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Rho Signaling in Synaptic Plasticity, Memory, and Brain Disorders

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Memory impairments are associated with many brain disorders such as autism, Alzheimer's disease, and depression. Forming memories involves modifications of synaptic transmission and spine morphology. The Rho family small GTPases are key regulators of synaptic plasticity by affecting various downstream molecules to remodel the actin cytoskeleton. In this paper, we will review recent studies on the roles of Rho proteins in the regulation of hippocampal long-term potentiation (LTP) and long-term depression (LTD), the most extensively studied forms of synaptic plasticity widely regarded as cellular mechanisms for learning and memory. We will also discuss the involvement of Rho signaling in spine morphology, the structural basis of synaptic plasticity and memory formation. Finally, we will review the association between brain disorders and abnormalities of Rho function. It is expected that studying Rho signaling at the synapse will contribute to the understanding of how memory is formed and disrupted in diseases.

Keywords: Rho GTPases, long-term potentiation, dendritic spine, long-term depression, brain disorders

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INTRODUCTION

The synapse is a highly specialized structure connecting two cells and it serves as the main site where neurons communicate and transmit signals between each other. Synaptic connections allow the formation and function of neuronal circuits that underlie our emotions, behaviors and memories. Synaptic structure was firstly described by Gray (1959), and it consists of vesicle-bearing pre-synaptic terminals that originate from axons and the post-synaptic component found on the cell body, dendrites or a dendritic spines. Many synaptic proteins such as neuroligins and neurexins were identified to maintain the connectivity and precise alignment of pre- and post-synaptic membrane that shapes the amplitude and reliability of neurotransmission (Missler et al., 2012; Tang et al., 2016; Hass et al., 2018). In the mammalian central nervous system, dendritic spines are the primary sites of excitatory inputs (Bourne and Harris, 2008). Morphological changes of dendritic spines in the pre-existing synapses, as well as the *de novo* growth or retraction of dendritic spines, are closely linked to the functional and structural plasticity of the synapse, which is widely believed to be the basis of memory and cognition (Yuste and Bonhoeffer, 2001; Yang et al., 2008; Holtmaat and Svoboda, 2009; Kasai et al., 2010; Chidambaram et al., 2019).

Dendritic spines are small protrusions that emerge on dendritic processes, and they occur at a density of 1–10 spines/μm (Sorra and Harris, 2000). Dendritic spines are heterogenous in size and shape and can be classified into several categories based on their morphological differences. Typically, a dendritic spine consists of three basic compartments: (1) a delta-shaped base which contains branched actin filaments residing on the microtubule network in the dendrites, (2) a constricted neck in the middle which contains branched and linear longitudinal actin filaments, and (3) a bulbous head contacting the axon, which usually contains a dense

network of short cross-linked branched actin filament (Hotulainen and Hoogenraad, 2010). Based on the size of spine heads and the length of spine necks, dendritic spines can be subdivided into mushroom, thin, branched, and stubby spines (Peters and Kaiserman-Abramof, 1970; Harris et al., 1992). A mushroom spine contains a narrow neck with a large head. Since the synaptic connections formed on the mushroom spine can usually last for a long time, it is also considered to be a mature "memory spine," where the long-term memory is stored (Bourne and Harris, 2007). Thin spines have a similar structure as mushroom spines, except that the spine head is smaller. Thin spines are more dynamic in their structure, having the capacity to transform into mushroom spines and therefore, are considered to be "learning spines" (Bourne and Harris, 2007). Stubby spines typically have an indiscernible neck and mainly exist during the early stage of postnatal development (Fiala et al., 1998). Branched or cup-shaped spines have multiple heads that originate from a single neck. It is important to note that this classification of spines may underrate the morphological heterogeneity of spines because there is a continuum of spine sizes and shapes that are also highly dynamic (Fiala et al., 1998; Parnass et al., 2000; Alvarez and Sabatini, 2007; Pchitskaya and Bezprozvanny, 2020).

