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Dendritic Spine Elimination: Molecular Mechanisms and Implications

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Abstract

Dynamic modification of synaptic connectivity in response to sensory experience is a vital step in the refinement of brain circuits as they are established during development and modified during learning. In addition to the well-established role for new spine growth and stabilization in the experience-dependent plasticity of neural circuits, dendritic spine elimination has been linked to improvements in learning, and dysregulation of spine elimination has been associated with intellectual disability and behavioral impairment. Proper brain function requires a tightly regulated balance between spine formation and spine elimination. Although most studies have focused on the mechanisms of spine formation, considerable progress has been made recently in delineating the neural activity patterns and downstream molecular mechanisms that drive dendritic spine elimination in the cerebral cortex. Here, we review the current state of knowledge concerning the signaling pathways that drive dendritic spine shrinkage and elimination and we discuss their implication in neuropsychiatric and neurodegenerative disease.

Keywords

dendritic spine; structural plasticity; long-term depression; glutamate receptor; heterosynaptic plasticity; synapse elimination

Introduction

Animals have a remarkable capacity to learn and integrate new information. The formation, stabilization and elimination of neural connections are thought to be critical for learning and are vitally important for the formation and fine tuning of neural circuits during development. Perturbation in the development and plasticity of neuronal connections results in neurological disorders associated with cognitive impairment. In the mammalian cerebral cortex, most excitatory synaptic connections are glutamatergic and are formed on dendritic spines, microscopic protrusions from neuronal dendrites (Harris and Kater 1994; Yuste and

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others 2000). Spines act to compartmentalize the signaling molecules and machinery required for synaptic transmission and plasticity (Colgan and Yasuda 2014). Spine size is correlated with synaptic strength, and spine addition and elimination contribute to the refinement of neural networks during development and throughout adulthood (Grutzendler and others 2002; Kasai and others 2010; Trachtenberg and others 2002). Notably, a recent study provided evidence that new spine growth and stabilization are essential for learning (Hayashi-Takagi and others 2015).

Although most studies have focused on the outgrowth and stabilization of dendritic spines, spine shrinkage and elimination also play a vital role in the neural circuit plasticity that underlies learning (Fig. 1A, B). Indeed, the formation and stabilization of new dendritic spines as new circuits are formed during learning is accompanied by elimination of pre-existing spines (Chen and others 2015; Lai and others 2012; Nakayama and others 2015; Sanders and others 2012; Xu and others 2009; Yang and others 2009); a subset of these studies in addition reported that the effectiveness of learning was correlated with the observed degree of spine elimination (Lai and others 2012; Yang and others 2009). Notably, induction of complete hearing loss in zebra finches resulted in decreased spine size and subsequent stability, and larger decreases in spine size accompanied stronger vocal deterioration of pre-learned songs (Tschida and Mooney 2012). In addition, spines gained in the visual cortex following monocular deprivation (MD) shrank after binocular vision had been restored, suggesting that the decrease in spine size reflected the de-activation of neural circuits established during MD (Fig. 1C) (Hofer and others 2009). Furthermore, during development, an early phase of dendritic spine addition and synaptogenesis is followed by a period of spine pruning and synaptic refinement, during which inappropriate and redundant spiny synapses are eliminated (De Felipe and others 1997; Holtmaat and others 2005; Rakic and others 1986; Wise and others 1979; Zuo and others 2005). Thus, spine shrinkage and elimination appear to be essential for fine tuning of neural circuits both when they are established during development and during learning in adults.

A series of recent papers also highlighted a vital daily, or better nightly, role for spine shrinkage mechanisms in restoring synaptic homeostasis. Global synaptic downscaling during sleep is thought to be important to counterbalance the increases in spine size and density that are occurring during sensory processing and learning in the wake state, renormalizing synaptic strength and spine size to allow for new learning on the next day (de Vivo and others 2017; Diering and others 2017; Li and others 2017; Maret and others 2011; Tononi and Cirelli 2014). Interestingly, this synaptic downscaling was restricted to small spines and spared larger spines (de Vivo and others 2017), which have been associated with memory. These results are consistent with those from recent *in vivo* imaging studies, where the authors found that spine pruning during REM sleep balanced the number of motor skill learning-induced new spines that were strengthened and maintained (Li and others 2017; Yang and others 2014).

What are the specific neural activity patterns and downstream signaling mechanisms that selectively drive the shrinkage and subsequent loss of those dendritic spines which are no longer required for the functional circuit? Here, we review the recent literature devoted to delineating the cellular and molecular mechanisms of dendritic spine elimination in the

cerebral cortex. Mechanisms of spine structural plasticity and synapse elimination in the cerebellum have been reviewed recently elsewhere (Hashimoto and Kano, 2013; Nishiyama 2014). We have highlighted those studies that investigate the molecular mechanisms driving the dynamic processes of spine shrinkage and elimination, rather than those that report static spine density changes, as it is not possible to link decreases in spine density to increased spine elimination rather than to decreased spine formation or stabilization, and vice versa. We have grouped the studies into three major areas: (1) those that focus on mechanisms associated with long-term depression (LTD) of synaptic strength, (2) those that examine competitive mechanisms between neighboring spines, and (3) those that focus on the role of non-neuronal cells. Finally, we end with a discussion of disease-associated dysregulation of dendritic spine elimination.

LTD-associated mechanisms of dendritic spine elimination

Several of the initial studies on the mechanisms of dendritic spine elimination focused on the hypothesis that eliminating spines would share cellular mechanisms and molecular signaling pathways with weakening synapses, as it had been well-established through multiple approaches that dendritic spine size and synaptic strength were tightly correlated (Harris and Stevens 1989; Matsuzaki and others 2001; Schikorski and Stevens 1997). Indeed, multiple groups went on to demonstrate that decreases in synaptic strength induced by long-term depression (LTD) are associated with spine shrinkage and loss, and that signaling pathways originally identified as driving LTD also contribute to driving the shrinkage and elimination of dendritic spines. As multiple, mechanistically distinct forms of LTD have been described, including N-methyl-D-aspartate glutamate receptor (NMDAR)-dependent, metabotropic glutamate receptor (mGluR)-dependent, and heterosynaptic LTD, we consider each of these separately.

NMDAR-dependent mechanisms—Initial studies of the activity-dependent mechanisms that drive spine elimination examined the consequences of LTD-inducing Low Frequency Stimulation (LFS; 900 stimuli at 1 Hz;(Dudek and Bear 1992) on the size and stability of dendritic spines. Using a local stimulating electrode placed within ~10–30 μm of a fluorescently labeled dendrite, three independent studies published in 2004 found that LFS induced shrinkage and elimination of dendritic spines (Nagerl and others 2004; Okamoto and others 2004; Zhou and others 2004), supporting the hypothesis that synaptic weakening is associated with a reduction in spine size (Fig. 2A, 3A). Importantly, Zhou and colleagues showed that shrinkage was observed only in dendritic spines near to the stimulating electrode (<30 μm); distant spines (>90 μm) did not shrink. LFS-induced spine shrinkage and elimination (Zhou and others 2004), like LFS-induced LTD (Dudek and Bear 1992), required activation of NMDARs and the downstream Ca^{2+}-dependent activation of calcineurin (protein phosphatase 2B, PP2B). Unlike synaptic depression, spine shrinkage was independent of protein phosphatase 1 (PP1), a downstream effector of calcineurin; instead, spine shrinkage was mediated by the actin severing protein cofilin and the shift of the F-actin/G-actin equilibrium towards G-actin (Fig. 3A) (Okamoto and others 2004; Zhou and others 2004). A subsequent study in hippocampal pyramidal neurons furthermore showed that intracellular perfusion of activated cofilin was sufficient to induce dendritic spine shrinkage (Noguchi and others 2016).

