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Morphogenic Signaling in Neurons Via Neurotransmitter Receptors and Small GTPases

Evgeni Ponimaskin · Tatyana Voyno-Yasenetskaya · Diethelm W. Richter · Melitta Schachner · Alexander Dityatev

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Abstract Several neurotransmitters including serotonin and glutamate have been shown to be involved in many aspects of neural development, such as neurite outgrowth, regulation of neuronal morphology, growth cone motility and dendritic spine shape and density, in addition to their well-established role in neuronal communication. This review focuses on recent advances in our understanding of the molecular mechanisms underlying neurotransmitter-induced changes in neuronal morphology. In the first part of the review, we introduce the roles of small GTPases of the Rho family in morphogenic signaling in neurons and discuss signaling pathways, which may link serotonin, operating as a soluble guidance factor, and the Rho GTPase machinery, controlling neuronal morphology and motility. In the second part of the review, we focus on glutamate-induced neuroplasticity and discuss the evidence on involvement of Rho and Ras GTPases in functional and structural synaptic plasticity triggered by the activation of glutamate receptors.

Keywords GTPase · GEF · GAP · Neurite outgrowth · Synaptic plasticity · Long-term potentiation · Long-term depression · Depotentiation · NMDA receptor · 5-HT receptor · Dendritic spine

E. Ponimaskin (✉) · D. W. Richter
Abteilung Neuro- und Sinnesphysiologie,
Physiologisches Institut, Universität Göttingen,
Humboldtallee 23,
37073 Göttingen, Germany
e-mail: eponima@gwdg.de

T. Voyno-Yasenetskaya
Department of Pharmacology, University of Illinois,
Chicago, IL 60612, USA

M. Schachner · A. Dityatev
Zentrum für Molekulare Neurobiologie,
Universitätsklinikum Hamburg-Eppendorf,
Hamburg, Germany

A. Dityatev
Institut für Neurophysiologie und Pathophysiologie,
Universitätsklinikum Hamburg-Eppendorf,
Martinistr. 52,
20246 Hamburg, Germany

Introduction

Several neurotransmitters including serotonin and glutamate have been shown to be involved in many aspects of neural development, such as neurite outgrowth, regulation of neuronal morphology, growth cone motility, and dendritic spine shape and density, in addition to their well-established role in neuronal communication. The main goal of this review is to go beyond a simple description of neurotransmitter-mediated morphological changes and to highlight multiple signaling processes initiated by binding of the cognate ligand to its receptor and leading to the modulation of the signaling effector molecules controlling neuronal morphology and motility. This control is commonly mediated by the members of small G protein superfamily. It contains over 100 members that are generally classified into 5 subfamilies: Rho, Ras, Arf, Rab and Ran families of GTPases. The small GTPases are monomeric G proteins with molecular masses over the range 20–30 kDa. The functions of the many small G proteins are still being elucidated and have been recently reviewed in detail [1, 2]. In this review, we focus on role of small GTPases of Rho and Ras families in mediating morphogenic activities of two neurotransmitters, serotonin and glutamate.

The Rho Activation/Inactivation Cycle

The Rho GTPase family comprises some 21 genes in humans, encoding at least 23 signaling proteins [3]. The best-studied members of the Rho family are RhoA, Rac1 and Cdc42 proteins. Although Rho GTPases are involved in the control of a wide range of cellular activities [2], their most prominent function is the regulation of cellular morphogenesis and motility through dynamic regulation of the actin cytoskeleton.

Rho GTPases act as molecular switches, which can reside in two states: an inactive GDP-bound form or an active GTP-bound form [4]. Activity of the Rho GTPases is regulated by three classes of proteins, including guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs) (Fig. 1). Conversion of the GDP-bound form to the active state is catalyzed by different GEF proteins that facilitate release of GDP [5, 6]. This results in the activation of Rho GTPases because of the binding of GTP, the intracellular concentration of which is higher than that of GDP. The classical GEFs for Rho GTPases contain a Dbl homology (DH) domain, which mediates the nucleotide exchange [7]. In addition, members of Ced-5, Dock180 and myoblast city (CDM) proteins–zizimin homology (CZH) family can also promote guanine nucleotide exchange, although they do not contain the DH domain [5]. Inactivation of the Rho GTPases occurs via hydrolysis of the bound GTP because of the intrinsic GTPase activity of the Rho GTPases, which is accelerated by the action of specific GAPs [8] (Fig. 1). In addition, the activity of Rho GTPases is regulated by the GDIs. These proteins bind to inactive Rho GTPases and thus compete with GEFs. GDIs also keep Rho GTPases in cytoplasm, removed from the plasma membrane, thus allowing for GEF-mediated activation in a regulated manner (Fig. 1) [9]. Several GDI dissociation factors (GDFs), such as ezrin/radixin/moesin (ERM) family proteins, can release GDP-bound Rho from GDI proteins, and thereby enhance the ability of GEFs to induce GDP to GTP exchange [10, 11] (Fig. 1). Because this simple activation/inactivation cycle implies multiple Rho GTPases, GAPs, GEFs and GDIs, the Rho pathway is well suited to integrate multiple inputs (Fig. 1) and to regulate different aspects of cellular and neuronal morphogenesis in a spatially and temporally appropriate manner.