A complex actin network that is associated with various actin regulators, including the Arp2/3 complex and other actin-binding proteins, constitutes the main component of spine cytoskeletal architecture, and therefore, the formation, maturation and plasticity of the spine are highly dependent on the remodeling of the actin cytoskeleton (Cingolani and Goda, 2008; Hotulainen and Hoogenraad, 2010; Korobova and Svitkina, 2010). The Rho family small GTPases are the central mediators of actin reorganization in many cell types, including neurons. They are molecular switches cycling between the active GTP-bound and inactive GDP-bound form, and their activities are tightly regulated by guanine nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs) (Govek et al., 2005; Vega and Ridley, 2007). Among Rho family members, RhoA, Rac1, and Cdc42 are the most extensively studied in the brain and are the focus of this review. RhoA, Rac1, and Cdc42 were initially found to be required in the growth factor-induced formation of focal adhesions and actin stress fibers (contractile actin and myosin filaments), membrane ruffles/lamellipodia (meshwork of newly polymerized actin filaments), and filopodia (actin-rich and finger-like membrane extension structures) in fibroblast cells (Ridley et al., 1992; Ridley and Hall, 1992; Nobes and Hall, 1995). Subsequent studies have shown that these Rho GTPases are crucial in the regulation of various aspects of cell morphology such as cell polarity and shape as well as several cellular processes such as exocytosis, endocytosis and proliferation, in a wide variety of mammalian cell types, including neurons (Etienne-Manneville and Hall, 2002; Govek et al., 2005).

RHO GTPases IN SPINE FORMATION

Ample studies indicate that altering the activity of Rho GTPases can affect spinogenesis in developing neurons (Tashiro et al., 2000; Scott et al., 2003; Tashiro and Yuste, 2004; Zhang and Macara, 2006; Kang et al., 2008; Impey et al., 2010; Um et al., 2014; Orefice et al., 2016; Valdez et al., 2016; Moutin et al., 2017; **Figure 1**). Overaction of Rho GTPases in cultured primary neurons by cytotoxic necrotizing factor 1 (CNF-1) treatment resulted in enrichment in the actin cytoskeleton and dendritic processes (Diana et al., 2007). Suppression of Rac1 activity *in vitro* through the manipulations of dominant negative Rac1, the Rac1-GAP breakpoint cluster region protein (Bcr), or the Rac-GEF beta p21-activated protein kinase exchange factor (betaPIX), in cultured hippocampal neurons, decreased dendritic spine density (Nakayama et al., 2000; Penzes et al., 2003; Tashiro and Yuste, 2004; Zhang and Macara, 2006; Impey et al., 2010; Fiuza et al., 2013; Um et al., 2014). Genetic knockout (KO) of Rac1 in excitatory neurons in mice resulted in decreased spine and synapse numbers accompanied by increased postsynaptic density (PSD) (Haditsch et al., 2009). In contrast, elevating Rac1 activity *in vitro* through constitutively active Rac1 in cultured hippocampal slices leads to increased spine density but reduced spine size (Nakayama et al., 2000; Tashiro et al., 2000). In CA1 pyramidal neurons of Bcr KO mice, the spine density was higher compared to WT mice (Um et al., 2014). Similar to Rac1, conditional KO of Cdc42 in the hippocampus also resulted in decreased spine density and impaired spine size enlargement in response to glutamate uncaging (Kim et al., 2014). The expression of constitutively active Cdc42 increased spine density in cultured hippocampal neurons (Kang et al., 2008) and rescued the spine density deficit in LgDel mice, a model for 22q11.2 deletion syndrome with altered Cdc42 signaling (Moutin et al., 2017). The expression of loss-of-function Cdc42 mutants in *Drosophila* vertical system neurons led to similar deficits in spine density observed in mammals (Scott et al., 2003). In contrast to the effects of Rac1 and Cdc42, the expression of constitutively active RhoA in hippocampal neurons or slice cultures consistently resulted in simplified dendritic trees and reduced spine density (Nakayama et al., 2000; Tashiro et al., 2000; Impey et al., 2010). Inhibition of RhoA with C3 transferase, dominant negative RhoA, shRNA knockdown or other strategies, all resulted in increased spine density and impaired activity-dependent spine pruning (Tashiro et al., 2000; Kang et al., 2009; Impey et al., 2010; Orefice et al., 2016). It is important to note that the increased spines caused by RhoA inhibition or Rac1 activation appeared to be immature spines with filopodia-like or lamellipodia-like morphology (Nakayama et al., 2000; Tashiro et al., 2000). These results suggest that Rac1 and Cdc42 are involved in the induction and maintenance of spines, whereas RhoA plays a role in the elimination or pruning of immature spines. Therefore, the balance of Rac1/Cdc42 and RhoA activity may be critical for establishing and maintaining the homeostasis of dendritic spine density in the brain.