These initial studies found widespread spine shrinkage and loss on stimulated dendritic segments. However, because they relied upon broad synaptic stimulation, it was not possible to determine whether the widespread spine shrinkage observed was due to input-specific mechanisms operating at several simultaneously stimulated spines, or rather due to spreading depression to nearby unstimulated spines. Oh and colleagues recognized that this issue could be addressed using two-photon glutamate uncaging (Matsuzaki and others 2001), which allows for targeted activation of individual dendritic spines. Low-Frequency Uncaging of glutamate (LFU) at a single dendritic spine induced input-specific long-lasting synaptic weakening and spine shrinkage at individual dendritic spines, but not at neighboring unstimulated spines (Fig. 2B, 3B) (Oh and others 2013). This input-specific spine shrinkage was dependent on NMDAR activation and, intriguingly, was differentially regulated in small and large spines; shrinkage of large spines also required signaling through group I mGluR activation and the activation of inositol trisphosphate receptors (IP_3Rs) (Fig. 3B) (Oh and others 2013). Thus, LTD-inducing stimulation at individual dendritic spines was sufficient to drive input-specific spine shrinkage and synaptic weakening.

Another study published in the same year identified a role for GABA signaling in spine shrinkage and elimination (Hayama and others 2013). Using a spike-timing dependent stimulation protocol that paired glutamate uncaging with back propagating action potentials (bAPs) and $GABA_A$ receptor ($GABA_AR$) activation, the authors found that $GABA_AR$ activation before the initiation of bAPs resulted in shrinkage of spines activated by glutamate uncaging (Fig. 3C). Notably, in this case, spine shrinkage was not input-specific, as it was also observed for all neighboring unstimulated spines within ~15 μm of the stimulated spine. This spine shrinkage was also shown to rely upon downstream activation of NMDARs, calcineurin and cofilin (Hayama and others 2013; Noguchi and others 2016). Shrinkage of both stimulated and neighboring dendritic spines was tightly correlated with synaptic weakening, thus further linking functional plasticity to structural reorganization.

A third study published in the same year found, in contrast, that synaptic depression and spine structural changes were uncoupled in time (Wiegert and Oertner 2013). In this study, synaptic weakening was induced by low-frequency optogenetic stimulation of channelrhodopsin-2 (ChR2)-expressing CA3 pyramidal cells. ChR2-mediated low-frequency stimulation of CA3 neurons reduced both the amplitude and success rate of postsynaptic Ca^{2+} transients (I_{Ca}) in CA1 neurons, suggesting both postsynaptic and presynaptic components of this optically-induced LTD (I_{Ca}-LTD) (Fig. 3D). Induction of I_{Ca}-LTD was blocked by inhibiting activation of NMDARs. Surprisingly, I_{Ca}-LTD was not correlated with shrinkage of activated spines on CA1 pyramidal cells; instead, I_{Ca}-LTD was followed by elimination of a subset of depressed spines and their neighbors a few days later. Delayed spine elimination was independent of the extent of I_{Ca}-LTD at individual spines, but rather was observed preferentially in spines associated with synapses exhibiting an initially low probability of neurotransmitter release (Wiegert and Oertner 2013); suggesting that unreliable spine synapses are more prone to selective removal from a functional circuit. Intriguingly, other groups had observed remodeling of presynaptic boutons and dissociation of synaptic contacts in response to LFS without effects on dendritic spine size at early time points (Bastrikova and others 2008; Becker and others 2008).

In summary, the studies described in detail above all demonstrate that patterns of synaptic activity that normally lead to NMDAR-mediated synaptic depression also are ultimately coupled to the shrinkage and elimination of dendritic spines. However, LTD-induced spine structural rearrangements were surprisingly quite different across the various studies, including (1) input-specific shrinkage and (2) spreading shrinkage and elimination that were time-locked with synaptic depression, and (3) delayed spreading elimination that occurred not until days after the stimulus. In addition to the varying induction protocols of synaptic depression, developmental stage, culture conditions, and experimental preparation of the hippocampal slices could all contribute to these differences.

Non-ionotropic NMDAR signaling in dendritic spine shrinkage—Conventionally, the induction of LTD has been attributed to the prolonged, low level influx of calcium through NMDARs driving the activation of calcineurin and the removal of AMPARs from the synapse. However, more recently, it was reported that the NMDAR also can signal independent of ion flow through the receptor to induce LTD (Nabavi and others 2013). This conformationally-induced signaling of the NMDAR relied upon basal activity of calcineurin to drive LTD, but was independent of calcium influx through the NMDAR. Supporting this novel non-ionotropic NMDAR signaling, it was later shown that NMDA or glutamate binding in the absence of ion flow through the receptor indeed induce conformational changes in the NMDAR intracellular domains (Dore and others 2015; Ferreira and others 2017), leading to changes in the interactions with PP1 and CaMKII (Aow and others 2015).

A recent study demonstrated that non-ionotropic NMDAR signaling can also drive dendritic spine shrinkage (Stein and others 2015). Application of the NMDAR glycine/D-serine binding site antagonist 7-CK, which blocked ion flow through the NMDAR, did not prevent spine shrinkage induced by Low-Frequency Uncaging (LFU) of glutamate (Fig. 3E). In contrast, LFU-induced spine shrinkage was blocked by the competitive glutamate binding site antagonist CPP (Oh and others 2013), supporting that glutamate binding, but not ion flux, is required for LFU-induced spine shrinkage. Furthermore, block of ion flow through the NMDAR also converted High-Frequency Uncaging (HFU)-induced spine enlargement into spine shrinkage (Stein and others 2015), suggesting that glutamate binding during HFU leads to conformationally-induced NMDAR signaling that, in the absence of Ca^{2+} influx, drives spine shrinkage. Importantly, non-ionotropic NMDAR signaling was independent of group I mGluRs (Stein and others 2015), but required signaling through p38 MAPK (Fig. 3E) (Stein and others 2015), which has been implicated in both NMDAR- and mGluR-dependent LTD (Bolshakov and others 2000; Nabavi and others 2013; Zhu and others 2002).

mGluR-dependent mechanisms—Dendritic spine shrinkage and elimination has also been shown to occur downstream of activation of group I mGluRs, which have been well-established to drive a form of LTD that co-exists with NMDAR-dependent LTD (Fitzjohn and others 1999; Huber and others 2001; Oliet and others 1997). mGluR-dependent LTD is commonly induced by paired-pulse low-frequency stimulation (PP-LFS) or by brief application of the group I mGluR-specific agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) (Fitzjohn and others 1999; Huber and others 2000; Palmer and others 1997).

DHPG-induced mGluR-LTD has been shown to drive a robust and persistent shrinkage and elimination of dendritic spines in hippocampal pyramidal neurons (Ramiro-Cortes and Israely 2013). DHPG bath application induced robust shrinkage in spines of all sizes that lasted at least 24 hours and involved new protein synthesis, but was independent of NMDAR activation (Ramiro-Cortes and Israely 2013). Low-frequency uncaging (LFU) experiments also supported a role for mGluRs in spine shrinkage, but instead suggested that mGluR- and IP_3R-dependent signaling selectively drives spine shrinkage in large spines (Fig. 3B) (Oh and others 2013). Consistent with a size selective role for mGluR signaling through IP_3Rs in shrinkage of large spines, only a subset of dendritic spines (~20%) contain ER structures, which are selectively localized to large spines (Cooney and others 2002) and DHPG- and uncaging-induced mGluR-dependent LTD were restricted to big, ER containing, spines (Holbro and others 2009; Oh and others 2013). Unexpectedly, a recent additional study reported no effects on spine elimination after a single DHPG application, but instead observed increased spine elimination in response to repeated DHPG stimulations given on consecutive days (Hasegawa and others 2015). Despite inconsistencies in the induction protocols and the downstream signaling mechanisms required to induce spine shrinkage and elimination, mGluR-dependent LTD has been clearly associated with dendritic spine shrinkage and elimination.