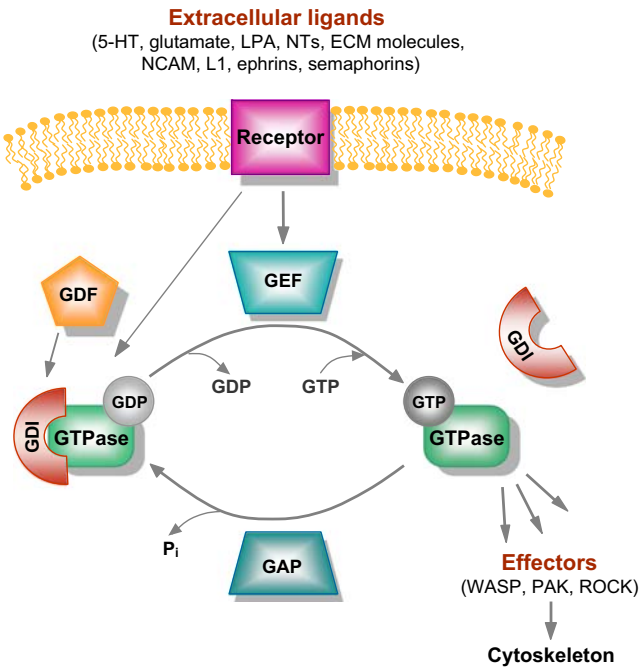

Fig. 1 Mode of activation of the small GTPases and the GTPase cycle. *GEF* guanine nucleotide exchange factors, *GAP* GTPase-activating proteins, *GDI* GDP dissociation inhibitor, *GDF* GDI dissociation factor, *NTs* neurotrophins, *LPA* lysophosphatidic acid, *ECM* extracellular matrix, *NCAM* neural cell adhesion molecule

Roles of Rho GTPases in Regulation of Actin Cytoskeleton

In their active, GTP-bound form, Rho members can initiate multiple signaling pathways through a conformation-specific interaction with a variety of downstream target proteins, called effectors (Fig. 1). Over 50 different effectors have been identified so far for Rho GTPases [12].

Many of the proteins, which have been identified as targets for Rac1 and Cdc42 GTPases contain the Cdc42/Rac1-interactive binding (CRIB) domain responsible for the interaction with these proteins in the GTP-bound state [13]. The best characterized common effector for Rac1 and Cdc42 is the p21 activated kinases (PAK) family of serine–threonine kinases. PAKs are components of signaling pathways that regulate cell morphology, including formation of lamellipodia and disassembly of stress fibers and focal adhesions. One of the important downstream targets of PAKs is the serine/threonine kinase LIMK1, which modulates the activity of the actin-binding protein cofilin [14], thus linking the activation of RhoA family of small GTPases to the changes in cytoskeleton dynamics. Cofilin is a ubiquitous actin-binding factor required for the reorganization of actin filaments in eukaryotes. The dephosphorylation of cofilin enables its actin severing and depolymerizing activity providing a simple phosphoregulatory mechanism for actin reorganization [15]. Once phosphorylated, the affinity of cofilin for actin is significantly decreased, resulting in stabilizing polymerized actin filaments (Fig. 2). On the other hand, microtubule (MT) destabilization also promotes the formation of actin stress fibers and enhances the contractility of cells. However, the mechanism involved in such coordinated regulation of MTs and the actin cytoskeleton is poorly understood. We have

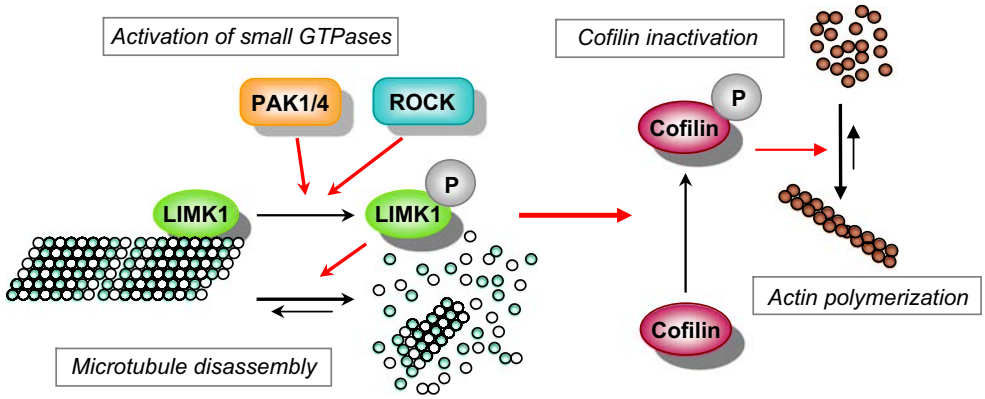

Fig. 2 A model of LIMK1 regulation of the actin cytoskeleton and microtubules. Ligand-induced activation of the Rho-ROCK or PAK1/4 pathway activates LIMK1, which in turn causes microtubules destabilization and release of LIMK1. Consequently, activated LIMK1 associates with actin, thereby inducing its polymerization via cofilin phosphorylation

