a balance between stabilization and destabilization. In the normal healthy brain, this balance is predominantly tipped in favour of stabilization, but the loss of this balance towards excessive and unregulated destabilization is a major factor in various brain disorders. The identification of pathways that mediate stabilization of spines and dendrites and the evidence that they are disrupted in brain diseases raise their profiles as possible targets for therapeutic intervention. However, our understanding of these mechanisms, which are vital for our brain function over several decades, is far from complete.

In the future, one challenge will be to understand how different activity patterns influence these stabilization mechanisms at the biochemical and physiological level and whether these can be manipulated to increase stability. Genome-wide searches for genes associated with psychiatric and neurodegenerative disorders will continue to identify mutations and polymorphisms in genes associated with dendrite instability. Understanding how these genes contribute to stabilization and how their functions are altered in susceptible individuals should reveal new methods to identify those at risk of disease and also suggest preventive or therapeutic approaches for these diseases.

Fortunately, our understanding of the biochemical and cellular mechanisms that shape and maintain dendrite structure is accelerating, as methods to control neuronal activity and fluorescent probes for measuring biochemical events in neurons become even more widely used. The goal of these studies should be to both reveal the timing and distribution of key signalling events and causally relate them to changes in dendritic spine and dendrite structure and function. Along the way, we should learn why different diseases are associated with different patterns of dendritic spine and dendrite destabilization. It is possible that in diseases in which multiple pathways are disrupted (for example, Alzheimer's disease), 'combination therapies', such as those increasingly used to treat cancer, may prove especially efficacious to treat some diseases.

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