Competition-based mechanisms of dendritic spine elimination

Homeostatic regulation of synaptic weights and cellular activity levels are thought to keep neuronal circuits in a balanced and working state (Turrigiano 2012). Maintaining total excitatory input in a dynamic range when new circuits are forming or strengthening might be expected to rely upon competitive interactions, in which newly formed and growing spines drive the shrinkage and elimination of inactive neighboring spines. In support of this hypothesis, Zito and colleagues demonstrated in 2004 that increased F-actin polymerization, which resulted in increased spine density, also decreased spine volume and individual synaptic response size, keeping total synaptic input constant (Zito and others 2004). Furthermore, ultrastructural studies have shown that induction of LTP with theta-burst stimulation (TBS) in hippocampal CA1 pyramidal neurons leads not only to increased spine size, but also to an overall decreased spine density relative to control, and thus the total PSD size (and presumably synaptic input) was conserved before and after LTP (Bourne and Harris 2011). The mechanisms driving competitive interactions between neighboring spines are currently beginning to be delineated.

Local competition between stimulated spines and their inactive neighbors on dendritic segments in the hippocampus has been directly demonstrated (Oh and others 2015). Based upon the existence of heterosynaptic LTD, in which LTP induction at a subset of synapses can drive synaptic depression at inactive synapses on the same cell (Abraham and Goddard 1983; Lo and Poo 1991; Lynch and others 1977), Oh and colleagues hypothesized that LTP-induced growth of spines on a dendritic segment would lead to heterosynaptic shrinkage of inactive neighboring spines. Indeed, they found that uncaging induced structural growth of a local cluster of dendritic spines leads to the shrinkage and synaptic weakening of the nearby unstimulated neighbors (Oh and others 2015). This local heterosynaptic spine shrinkage was dependent on group I mGluRs, inositol trisphosphate receptors (IP_3Rs) and calcineurin (Fig.

4A). Furthermore, inhibition of CaMKII prevented structural LTP of activated spines, but did not block shrinkage of unstimulated neighboring spines (Oh and others 2015), suggesting that a local spread of shrinkage-inducing signaling molecules from the stimulated spines to the nearby inactive neighbors, rather than a competition for limited structural resources, is responsible for heterosynaptic spine shrinkage. Heterosynaptic shrinkage of inactive neighboring spines has also been observed in basolateral amygdala principal neurons (Power and Sah 2014), where an increase in size of activated spines was accompanied by a decrease in size of non-activated neighboring spines that required the mGluR- and IP_3R-dependent propagation of a dendritic calcium wave.

What might be the downstream molecular signaling pathways that lead to heterosynaptic shrinkage of unstimulated spines? LTP-inducing stimulation patterns lead to the local dendritic translation of the neuronal activity-regulated protein, Arc, which subsequently accumulates at inactive spines in the stimulated area (Okuno and others 2012). This selective targeting of Arc, a molecule known to drive synaptic depression (Chowdhury and others 2006; Rial Verde and others 2006; Shepherd and others 2006), together with activity-dependent degradation of Arc at active synapses (Greer and others 2010), could provide a mechanism to locally control synaptic strength and spine size, and therefore tune synaptic weights based on previous activity. In addition, analysis of naturally occurring changes in spontaneous synaptic transmission in the cerebral cortex and hippocampus showed that the efficacy of individual synapses was regulated by the extent of co-activity with nearby neighbors (Winnubst and others 2015). Synapses with a low synchronicity of activity became depressed in their transmission frequency (Winnubst and others 2015), which potentially could lead to their subsequent elimination.

Another study by Bian and others showed that synaptic activity drives local competition for a limited number of cadherin/catenin complexes during developmental refinement of cortical circuits (Bian and others 2015). In response to photostimulation of channelrhodopsin (ChR) expressing neurons, cadherin/catenin complexes were redistributed to ChR-activated spines, which increased in size, while neighboring inactive spines were depleted of complexes and subsequently shrank (Fig. 4B). As expected for an activity-driven competition, the extent of spine shrinkage and the observed decrease in β-catenin content of the unstimulated neighboring spines was dependent on inter-spine distance; spines needed to be within a 10 μm radius, and the closer the neighboring spines, the greater the shrinkage (Bian and others 2015). These findings suggest that activity-driven competition for cadherin/catenin complexes is vital for the coordinated local maturation and pruning of dendritic spines during development.

It remains to be demonstrated that local competitive mechanisms are at play on dendritic segments in the hippocampus or cerebral cortex *in vivo*. Some evidence supports the existence of competitive mechanisms of spine shrinkage in the cerebellum *in vivo*: Lee and colleagues have shown that motor skill learning increases the number of multi-synapse boutons (MSBs) contacting spines and at the same time decreases the size of the neighboring spines (Lee and others 2013). Notably, new spines in the cerebral cortex have also been shown to preferentially contact MSBs (Knott and others 2006). Heterosynaptic spine elimination could contribute to the clustering of synaptic inputs, and therefore to the

development of functional compartmentalization of local dendritic segments, which enhances the computational capability of the neuron (Branco and Hausser 2010; Govindarajan and others 2006; Kastellakis and others 2015; Larkum and Nevian 2008).

Non-neuronal mechanisms of dendritic spine elimination

Astrocyte-dependent mechanisms—Astrocytes are closely associated with spine synapses and are actively involved in the control of synapse formation and elimination (Barres 2008; Chung and others 2015). The molecular mechanisms via which glia regulate neuronal synapse number and function have begun to come to light over the past few decades.

One molecular mechanism through which astrocytes control spine morphology and synaptic function is via ephrin signaling through the Eph receptor tyrosine kinases (Fig. 5A). Murai and colleagues showed that EphA4 is enriched on dendritic spines of pyramidal neurons in the mouse hippocampus, ephrin-A3 is localized on astrocytic processes that envelop spine synapses, and activation of EphA4 by ephrin-A3 induces spine retraction (Murai and others 2003). A subsequent study found that the ephrin-A3/EphA4-dependent decrease in spine density relied on inhibition of β1-integrin activity and its downstream signaling via Crk-associated substrate (Cas), the tyrosine kinase focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2) (Bourgin and others 2007). This ephrin-A3-induced signaling causes an initial phase of spine elongation and synaptic reorganization, which involves activation of the actin filament severing factor cofilin by the phosphatases slingshot 1 (SSH1) and calcineurin, and is ultimately followed by spine retraction (Zhou and others 2012). Notably, EphA4 signaling was also shown to be required for homeostatic synaptic downscaling and spine loss via interactions with ephrin-A1 (Fu and others 2011; Fu and others 2007), thus further linking spine elimination with mechanisms of synaptic weakening. EphA4-induced spine loss was inhibited by block of cyclin-dependent kinase 5 (Cdk5) activity and, furthermore, involved the Cdk5-dependent activation of ephexin1 (Fu and others 2007), a guanine nucleotide exchange factor (GEF) that specifically activates the small GTPase RhoA and thus regulates reorganization of the actin cytoskeleton (Fig. 5A).

Astrocytes have also recently been implicated in pruning of retinogeniculate synapses during development. Astrocytes control C1q messenger RNA (mRNA) expression in retinal ganglion cells (RGCs) through the release of transforming growth factor-β (TGF-β) (Bialas and Stevens 2013; Stevens and others 2007). Released TGF-β is sensed by the TGF-β receptor II (TGFβRII) in RGCs, which is specifically upregulated during this critical developmental period. TGFβRII-deficient mice show reduced C1q expression and exhibit impaired complement- and microglia-dependent pruning of retinogeniculate synapses (Bialas and Stevens 2013). Interestingly, C3 and other members of the complement cascade are also upregulated in aging astrocytes (Boisvert and others 2018), suggesting a similar involvement of astrocytes in the age-dependent decline in spine density. In addition, astrocytes also have been shown to be directly involved in activity-dependent phagocytosis and elimination of retinogeniculate synapses (Chung and others 2013). This astrocyte-dependent phagocytosis and synapse elimination was dependent on the two phagocytic receptors MEGF10 and MERTK (Fig. 5B), which are enriched in astrocytes and normally

function to recognize engulfment signals like phosphatidylserine on cell debris. Mice deficient for both phagocytic receptors failed to eliminate retinogeniculate synapses and retained excessive functional connections in the dLGN (Chung and others 2013).