recently reported that LIMK1 is also involved in the MTs destabilization and shown that endogenous LIMK1 colocalizes with MTs and forms a complex with tubulin via PDZ domain [16]. Overexpression of wild type LIMK1 resulted in MTs destabilization, whereas kinase-dead mutant of LIMK1 did not affect MTs stability. Thus, LIMK1 may coordinate both microtubules and actin cytoskeleton (Fig. 2).

In addition to the PAKs, the Wiskott–Aldrich syndrome protein (WASP) has been identified as a specific effector of Cdc42. WASP and its neural isoform N-WASP interact with GTP-bound Cdc42 and are involved in regulation of actin polymerization and filopodia formation both by interaction with profilin and by direct interaction with actin [17]. Other groups of Rho GTPase effectors, WAVE (Wiskott–Aldrich syndrome protein verprolin homologous)-1, WAVE-2 and WAVE-3, function as molecular platforms for the coordination of actin polymerization and branching catalyzed by the Arp2/3 (actin-related protein 2/3) complex [18]. The major effector for RhoA GTPases with respect to actin and microtubule reorganization is the Rho-kinase family (ROCK, also referred as ROK), which mainly consists of ROCK1, ROCK2, ROKα and ROKβ proteins. Activation of these serine–threonine kinases results in the formation of stress fibers and focal adhesions [19, 20].

The signal triggering formation of stress fibers results from phosphorylation of the regulatory part of the myosin molecule, the myosin light chain (MLC), which enables myosin to change conformation, interact with actin, and slide along actin filaments causing contractility [21]. The phosphorylation status of MLC is regulated by a set of Ca^{2+}/calmodulin-dependent kinases, called myosin light chain kinases (MLCKs) [22]. However, whereas MLCKs' primary function is phosphorylation of the regulatory MLC, ROCK may contribute to stress fiber formation in several ways: (1) ROCK can directly phosphorylate MLC, although so far only demonstrated in vitro [23]. (2) ROCK can regulate MLC phosphate levels by inhibition of MLC dephosphorylation. MLC dephosphorylation is accomplished by a specific myosin phosphatase, myosin phosphatase type 1 (PP1M) [24], and activity of PP1M is inhibited via phosphorylation of the myosin binding subunit of PP1M by ROCK [19, 25]. In many cases, inhibition of PP1M accounts for the major contribution of ROCK to the increase in MLC phosphorylation [26, 27]. (3) Finally, ROCK regulates phosphorylation and activation of LIMK1, which in turn phosphorylates and inactivates cofilin.

Functional Roles of Rho Activation in the Nervous System

The most prominent function of small GTPases is the regulation of the actin and microtubule cytoskeleton. Experiments in fibroblasts have demonstrated that RhoA, Rac1 and Cdc42 GTPases are responsible for the formation of distinct F-actin-based structures such as stress fibers and focal adhesions, lamellipodia and filopodia [28]. In yeast, multiple Rho proteins are required for polarized membrane growth during budding and mating [29].

Over the past years, it has become evident that members of the Rho family are widely expressed in multiple neural tissues and appear to function as key mediators that link the extracellular signals to cytoskeletal rearrangements [30, 31]. Marked changes in morphology, motility, and guidance of axons have been observed in response to activation of Rho family GTPases both in vitro and in vivo [32–34]. The combined studies suggest that Rac1 and Cdc42 are positive regulators promoting neurite extension and growth cone protrusion. Conversely, activation of RhoA induces stress fiber formation, leading to the growth cone collapse and neurite retraction [35, 36]. As key regulators of both actin and microtubule cytoskeleton, the Rho GTPases have also emerged as important regulators of dendrite and spine structural plasticity [37] and appear to be a part of the initial molecular cascade required for the growth of new synapses associated with long-term memory [38]. Although the importance of Rho GTPases in neuronal morphogenesis is widely accepted, the upstream signaling components including extracellular ligands and receptors involved in

regulation Rho-mediated pathways through the lifetime of a neuron are not fully characterized.

Serotonin and Modulation of Neuronal Morphology

Five-hydroxytryptamine (5-HT or serotonin) is a neuromodulator involved in a wide range of physiological functions via activation of a large family of specific receptors. With the exception of the 5-HT_3 receptor, which is a cation channel, all other 5-HT receptors are members of the superfamily of seven transmembrane-spanning G protein-coupled receptors (GPCRs) that are coupled to multiple heterotrimeric G proteins [39].