RHO GTPases IN SPINE MORPHOLOGY

Rho GTPases are critically involved in the regulation of spine morphology. For example, while spine density was found to decrease by blocking Rac1 activity, reduced spine head was also observed (Tashiro and Yuste, 2004), suggesting that Rac1 is

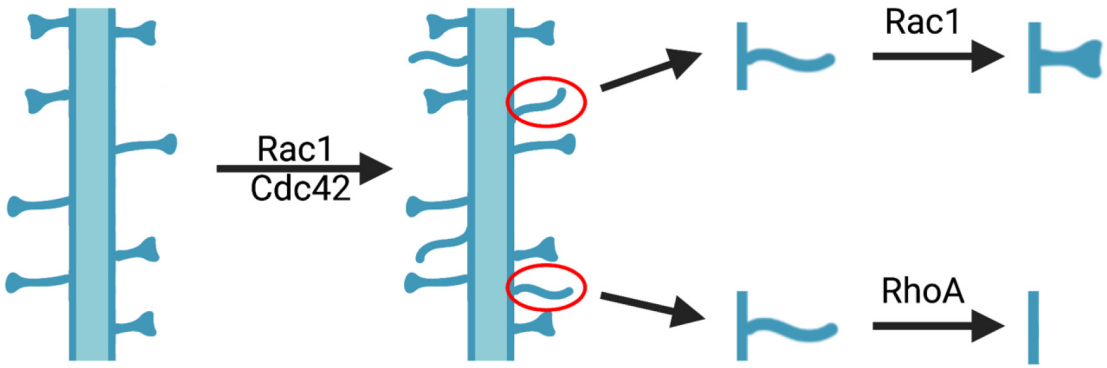

FIGURE 1 | Role of Rho GTPases in modulating dendritic spine density and morphology. The activation of Rac1 and Cdc42 leads to increased immature spines, some of which will undergo morphological changes to obtain mature morphology through Rac1-dependent mechanisms, whereas others will be eliminated by RhoA-dependent processes.

essential for the enlargement or maturation of spines. The role of Rac1 in spine morphology was also shown in many other studies by increasing Rac1 activity via knockdown of upstream negative regulators (e.g., Bcr, alpha-chimaerin and p250GAP), expression of constitutively active Rac1, or neurotrophic factor BDNF treatment (Tashiro and Yuste, 2004; Wiens et al., 2005; Impey et al., 2010; Um et al., 2014; Orefice et al., 2016; Valdez et al., 2016). Interestingly, as mentioned earlier, Rac1 activation has also been shown to decrease spine size during early development, suggesting that the effect of Rac1 is age -dependent. In contrast to Rac1, the effect of RhoA on spine size is less clear (Tashiro and Yuste, 2004; Impey et al., 2010; Orefice et al., 2016).

Other than affecting basal spine morphology, Rho GTPases also participate in the activity-dependent remodeling of spine morphology. For example, during structural LTP (sLTP) induced by the glutamate uncaging at single spines of cultured hippocampal neurons, RhoA and Cdc42 activity was transiently increased and then decayed to a slight but stable elevation, coinciding with spine volume enlargement during sLTP (Murakoshi et al., 2011). Inhibition of RhoA activity via shRNA or C3 transferase, decreased both transient and sustained spine enlargement, while the inhibition of Cdc42 only impaired the sustained spine enlargement (Murakoshi et al., 2011). In this study, the investigators created novel sensors to measure RhoA and Cdc42 activity using the fluorescence resonance energy transfer (FRET) technique, allowing live imaging of the activity of these GTPases in live neurons. In a later study, similar sensors were made to image Rac1 activity during sLTP in hippocampal neurons, and it was found that Rac1 was also required for the transient and sustained spine enlargement (Hedrick et al., 2016). Interestingly, it was found that the active Rac1, Cdc42, and RhoA not only affected the morphological plasticity of activated spines, but also could diffuse to nearby spines and lower the threshold of sLTP induction in these spines, a phenomenon typically referred to as heterosynaptic plasticity or synaptic crosstalk. To explore the mechanism behind this crosstalk, the researchers examined the diffusivity of Rho GTPases during sLTP and showed that RhoA and Rac1 had a higher diffusion distance than Cdc42, that is, the active RhoA, and Rac1 diffused to the nearby spines whereas the active Cdc42 remained locally in the activated spine. Preventing the diffusion of active RhoA and Rac1 by an inhibitor protein that was fused with the microtubule binding domain of microtubule-associated protein 2 (MAP2) abolished the effect on heterosynaptic plasticity (Hedrick et al., 2016). Therefore, although Rac1, Cdc42, and RhoA all are involved in spine plasticity, they play differential roles, with spine-specific Cdc42 plus RhoA and Rac1 being all important for spine-specific homosynaptic sLTP, and the diffusion of the active RhoA and Rac1 being important for heterosynaptic sLTP.