Microglia-dependent mechanisms—Microglia are the resident immune cells of the brain. They are derived from myeloid progenitors and enter the brain early during development (Ginhoux and others 2010). Microglia are active and highly motile in the developing uninjured brain, where microglial processes make frequent, short-lived contacts with synapses (Davalos and others 2005; Nimmerjahn and others 2005).

Microglia have been implicated in spine shrinkage and elimination both during developmental and during activity-dependent circuit refinement. Intriguingly, microglia appear to preferentially contact a subset of spines which are smaller, structurally dynamic and more frequently lost (Tremblay and others 2010). Notably, during manipulation of visual experience through light deprivation and re-exposure, microglia changed their sampling and contact dynamics (Tremblay and others 2010). During light deprivation, a paradigm that promotes synaptic remodeling, microglia became less mobile and started contacting preferentially larger spines, which subsequently shrank over time, and showed an increased occurrence of cellular inclusions (hypothesized to be engulfed synapses). These changes were reversed by light re-exposure, supporting an experience- and activity-dependent role for microglia in driving dendritic spine shrinkage and elimination.

How are microglia-neuron interactions mediated, and what are the molecular pathways leading to the activity-dependent pruning of spiny synapses? In the hippocampus, it was shown that the fractalkine receptor (CX_3CR1) is vital for the engulfment of synaptic material by microglia during spine pruning (Fig. 5C) (Paolicelli and others 2011). High-resolution imaging and EM data identified PSD95-positive puncta within microglia processes of WT mice (Paolicelli and others 2011). *Cx3cr1* knockout mice showed a significantly higher spine density in CA1 pyramidal neurons compared to wild-type. Furthermore, *Cx3cr1* knockout mice contained fewer microglia during development, suggesting that the synaptic pruning deficit and the resulting behavioral phenotype, which is associated with neurodevelopmental and neuropsychiatric disorders, was potentially due to reduced microglia interactions (Paolicelli and others 2011; Zhan and others 2014). However, CX_3CR1-dependent signaling does not seem to be required for the normal functional and structural development of the visual cortex and experience-dependent plasticity induced through monocular deprivation (Schecter and others 2017). Thus, CX_3CR1 appears to be vital in the hippocampus, but not in the visual cortex, for experience-dependent microglial-mediated pruning of dendritic spines.

Microglia also express the complement receptor 3 (CR3), and exhibit CR3-dependent phagocytic signaling with the classical complement proteins C1q and C3 (Fig. 5C), which have recently been identified in the regulation of retinogeniculate synapse elimination during eye-specific segregation (Schafer and others 2012; Stevens and others 2007). Furthermore, C3 KO mice did not exhibit age-dependent decrease in spine density in the hippocampus, which was accompanied by a significantly reduced cognitive decline and spatial memory deficit (Shi and others 2015). In line with this unexpected role for microglia and the

complement system in age-dependent spine elimination, inhibition of C1q, C3 or the microglia complement receptor CR3 also reduced Aβ-induced synapse loss and decreased the amount of phagocytic microglia (Hong and others 2016). Notably, a recent study also identified C1q and microglia activation as an important factor in frontotemporal dementia (FTD) (Lui and others 2016) and activation of the complement system has recently been implicated in the development of schizophrenia, the pathogenesis of a Rett syndrome mouse model, and the synapse loss and cognitive dysfunction following West Nile virus infection (Schafer and others 2016; Sekar and others 2016; Vasek and others 2016).

Dendritic spine elimination associated with disease

Because spine size is tightly coupled with synaptic function and the shrinkage and elimination of dendritic spines is vitally important for refinement of neuronal circuits, it is not surprising that dysregulation of spine structural and functional plasticity mechanisms is associated with neuropsychiatric and neurodegenerative diseases. Neuropathological evidence points to increased rates of dendritic spine loss in schizophrenia, depression and Alzheimer's disease, and to decreased rates of spine elimination in Fragile X syndrome (Fig. 6).

D-serine and schizophrenia-associated spine loss—Schizophrenia is a complex, heterogeneous neuropsychiatric disorder with deficits in executive and cognitive function. One pathological hallmark of schizophrenia is the reduced density and size of dendritic spines in specific cortical regions, including the hippocampus (Glausier and Lewis 2013; Konopaske and others 2014). Abnormalities in excitatory neurotransmission, specifically a dysregulation or hypofunction of NMDARs, have been implicated in the etiology of schizophrenia (Coyle 2006). Indeed, reduced levels of the requisite NMDAR co-agonist D-serine and polymorphisms of genes involved in the regulation of endogenous D-serine levels have been found in schizophrenic patients (Fig. 6A) (Balu and others 2013; Bendikov and others 2007; Goltsov and others 2006; Hashimoto and others 2005; Morita and others 2007). Furthermore, increased levels of the endogenous NMDAR D-serine binding site antagonist kynurenic acid also have been reported in patients with Schizophrenia (Plitman and others 2017). Notably, this limited availability or access of the NMDAR co-agonist D-serine results in reduced Ca^{2+}-influx through the receptor, which will lead to a shift in the downstream signaling mechanisms favoring non-ionotropic NMDAR signaling (Fig. 7A); and could convert Ca^{2+}-dependent synaptic stabilization and spine growth pathways into spine shrinkage and synaptic depression. Thus, changes in D-serine availability and consequent NMDAR activation could contribute to the observed decrease of dendritic spine number and cognitive deficits in patients with schizophrenia.

Ketamine and depression-associated spine loss—Chronic stress induces symptoms of depression and is also associated with an increased loss of dendritic spines in rodent models (Gerhard and others 2016; Li and others 2011). Notably, a reduction in volume of the prefrontal cortex (PFC) and hippocampus and a markedly reduced number of spine synapses has also been found in patients with major depressive disorder (Gerhard and others 2016; Kang and others 2012). Remarkably, the NMDAR antagonist ketamine has been shown to act as a rapid antidepressant and to rescue the stress-induced reduction in spine

density back to normal levels (Fig. 6B) (Li and others 2010a; Li and others 2011). Ketamine has been proposed to act on NMDARs at inhibitory synapses, resulting in disinhibition of excitatory neurons and subsequent increased BDNF release and mTOR-dependent new spine formation, which has been proposed to reverse the chronic stress-induced depressive symptoms (Fig. 6B, 7B) (Gerhard and others 2016; Li and others 2010a). Alternatively, ketamine has been shown to increase BDNF levels by directly blocking pyramidal NMDARs at rest. Activation of NMDARs during spontaneous glutamate release in the absence of neuronal activity was found to suppress BDNF translation, which was subsequently disinhibited by ketamine application, resulting in potentiation of synaptic transmission (Autry and others 2011; Nosyreva and others 2013). However, the exact mechanisms through which ketamine, or its metabolites, exerts its antidepressant effects and increases spine density (Phoumthipphavong and others 2016; Ruddy and others 2015) are still up to debate (Collingridge and others 2017; Suzuki and others 2017; Yang and others 2018; Zanos and others 2016).

Caspases and Alzheimer's disease-associated spine loss—Alzheimer's disease (AD) is a progressive neurodegenerative disorder that results in devastating mental dysfunction, including loss of memory (Selkoe 2002). Dendritic spine loss is one of the first structural changes that can be observed in the brains of AD patients, and the amount of spine loss is closely correlated with cognitive decline (DeKosky and Scheff 1990; Selkoe 2002; Terry and others 1991). One hallmark of early synaptic dysfunction in AD is increased levels of oligomeric amyloid-beta (Aβ) protein, which causes a rapid synaptic depression and spine elimination (Fig. 6C), which is dependent upon signaling through NMDARs (Hsieh and others 2006; Shankar and others 2007; Wei and others 2010).