Serotonin is involved in many aspects of neuronal development, such as neurite outgrowth, growth cone motility, and dendritic spine density, in addition to its well-established role in neuronal communication via synaptic neurotransmission [40]. For instance, depletion of 5-HT results in a reduction of dendritic length and decreased spine formation in hippocampal neurons of rats [41, 42]. Application of 5-HT increases dendritic differentiation in the rat cerebral cortex [43] and promotes neurite outgrowth from thalamic neurons [44]. Prolonged exposure of rats to a 5-HT reuptake inhibitor significantly increases dendritic spine density and the length of dendrites in the stratum radiatum [45]. Also in invertebrates, exposure to 5-HT enhances neurite growth from antennal lobe neurons in the sphinx moth [46]. Whereas these observations are consistent with the view that 5-HT acts to promote neurite outgrowth, other experiments have provided opposing results. For example, serotonin induces growth cone collapse and neurite retraction of chick dorsal root ganglion neurons [47]. Moreover, application of 5-HT to the growing neurites from the *Helisoma* neurons causes an abrupt cessation of their elongation [48]. Decreasing 5-HT levels increase distal axon outgrowth and branching in ENC1 neurons from embryonic *Helisoma*, whereas increasing 5-HT level reduces the number of neurite branch points [49].

Although several serotonin receptors, including 5-HT1A, 5-HT2A, and 5-HT2B have been proposed to be involved in modulation of such morphogenic events elicited by 5-HT [50–52], the molecular downstream mechanisms underlying such opposite effects of serotonin on neuronal morphology are still poorly understood.

Molecular Mechanisms Underlying Serotonin-Mediated Effects on Neuronal Outgrowth

We have recently shown that the 5-HT4 receptor is coupled not only to the heterotrimeric G_s, but also to the G_{13} protein [53]. We have also identified heterotrimeric G_{12} protein as an additional G protein that is activated by another member of serotonin receptors, the 5-HT7 receptor [54]. Both $G\alpha_{12}$ and $G\alpha_{13}$ proteins belong to the G_{12} family of heterotrimeric G proteins [55]. These G proteins are ubiquitously expressed and regulate a variety of cellular responses, including neoplastic transformation of fibroblasts [56, 57], activation of c-*Jun* N-terminal kinase and serum response element [58, 59], stress fiber formation [60], and neurite retraction of PC12 cells [61]. Although $G\alpha_{12}$ and $G\alpha_{13}$ proteins share a high amino acid sequence homology (67%), their functional properties are not completely overlapping. For example, $G\alpha_{13}$ knockout mice die during early embryonic development [62], whereas mice with a disrupted $G\alpha_{12}$ gene are viable and fertile [63].

The small GTPases of the Rho family are the most common effectors of $G\alpha_{12}$ and $G\alpha_{13}$ proteins. One mechanism by which G_{12} proteins communicate with Rho GTPases involves the Rho-specific guanine nucleotide exchange factor, p115RhoGEF [64–66]. $G\alpha_{12}$ and p115 communicate in bidirectional manner: On one hand, activated G_{12} proteins directly interact with p115RhoGEF and stimulate its ability to catalyze nucleotide exchange on Rho GTPases [64]. On the other hand, the regulator of G protein signaling domain of p115RhoGEF specifically stimulates the intrinsic GTPase activity of $G\alpha_{12}$ and $G\alpha_{13}$ [64, 66]. Thus, p115RhoGEF serves as an adaptor between receptor-activated G_{12} proteins and small GTPases, providing a link to extracellular signals. More recent studies show that the $G\alpha_{13}$ protein also interacts with and regulates the function of the ERM family of proteins [67]. ERM proteins are cross-linkers between the actin cytoskeleton and the plasma membrane to regulate the organization of cortical actin [68], and often operate as GDI dissociation factors [11].

Using different experimental approaches, we observed that transient expression of 5-HT4 receptor in neuroblastoma–glioma hybrid cells induces constitutive and serotonin-promoted activation of the serum response element (SRE)-mediated gene transcription through the activation of the $G\alpha_{13}$ subunit and small GTPase RhoA [53]. Moreover, the activation of 5-HT4 receptors causes RhoA-mediated reorganization of the actin cytoskeleton resulting in neurite retraction and in cell rounding (Fig. 3). It is interesting to note that the expression of the 5-HT7 receptor in mammalian cells also induces agonist-dependent activation of the SRE. However, differently to the 5-HT4R/$G\alpha_{13}$ pathway, this occurs through a G_{12}-mediated activation of the small GTPases RhoA and Cdc42 (Fig. 3). Also, the consequences of the 5-HT7/$G\alpha_{12}$ signaling for the cellular morphology are different. Agonist-dependent activation of the transiently expressed 5-HT7 receptor induces pronounced formation of filopodia in neuroblastoma cells via the Cdc42-mediated pathway, which is accompanied by RhoA-dependent cell rounding (Fig. 3).