RHO GTPases IN SYNAPTIC PLASTICITY

In addition to affecting spine properties, many studies have shown that Rho GTPases are potent regulators of synaptic transmission and plasticity (**Figure 2**). Long-term potentiation (LTP) and long-term depression (LTD) are the two most extensively studied forms of long-lasting synaptic plasticity considered to be the basis for learning and memory (Bliss and Collingridge, 1993; Malenka and Bear, 2004; Citri and Malenka, 2008; Collingridge et al., 2010; Kandel et al., 2014). In the CA1 region of the hippocampus, the development of LTP and LTD depends on glutamatergic receptors in the postsynaptic density: α-amino-3-hydroxy-5-methyl-4-isoxazole-proponic acid receptors (AMPARs), and N-methyl-D-aspartate receptors (NMDARs) (Bliss and Collingridge, 1993; Kennedy, 2000; Bredt and Nicoll, 2003; Malenka and Bear, 2004; Huganir and Nicoll, 2013). Under resting membrane potential, AMPARs mediate the majority of synaptic transmission because NMDARs are inhibited by a voltage-dependent extracellular magnesium blockade. However, during intense neuronal activities and learning experience, activation of AMPARs causes sufficient postsynaptic depolarization that leads to the release of magnesium blockade of NMDARs and the influx of calcium through these receptors. Depending on the concentration and kinetics of calcium in the neurons, it can initiate two distinct signaling pathways via low-calcium-affinity kinase, CaMKII, or high-calcium-affinity phosphatase, calcineurin (Malenka and Bear, 2004;