How does excess Aβ protein lead to increased spine elimination? Recent studies have identified a role for caspases, enzymes with well-established roles in apoptosis (McIlwain and others 2013). Caspases had been initially identified as downstream mediators of NMDAR-dependent LTD (Li and others 2010b) and later of spine shrinkage and synapse elimination (Erturk and others 2014; Henson and others 2016). Elevated levels of caspase-3 activity have been found in spines of a transgenic mouse model of AD (D'Amelio and others 2011) and inhibition of caspase activity blocked the Aβ-induced dendritic spine shrinkage and loss (D'Amelio and others 2011; Tackenberg and others 2013) (Fig. 7C). Activation of caspase-3 during LTD is mediated by the intrinsic mitochondrial pathway, which requires the BAD-BAX cascade for formation of the mitochondrial permeability transition pore and subsequent cytochrome c release (Fig. 7C, **inset**) (Jiao and Li 2011; Li and others 2010b). Notably, local dendritic activation of the mitochondrial pathway is sufficient to induce spine elimination and dendritic pruning (Erturk and others 2014). In contrast to the high levels observed during apoptosis, caspase activation during LTD and spine elimination is moderate and transient (Erturk and others 2014; Jiao and Li 2011; Li and others 2010b). Intriguingly, caspase-3 also has been shown to be important for learning and memory in zebra finches and mice (Huesmann and Clayton 2006; Liu and others 2014; Lo and others 2015). Together these findings demonstrate a locally confined, non-apoptotic function of caspase-3 in driving dendritic spine loss in AD.

Non-ionotropic NMDAR signaling also has been implicated in dendritic spine elimination during AD (Fig. 7C). Several recent studies have reported that Aβ-induced synaptic depression and spine elimination are independent of ion flow through the receptor (Birnbaum and others 2015; Kessels and others 2013; Tamburri and others 2013). Although Aβ-induced spine elimination was blocked in the presence of the NMDAR glutamate binding site antagonist APV, it was unaffected by the channel pore blockers MK-801 or memantine (Birnbaum and others 2015). Furthermore, consistent with shared signaling mechanisms between LTD and Aβ-induced spine elimination, p38 MAPK activity was increased following Aβ treatment and block of p38 activity, which has been shown to prevent LTD-induced spine shrinkage (Stein and others 2015), also prevented Aβ-induced spine elimination (Birnbaum and others 2015).

In addition to effects on NMDAR signaling, Aβ oligomers also have been found to directly bind paired immunoglobulin-like receptor B (PirB) and its human ortholog leukocyte immunoglobulin-like receptor B2 (LilrB2) (Kim and others 2013). Intriguingly, PirB is a natural killer cell receptor known to interact with class I major histocompatibility complex (MHCI) proteins in the immune system. Like MHCI, PirB was originally thought to function exclusively in the immune system, but is now known to be also expressed in neurons (Corriveau and others 1998; Syken and others 2006), and together with MHCI, PirB is important for regulation of dendritic spine and synapse density in cortex and hippocampus (Adelson and others 2016; Bochner and others 2014; Djurisic and others 2013; Elmer and McAllister 2012; Vidal and others 2016).

Mef2 and Fragile X syndrome-associated dysregulation of spine pruning— Fragile X syndrome (FXS) is a leading monogenic cause of intellectual disability and the most common identified cause of autism (Bagni and Greenough 2005). FXS results from loss of function of the fragile X mental retardation 1 (*FMR1*) gene, which encodes the fragile X mental retardation protein (FMRP), a RNA-binding protein important for the regulation of local dendritic protein translation (Bagni and Greenough 2005; Darnell and Klann 2013). One characteristic pathological finding observed in FXS patients (Fig. 6D), and in the FXS mouse model *Fmr1* KO mice, is an increased spine density and a more immature spine morphology (Bagni and Greenough 2005; Hinton and others 1991; Irwin and others 2001; Pan and others 2010; Wisniewski and others 1991), suggesting an FXS-associated impairment of synaptic plasticity mechanisms. Indeed, FMRP has been identified to play a role in spine and synapse elimination downstream of activation of the myocyte enhancer factor 2 (MEF2) transcription factor (Fig. 7D).

The MEF2 family of transcription factors are regulated by neuronal activity and have important roles in activity-dependent signaling in neurons (Flavell and others 2006). Increased MEF2 levels lead to reduced spine and synapse density, and deletion of MEF2 leads to an increased spine and synapse density (Flavell and others 2006; Pfeiffer and others 2010; Rajkovich and others 2016), both of which have been reported to interfere with learning and memory (Barbosa and others 2008; Cole and others 2012). Notably, local dendritic translation of MEF2-dependent mRNA transcripts is regulated by FMRP and activation of synaptic group I mGluRs (Pfeiffer and others 2010; Tsai and others 2012; Wilkerson and others 2014; Zang and others 2013). Consistent with a role for FMRP in

MEF2-dependent developmental pruning of dendritic spine synapses, increased MEF2 levels no longer led to decreased spine density in *Fmr1* KO mice (Pfeiffer and others 2010; Tsai and others 2012; Zang and others 2013). Like mGluR LTD, the MEF2-induced decrease in spine and synapse number was dependent on local translation of Arc mRNA in response to synaptic mGluR activation (Jakkamsetti and others 2013; Waung and others 2008; Wilkerson and others 2014).

Concluding remarks

Considerable progress has been made in the identification of molecular signaling pathways controlling dendritic spine shrinkage and elimination; however, much remains unknown. Neuropathological evidence points to increased rates of dendritic spine loss in diseases including schizophrenia, depression, and Alzheimer's disease; therefore, a better understanding of the signaling mechanisms driving spine destabilization is not only important for understanding the mechanisms of normal brain circuit development and function, but also should provide new insights into how dysregulation of these mechanisms contributes to neuropsychiatric and neurodegenerative diseases. Recent advances in optical techniques, which allow the monitoring and manipulation of protein activity with high spatiotemporal resolution at the single spine level (Nishiyama and Yasuda 2015), in combination with cell-specific optogenetic manipulations (Bernstein and others 2012; Fenno and others 2011; Rost and others 2017; Sjulson and others 2016) and *in vivo* imaging of spine structural and functional plasticity during learning (Chen and others 2014; Grienberger and others 2015; Yang and Yuste 2017) or in disease models (Bhatt and others 2009; Sigler and Murphy 2010), will no doubt further transform our understanding of the underlying molecular signaling pathways and their ultimate role in behavior and disease.

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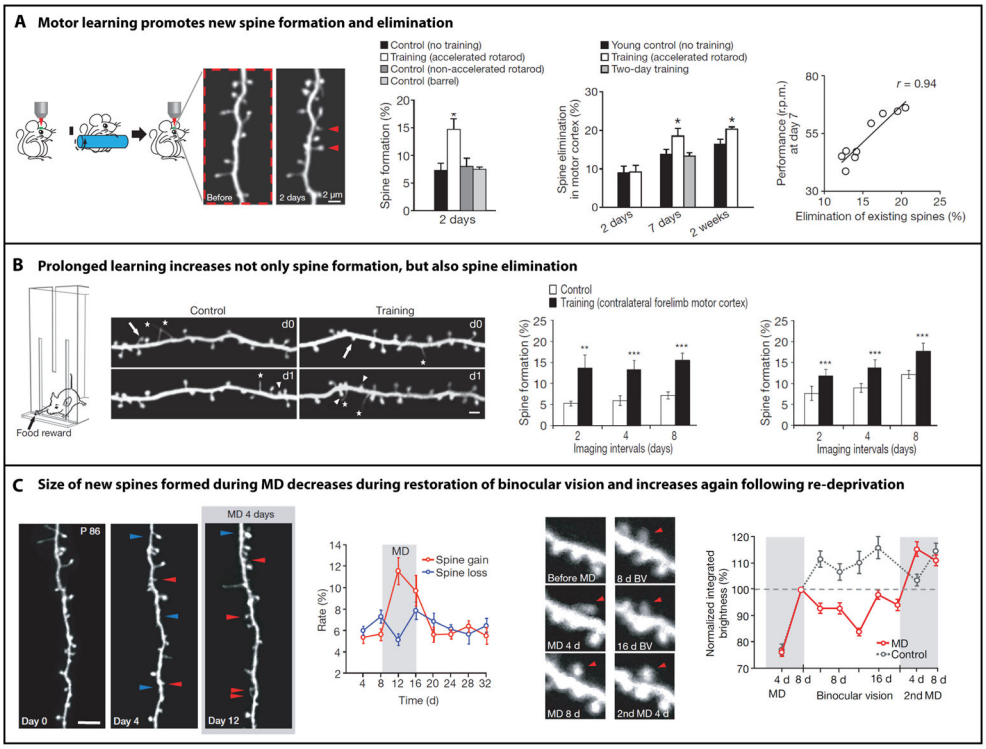