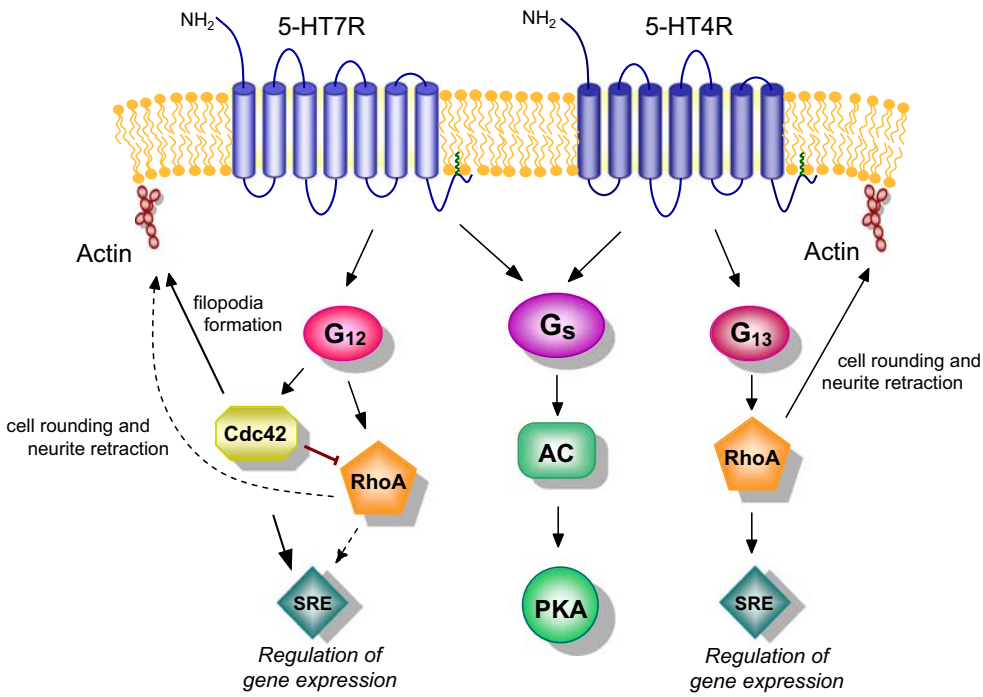

Fig. 3 Molecular model for the 5-HT4 and 5-HT7 receptor-mediated signaling. Both receptors couple to the stimulatory Gs protein leading to activation of different types of adenylyl cyclases. In addition, the 5-HT7 receptor couples to Gα12 protein resulting in activation of small GTPases of the Rho family, RhoA and Cdc42. In contrast, the 5-HT4 receptor binds to Gα13 protein leading to activation of RhoA. *Arrows* indicate activation and lines with a blunt end indicate inhibition. *AC* adenylyl cyclase, *PKA* protein kinase A, *SRE* serum response element

More importantly, activities of 5-HT7R/Gα_{12} and 5-HT4R/Gα_{13} signaling pathways in hippocampal neurons appear to be critically involved in the regulation of neuronal morphology. Treatment of hippocampal neurons with the specific 5-HT4 receptor agonist BIMU8 reduces both total length and number of neurites, whereas the 5-HT7 receptor agonist 5-CT leads to pronounced extension of neurite length [54]. We also observed a high degree of colocalization between Gα_{13} and the 5-HT4 receptor and between Gα_{12} and the 5-HT7 receptor, which may underlie a close molecular relationship for their functional effects in neurons [54]. Generally, our data demonstrate that a functionally meaningful coexistence of 5-HT7R/Gα_{12} and 5-HT4R/Gα_{13} signaling pathways in neurons may provide a molecular link between the serotonin ligand as an extracellular signal and the Rho GTPase intracellular signaling machinery, which controls neuronal morphology and motility. Our data also suggest that depending on the expression level and/or subcellular localization of the 5-HT4 and 5-HT7 receptors, serotonin may differentially regulate neuronal morphology because of the opposite effects of these receptors on neurite retraction and extension.

Another remarkable signaling pathway, which links serotonin with the small GTPases and actin network, was recently identified by Hiroshi Udo and colleagues using sensory and motor neurons from *Aplysia* as experimental model for their analysis in vivo [38]. Repeated application of 5-HT to these cell types enhances outgrowth of filopodia from presynaptic varicosities and results in formation of new synapses on sensory neuron. The authors thus propose that the application of serotonin stimulates 5-HT receptors coupled to G_q proteins. At the next downstream step, Gα_q and G$\beta\gamma$ subunits can activate the PLC and PI3 kinase pathways, which in turn upregulate the activity of Cdc42. The selective activation of Cdc42 leads to the formation of actin-based filopodia by activating downstream effector proteins, such as N-WASP and, to a lesser extent, also PAK. Presynaptic components, including synaptic vesicles, are then recruited to the newly formed filopodia in a Cdc42-dependent manner resulting in formation of new functional presynaptic varicosities. Thus, the 5-HT-induced molecular cascade appears to be a part of neuronal machinery required for the formation of synapses on sensory neurons associated with the storage of long-term memory.

Glutamate and Modulation of Neuronal Morphology by GTPases of the Rho Family

Glutamate is a major excitatory neurotransmitter in the mammalian CNS. It activates metabotropic G protein-coupled receptor and three types of ionotropic receptors, named after their agonists, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainic acid (KA). These functionally defined receptor classes are represented by distinct molecular families of receptor genes. Activation of either metabotropic or any of the ionotropic glutamate receptors may lead to functional and/or structural changes in neurons. Most information has been accumulated regarding the mechanisms of synaptic plasticity triggered by NMDA receptors. These are heteromeric channels assembled from the

NMDAR subunit NR1 and at least one type of NR2 subunit. CA1 pyramidal cells in the hippocampus of adult rodents express mostly the two NR2 subunits, NR2A and NR2B. It is believed that the different NR2 subunits confer distinct gating and pharmacological properties to NMDA receptors and couple them to distinct intracellular signaling machineries, which may shape their characteristic roles in synapse formation and synaptic plasticity. Mounting evidence indicates that long-lasting changes in synaptic efficacy, known as long-term potentiation (LTP) and long-term depression (LTD), are processes associated with redistribution of presynaptic and postsynaptic proteins and changes in presynaptic and/or postsynaptic morphology [69, 70]. LTP-inducing stimuli may cause enlargement of existing spines and formation of new spines [71, 72] and increase in the number of presynaptic boutons contacting several spines [73]. LTD-inducing events are associated with shrinkage and retraction of spines [71, 72, 74]. The signaling pathways underlying functional and structural modifications are distinct but overlapping, and involve several small GTPases.

Activation of NMDA receptors by application of glutamate in hippocampal cultures leads to a robust increase in the frequency of miniature excitatory postsynaptic currents (mEPSCs), suggesting an increase in the number of vesicles released in synapses expressing AMPA receptors. Indeed, these functional changes are accompanied by a rapid increase in (1) numbers of postsynaptic clusters of the AMPA receptor subunit GluR1, (2) clusters of the presynaptic proteins synaptophysin and synapsin I, and (3) sites where presynaptic and postsynaptic proteins colocalize [75]. Inhibitors of Rho GTPases and an inhibitor of RhoA-dependent kinase ROCK block potentiation of mEPSCs. Furthermore, recordings in pairs of synaptically coupled neurons expressing a dominant-negative form of RhoA show that both presynaptic and postsynaptic RhoA expressions is required for potentiation of synaptic responses. Inhibition of RhoA also blocks the increase in puncta of presynaptic and postsynaptic proteins and in number of sites where these coproteins localize at the onset of synaptic potentiation. These observations are consistent with the idea that potentiation involves a rapid structural modification of existing synapses or formation of new synapses, which require coordinated changes on both pre- and postsynaptic sites [76]. The contribution of Rho GTPases to increases in EPSCs and synaptic puncta during synaptic potentiation is related to Rho GTPase-mediated control over the localization of presynaptic guanylyl cyclase and cGMP-dependent protein kinase that in turn regulates cGMP levels during long-lasting potentiation.

NMDA receptor-dependent activation of Rho GTPases is mediated by specific GEFs (Fig. 4). One of them is Lfc. Under resting conditions, Rho GEF Lfc is primarily localized in the dendritic shaft through its interaction with microtubules. Upon NMDA receptor stimulation and Ca^{2+} influx, Lfc is released from microtubules and accumulates in spines through interaction with neurabin and/or spinophilin, two structurally related protein phosphatase 1-binding and actin-binding proteins. The neurabin/spinophilin/Lfc complex activates Rho through the GEF activity of Lfc, leading to stabilization of the F-actin cytoskeleton in spines and a reduction in dendritic arborizations and spine size [77]. By contrast, the Rac1 GEF Tiam1 positively influences the complexity of dendrites and elevates spine and synaptic density in vitro [78]. Importantly, Tiam1 associates with NMDA receptors and activation of NMDA receptors induces phosphorylation of Tiam1, increasing activity of