Figure 1. Examples of experience-dependent spine shrinkage and elimination

(A) *Left*, schematic depicting two-photon imaging of an adult Thy1-YFP mouse before and after rotarod training and images of a YFP-labeled dendritic segment in the motor cortex showing that new spines formed (red arrowheads) over 2 d of training. *Middle bar graphs*, the percentage of spines formed and eliminated within the motor cortex increased after training compared to controls. *Right*, Enhanced performance at day 7 is correlated with increased percent of spine elimination. Adapted from Yang and others 2009 with permission from Springer Nature. (B) *Left*, schematic of motor skill learning paradigm and images of YFP-labeled dendritic branches acquired in one day intervals from the motor cortex of a control and trained mouse showing spine formation (arrowheads) and elimination (arrows). Scale bar, 2 µm. *Right bar graphs*, percentage of spines formed and eliminated increased following training as compared to controls. Adapted from Xu and others 2009 with permission from Springer Nature. (C) *Left*, images of an EGFP-labeled dendritic segment in binocular visual cortex. Monocular deprivation (MD) in the contralateral eye was induced after imaging on day 8. Arrowheads show spines appearing (red) or disappearing (blue). Scale bar, 5 µm. *Middle*, the rate of spine formation increased following MD. *Right*, example of a spine that appeared during the first MD, shrank in size following restoration of binocular vision (BV), and grew again after a second MD. Graph shows the time course of the average brightness of the new spines formed during MD (red) or the non-deprived control animals (grey), normalized to the spine brightness at day 8. Adapted from Hofer and others 2008 with permission from Springer Nature.

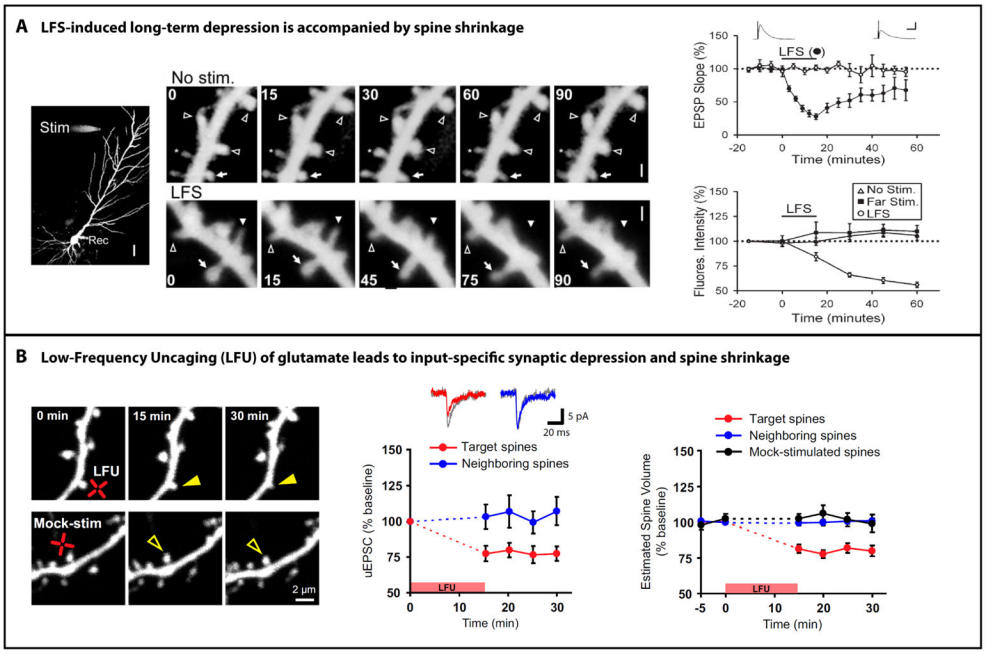

Figure 2. Examples of activity-dependent spine shrinkage and elimination

(A) *Left*, image of a hippocampal CA1 neuron with recording (Rec) and stimulating (Stim) electrodes. Scale bar, 20 μm. *Middle*, spine morphology is mostly stable (open arrowheads) in the absence of stimulation, whereas Low-Frequency Stimulation (LFS) leads to a persistent reduction in size of many spines (arrows) or complete spine retraction (filled arrowhead). Scale bar, 1 μm. *Right*, top graph shows LFS-induced reduction in synaptic responses (filled circles) compared to unstimulated controls (open circles) and bottom graph shows LFS-induced reduction in spine fluorescence intensity (open circles) compared to no stimulation (triangles) and LFS applied far away from the imaged dendritic segment (squares). Adapted from Zhou and others 2004 with permission from Elsevier. (B) *Left*, images of target spines exposed to Low-Frequency Uncaging (LFU, yellow arrowheads) or mock stimulation in the absence of caged glutamate (open arrowheads). *Middle*, LFU decreases the uncaging evoked EPSC (uEPSC) amplitude of target spines (red circles), but not of unstimulated neighboring spines. *Right*, LFU leads to a decrease in the volume of the stimulated target spines (red circles), but not in neighboring unstimulated (blue circles) or mock stimulated (black circles) spines. Adapted from Oh and others 2013 with permission from the National Academy of Sciences.

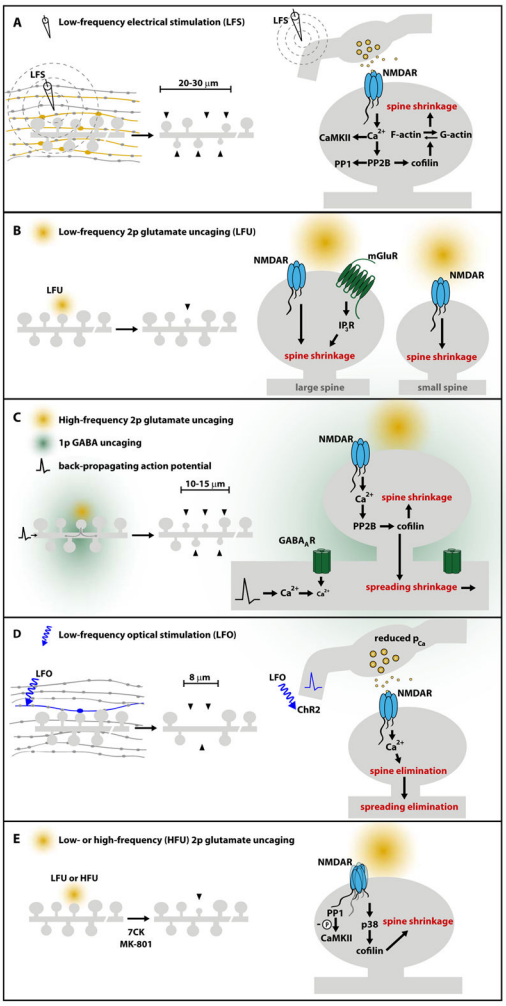

Figure 3. NMDAR-dependent mechanisms of dendritic spine shrinkage and elimination

(A) Low-frequency electrical stimulation (900 pulses at 1 Hz) induces wide spread spine shrinkage and elimination (arrowheads) proximal (20–30 µm) to the stimulating electrode (Nagerl and others 2004; Okamoto and others 2004; Zhou and others 2004). LFS-induced spine shrinkage requires signaling through NMDARs and PP2B (calcinuerin) and a cofilin-dependent (Zhou and others 2004) shift of the F-actin/G-actin equilibrium towards G-actin (Okamoto and others 2004). (B) Low-frequency uncaging of glutamate (LFU, 90 pulses at 0.1 Hz) leads to input-specific shrinkage of stimulated spines. Shrinkage of all spines requires NMDAR activation, and activation of group I mGluRs and IP3Rs contributes to shrinkage of large spines (Oh and others 2013). (C) $GABA_AR$ activation during a spike-timing dependent glutamate uncaging protocol modulates local NMDAR-dependent Ca^{2+}-influx and results in shrinkage of the stimulated spine and all neighboring non-stimulated spines within ~10–15 µm of the target, dependent upon activation of PP2B and cofilin (Hayama and others 2013). (D) Optogenetic low-frequency stimulation (900 light pulses at 1 Hz) of ChR2-expressing, presynaptic CA3 neurons induces depression of spine calcium transients (I_{Ca}-LTD), followed by elimination of a subset of depressed spines and their neighbors days later (Wiegert and Oertner 2013). (E) LFU leads to p38 MAPK-dependent,

input-specific shrinkage of stimulated spines even when ion flow through the NMDAR is blocked by the glycine/D-serine binding site antagonist 7-CK. Notably, application of 7CK or the open channel blocker MK-801 also converts spine enlargement resulting from a high-frequency uncaging stimulus (HFU) into spine shrinkage (Stein and others 2015).