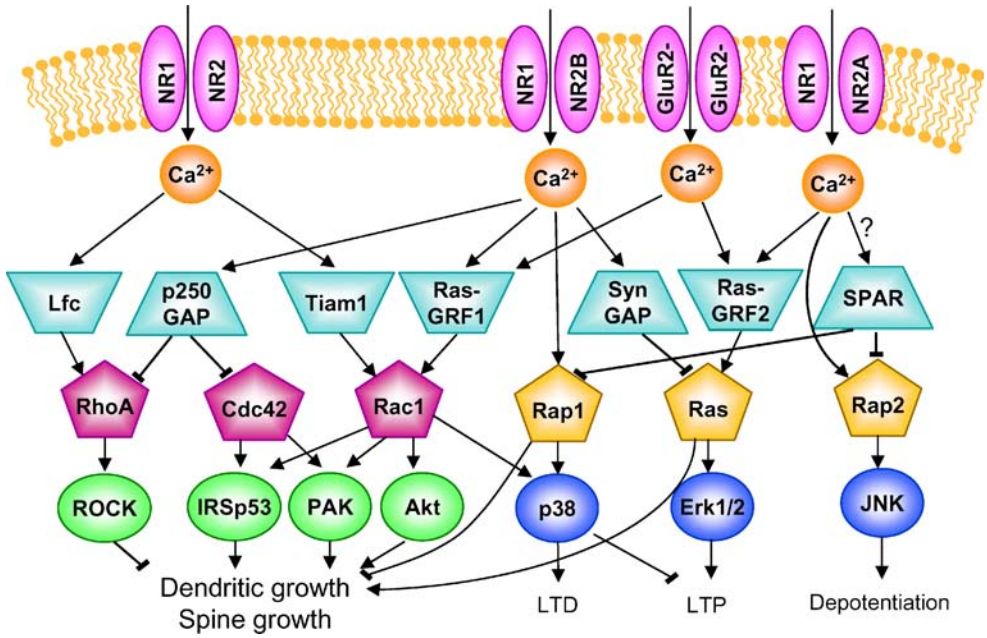

Fig. 4 Glutamate receptor signaling via small GTPases of the Rho (RhoA, Cdc42, Rac1) and Ras (Ras, Rap1, Rap2) families and their nucleotide exchange factors (Lfc, Tiam1, Ras-GRF1, Ras-GRF2), and the GTPase-activating proteins (p250GAP, SynGAP, SPAR). *Arrows* indicate direct or indirect activation, and lines with a blunt end indicate inhibition. *GluR2−* AMPA receptors lacking subunit GluR2; *IRSp53* insulin receptor substrate 53; *JNK* c-*Jun* NH2-terminal kinase; *NR1*, *NR2A* and *NR2B* subunits of NMDA receptors; *NR2*, either NR2A or NR2B; *ROCK*, Rho-associated kinase; *SPAR*, spine-associated RapGAP, *PAK* p21 activated kinases

Rac1 and protein kinase Akt (Fig. 4). Knockdown of Tiam1 expression inhibits NMDA receptor-dependent increase in spine density, suggesting that this Rac1 GEF is an important player in activity-dependent morphological changes.

The NR2B subunit of NMDA receptors colocalizes at the postsynaptic density with p250GAP, a GAP for Rho family proteins. NMDA receptor stimulation leads to dephosphorylation and redistribution of p250GAP in situ. It is important to note that p250GAP promotes GTP hydrolysis activity of Cdc42 and RhoA, but not Rac1, in vitro and in vivo. Overexpression of p250GAP in neural cells in vitro suppresses the activities of these GTPases resulting in the stimulation or inhibition of neurite outgrowth, depending on the culture conditions [79]. Which signaling cascades are activated by NMDA receptor stimulation via p250GAP downstream of Cdc42 and RhoA is not fully known. One attractive signaling candidate is the insulin receptor substrate 53 (IRSp53), which is highly expressed in the postsynaptic density and interacts with scaffolding proteins, such as shank, PSD-95, and chapsyn-110/PSD-93 [80, 81]. Overexpression of IRSp53 in cultured neurons increases the density of dendritic spines, not affecting their length or width, whereas knockdown of IRSp53 reduces the density, length, and width of spines [81].

The functional importance of small GTPases is underscored by alterations in signaling pathways involving the Rho family of small GTPases that contribute to both syndromic and nonsyndromic mental retardation disorders [37]. Although it is not known which of the molecules linked to mental retardation are regulated by neurotransmitters and their receptors, one attractive candidate is the Rho-GAP oligophrenin-1 which is not functional in a family affected with X-linked mental retardation. Oligophrenin-1 activity is required for dendritic spine morphogenesis, as knockdown of oligophrenin-1 in CA1 neurons in rat hippocampal slices decreases spine length [82]. In the context of this review, it is noteworthy that oligophrenin-1 associates with the postsynaptic adaptor protein Homer that serves as scaffolding protein for metabotropic glutamate receptors [82].

Regulation of Synaptic Plasticity by GTPases of the Ras Family

Other important players in synaptic plasticity are GTPases of the Ras family, which are under control of transmembrane recognition molecules, such as integrins and the cell adhesion molecules NCAM and L1 [83, 84], neurotrophins [85] and neurotransmitters [80, 81] (Fig. 1). The link between morphogenetically active neurotransmitter receptors and recognition molecules is interesting in view of their converging signaling pathways via Ras GTPases. They could be thus additive or neutralizing and even synergistic in these actions. Also, the functional link between neurotrophin receptors and recognition molecules adds another dimension to the way these cell surface receptors cooperate in signal transduction, leading to morphogenically finely tuned outcomes during development and at synapses in the adult.

Two seminal studies shed light on the mechanisms by which activation of NMDA receptors controls exocytocis and endocytocis of AMPA receptors via the small GTPases Ras, Rap1 and Rap2 [86, 87]. They show that Ras regulates activity-dependent synaptic delivery of AMPA receptors with long cytoplasmic tails (GluR1 and GluR2L) during long-term potentiation. Downstream effectors of Ras involves extracellular-regulated kinase 1 and 2 (Erk1/2). By contrast, Rap1 controls synaptic removal of AMPA receptors with short cytoplasmic tails (GluR2/3) during long-term depression [86]. This process involves 38 kDa mitogen-activated protein kinase (p38 MAPK). Rap2, which shares 60% identity with Rap1, regulates activity-dependent synaptic removal of AMPA receptors with long cytoplasmic tails during depotentiation, i.e., reversal of long-term potentiation. Rap2 activity depresses AMPA receptor-mediated synaptic transmission via activation of c-*Jun* NH2-terminal kinase (JNK), rather than Erk1/2 or p38 MAPK [87] (Fig. 4).

Not only the effectors involved in long-term potentiation, long-term depression and depotentiation, but also mechanisms regulating GTPase activation during these processes are distinct. Induction of potentiation and depotentiation in mature synapses appear to depend on NR2A-containing NMDA receptors, whereas NR2B-containing receptors are required for long-term depression [87–89]. The NR2A containing receptors are coupled to Ras via Ca^{2+}/calmodulin sensitive Ras-guanine nucleotide-releasing factor protein Ras-GRF2 [90]. The NR2B-containing NMDA receptors are coupled to Ras-GRF1 and an excitatory synapse-specific Ras GAP, SynGAP [90–92]. Ras-GRF1 mediates signaling to the Rac1 effector p38 MAPK. In addition to Ras, the NMDA receptor containing subunit NR2A activates Rap2 [87]. The underlying signaling mechanisms may involve spine-associated RapGAP (SPAR), which is known to inhibit activities of Rap1 and particularly Rap2 and to form a complex with NMDA receptors and PSD-95 [93].

Although several studies highlight the importance of NR2B-containing NMDA receptors for synaptic depression, the outcome of NR2B NMDA receptor activation may vary. Switching from synaptic NR2B-containing NMDA receptors that bind CaMKII with high affinity to those containing NR2A that bind CaMKII with low affinity, dramatically reduces long-term potentiation [94]. Also, age is an important factor in consideration of NMDA receptor activity. During early development, NR2B containing NMDA receptors are expressed more strongly than NR2A containing receptors and are located at synapses. Expres-

sion of NR2A increases in more mature neurons. These then apparently displace NR2B receptors in synapses. It is noteworthy that extrasynaptic and synaptic receptors play opposite roles in the regulation of neuronal survival, synaptic strength, and Erk1/2 activity [95–97]. It is therefore plausible to assume that synaptically located NR2B-containing NMDA receptors may promote strengthening of synaptic activity, whereas extrasynaptic NR2B-containing NMDA receptors may induce weakening of synapses via the signaling pathways outlined above.

Another emerging mechanism of glutamate-mediated signaling involves activation of Ras by Ca^{2+} influx via Ca^{2+} permeable AMPA receptors [98]. Activation of Ras and Erk in mature neurons occurs through the Ca^{2+}/calmodulin regulated Ras-GRF1 and Ras-GRF2 exchange factors, which form AMPA-induced complexes with Ca^{2+} permeable AMPA receptors (Fig. 4). As Ca^{2+} permeable AMPA receptors are highly expressed in interneurons, this signaling pathway may be critical for plasticity of excitatory synapses impinging on interneurons.

Ras and Rap signaling appears to regulate not only recycling of AMPA receptors but also spine morphogenesis. Transgenic mice expressing active Ras in postmitotic neurons show increased spine density and number of synapses in cortical pyramidal neurons [99, 100]. Neurons deficient in the major Ras inhibitor SynGAP show accelerated spine development and larger spines than those of wild-type mice [101]. By contrast, degradation of the Rap inhibitor SPAR induced by phosphorylation via serum-inducible kinases leads to loss of mature dendritic spines [102]. Overexpression of SPAR causes enlargement of spine heads [93]. Thus, Ras and Rap GTPases act in opposite directions regulating the synaptic strength and morphology of spines. However, it remains to be investigated which of the morphogenic effects are direct and which are indirect, being induced by changes in synaptic activity as a consequence of Ras and Rap activation.

In conclusion, similar mechanisms appear to be involved in morphogenic signaling via glutamate and 5-HT receptors, in the sense that both neurotransmitters may induce bidirectional structural changes in neurons via activation of different sets of small GTPases.

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