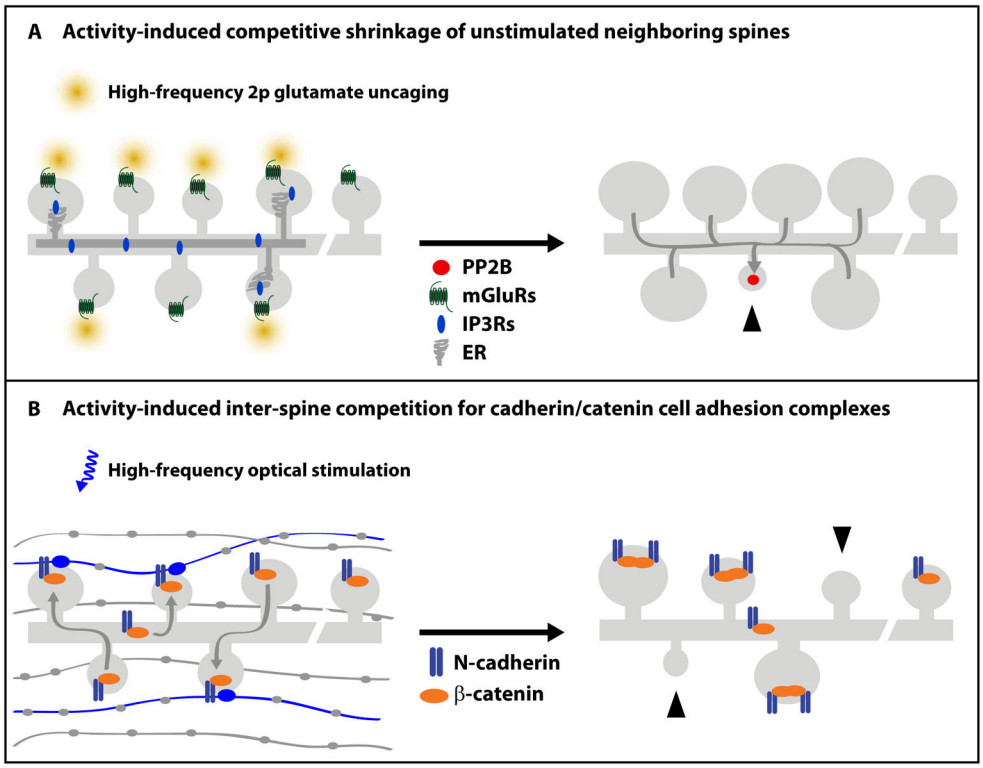

Figure 4. Competition-based mechanisms of dendritic spine shrinkage and elimination

(A) Stimulation of a local cluster of dendritic spines with high-frequency glutamate uncaging induces structural potentiation of the activated spines and causes shrinkage of nearby unstimulated neighboring spines (Oh and others 2015). This local heterosynaptic spine shrinkage requires signaling through group I mGluRs, IP3Rs and PP2B. (B) Optogenetic stimulation (10 light pulses at 4 Hz, every 60 s) of presynaptic channelrhodopsin (ChR)-expressing axons leads to the local redistribution of cadherin/catenin complexes to activated ChR-contacting spines, which increase in size. Unstimulated neighboring spines within a radius of ~10 μm, however, are depleted of cadherin/catenin complexes and shrink (Bian and others 2015).

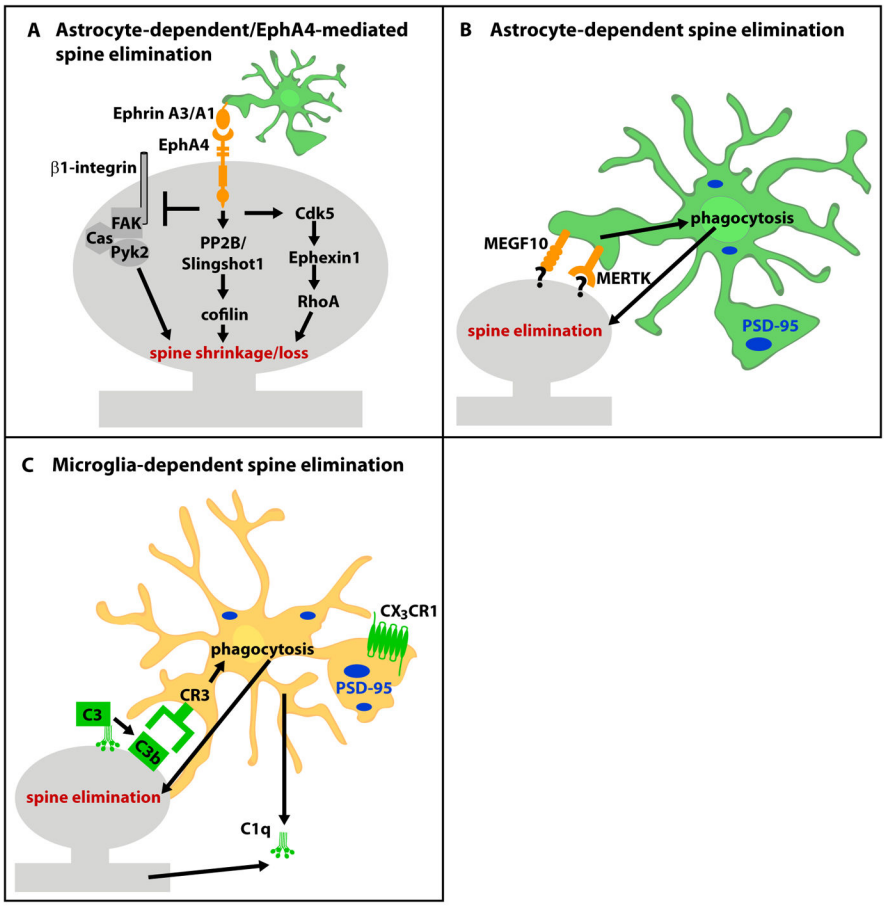

Figure 5. Non-neuronal mechanisms of dendritic spine shrinkage and elimination

(A) Activation of postsynaptic EphA4 receptors by astrocytic expressed ephrin A3 leads to a cofilin-dependent early phase of spine elongation and synaptic reorganization, which is followed by dendritic spine elimination via inhibition and disassembly of an integrin signaling complex consisting of Cas, FAK and Pyk2 (Bourgin and others 2007; Murai and others 2003; Zhou and others 2012). Furthermore, EphA4-induced signaling through Cdk5, ephexin 1 and RhoA is required for ephrin A1 induced spine loss (Fu and others 2007). (B) Astrocytes also participate in activity-dependent phagocytosis and elimination of retinogeniculate synapses during development, which requires the MEGF10- and MERTK-dependent phagocytic pathways (Chung and others 2013). (C) Microglia-released C1q targets dendritic spines for elimination through the classical complement pathway. C1q triggers cleavage of C3 to C3b, which leads to CR3-dependent phagocytic removal by microglia (Hong and others 2016; Schafer and others 2012; Shi and others 2015; Stevens and others 2007). In addition to CR3, microglia express the fractalkine receptor (CX_3CR1), which is vital for the engulfment of synaptic material and pruning of dendritic spines by microglia (Paolicelli and others 2011).

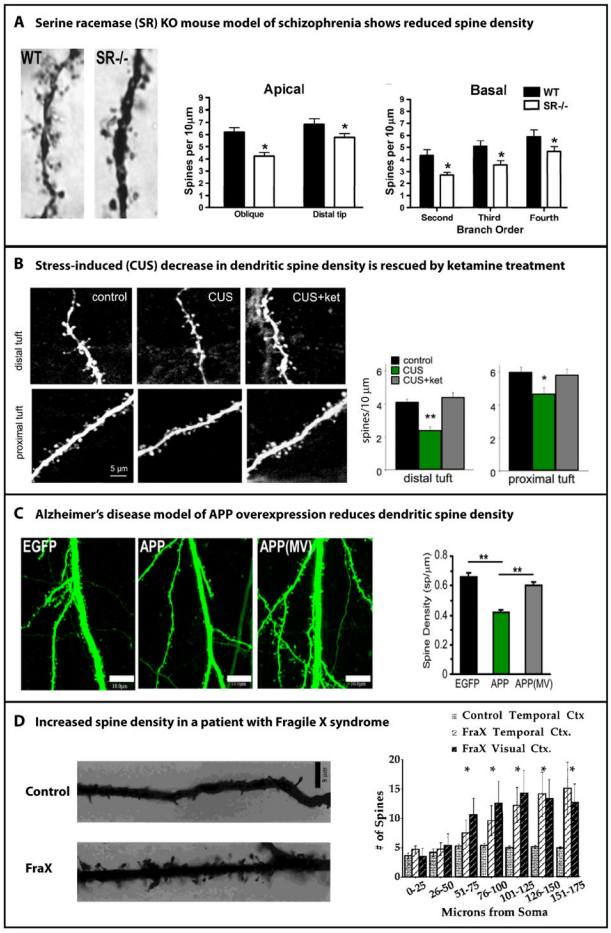

Figure 6. Examples of dendritic spine elimination in disease

(A) *Left*, images of apical dendritic spines on a Golgi-stained pyramidal neuron in the somatosensory cortex of a WT and serine racemase (SR) KO mouse (Balu and others 2012). *Right*, spine density is reduced on both apical and basal dendrites, including oblique and distal apical branches and all branch orders of basal dendrites. Adapted from Balu and others 2012 with permission from Elsevier. (B) *Left*, images of distal and proximal segments of layer V pyramidal cell apical tuft dendrites. Scale bar, 1 μm. *Right*, chronic unpredictable stress (CUS) decreases spine density on both distal and proximal dendritic segments, which is completely rescued by ketamine treatment. Adapted from Li and others 2011 with permission from Elsevier. (C) *Left*, images of CA1 pyramidal cells cotransfected with EGFP and either amyloid precursor protein (APP) or APP (MV), which is cleaved by α-secretase, but not by β-secretase and therefore cannot produce Aβ. *Right*, overexpression of APP and subsequent Aβ production decreases spine density. Adapted from Hsieh and others 2006 with permission from Elsevier. (D) *Left*, example images of dendritic spines on Golgi-stained dendrites from a Fragile-X patient and an unaffected control subject. *Right*, spine densities on distal dendritic segments along apical shafts, in both the visual and temporal cortices, are increased in Fragile-X patients compared to control subjects. Adapted from Irwin and others 2001 with permission from John Wiley and Sons.

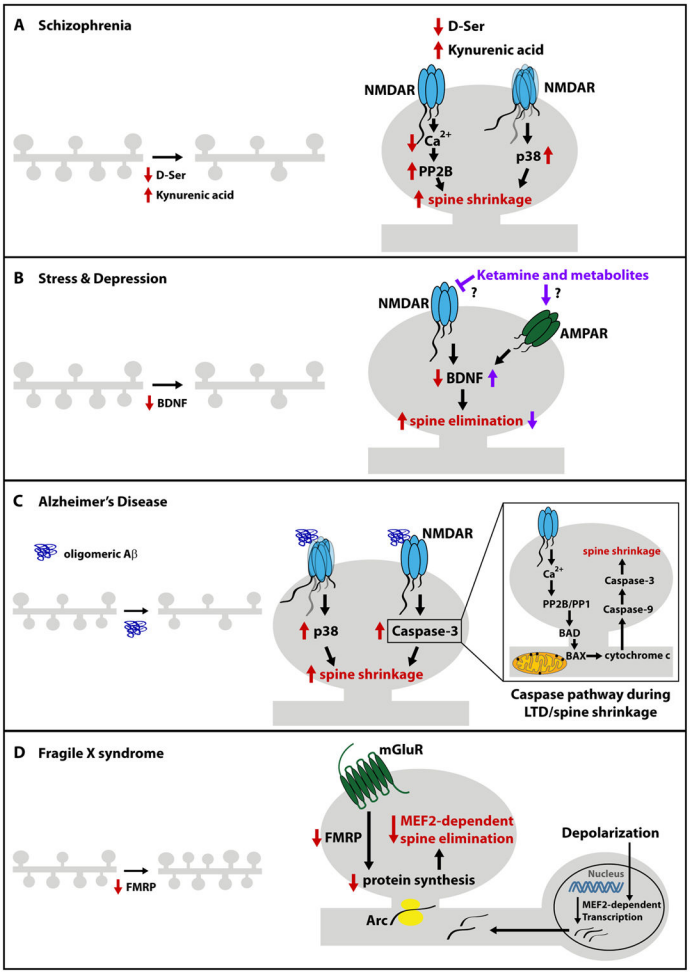

Figure 7. Mechanisms of dendritic spine shrinkage and elimination in disease

(A) Schizophrenia is associated with decreased spine densities (Glausier and Lewis 2013; Konopaske and others 2014). Reduced levels of the NMDAR co-agonist, D-serine, and increased levels of the endogenous NMDAR D-serine binding site antagonist, kynurenic acid, have been reported in schizophrenic patients (Bendikov and others 2007; Hashimoto and others 2005; Plitman and others 2017). Limited access of the NMDAR to the obligatory co-agonist will result in reduced Ca^{2+}-influx through the receptor and could cause a shift in the downstream signaling mechanisms favoring PP2B-dependent, and non-ionotropic NMDAR- and p38 MAPK-dependent signaling towards spine shrinkage and synaptic depression. (B) Stress-induced depression is associated with decreased spine densities (Gerhard and others 2016). The NMDAR antagonist ketamine is a fast acting antidepressant, which rescues the stress-induced reduction in spine density (Li and others 2010a; Ruddy and others 2015), possibly through inhibition of NMDARs (Autry and others 2011; Collingridge and others 2017; Suzuki and others 2017) or activation of AMPARs (Zanos and others 2016), leading to increased BDNF levels and mTOR-dependent new spine formation (Li and others 2010a). (C) Alzheimer's disease (AD) is associated with decreased spine densities (Selkoe 2002). Increased levels of oligomeric Aβ in AD cause NMDAR-dependent spine elimination and synaptic depression, which does not require ion influx through the receptor

(Kessels and others 2013; Tamburri and others 2013), but leads to activation of p38 MAPK (Birnbaum and others 2015). Inhibition of caspase-3 activity also blocks Aβ-induced dendritic spine shrinkage and loss (D'Amelio and others 2011; Tackenberg and others 2013). Inset shows the caspase-dependent signaling pathway during LTD. (D) Fragile X syndrome (FXS) is associated with immature spine morphology and increased spine densities due to dysfunctional spine elimination mechanisms (Bagni and Greenough 2005). In Fragile X syndrome, loss of Fragile X Mental Retardation Protein (FMRP) function prevents spine elimination that is driven by the activity-induced transcription factor, MEF2, protein-synthesis and mGluR- dependent local dendritic translation of these MEF2 transcripts, including Arc mRNA (Pfeiffer and others 2010; Tsai and others 2012; Wilkerson and others 2014).