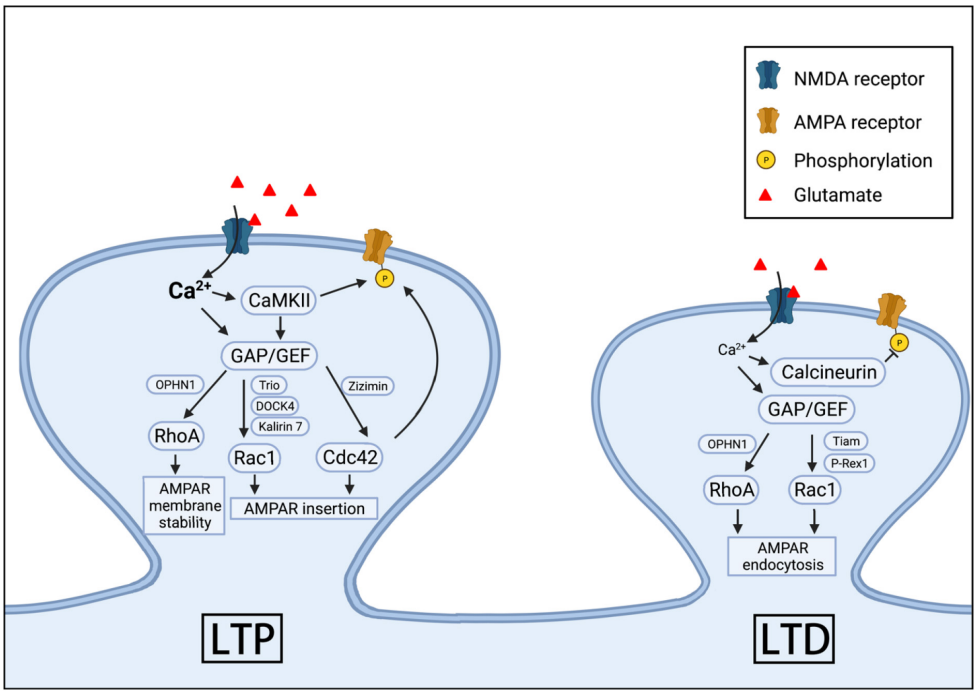

FIGURE 2 | Rho GTPases in AMPAR expression, LTP and LTD. RhoA, Cdc42, and Rac1 can participate in the regulation of the synaptic insertion, internalization and membrane stability of AMPARs during LTP and LTD. These GPTases are activated by NMDARs or other surface proteins through various GAPs and GEFs.

Collingridge et al., 2010; Huganir and Nicoll, 2013). These signaling molecules can then modulate many downstream effectors through various mechanisms to alter synaptic strength. For example, AMPAR can be directly phosphorylated by CaMKII to increase the channel conductance of AMPARs, thus potentiating synaptic transmission (Derkach et al., 1999; Kristensen et al., 2011; Huganir and Nicoll, 2013; Diering and Huganir, 2018). The regulation of AMPAR numbers at the postsynaptic membrane represents another key mechanism to modify synaptic strength during LTP and LTD. While the number of AMPARs at the synapse is maintained through constitutive recycling of the receptors for the stable basal synaptic transmission, the number can be drastically altered by activity-dependent endocytosis and exocytosis during LTD and LTP, respectively (Malinow and Malenka, 2002; Collingridge et al., 2004; Derkach et al., 2007; Anggono and Huganir, 2012). Rho GTPases may affect AMPARs at the postsynaptic membrane in several ways, including anchoring, clustering and trafficking of these receptors (Allison et al., 1998; Matsuzaki et al., 2001; Zhou et al., 2001; Wiens et al., 2005; Derkach et al., 2007; Zhou et al., 2011). For example, TC10, a member of Rho GTPases, was found to be involved in ADP-ribosylation factor 6 (Arf6)-mediated, clathrin-independent AMPAR constitutive translocation (Zheng et al., 2015). In this study, it was shown that TC10 existed in the Arf6-containing endosomes, and the expression of a dominant negative TC10 in the hippocampal neuron culture resulted in decreased surface/total AMPAR ratio, whereas constitutively active TC10 increased the ratio (Zheng et al., 2015). Cdc42 can also modulate AMPARs on the postsynaptic membrane. It was shown that Cdc42 participated in a signaling pathway that phosphorylates the AMPAR subunit, GluA1, at a novel phosphorylation site S863, which then facilitates the AMPAR expression on the postsynaptic surface (Hussain et al., 2015). In another study, it was found that LTP induction triggered cholesterol redistribution in the intracellular membrane, which was associated with the postsynaptic insertion of AMPARs and the activation of Cdc42 (Brachet et al., 2015). Cdc42 was required for the increased synaptic transmission induced by the cholesterol removal, and the expression of dominant negative Cdc42 abolished the increased AMPAR currents (Brachet et al., 2015). The effects of Rac1 on AMPAR trafficking appear to be complex. For example, in the hippocampal neurons of microtubule associated protein 1B (MAP1B) KO mice, endocytosis of AMPARs during LTD was impaired and this impairment was rescued by the overexpression of Rac1 (Benoist et al., 2013). Since MAP1B was known to facilitate the translocation of the Rac1-GEF protein, T-cell lymphoma invasion and metastasis protein 1 (Tiam1), to the synapses, the impaired endocytosis in MAP1B KO mice could be due to decreased Tiam1 expression at the synapse and hence reduced Rac1 activity. The role of Rac1 in AMPAR endocytosis during LTD is also supported by results from KO mice lacking the Rac1-GEF protein, phosphatidylinositol 3, 4, 5-trisphosphate-dependent Rac exchanger 1 (P-Rex1). It was reported that in hippocampal neurons of P-Rex1 KO mice, NMDA-induced reduction in surface AMPARs was impaired and overexpression of Rac1 rescued this impairment (Li et al., 2015). Furthermore, the pharmacological inhibition of Rac1 via NSC23766 in cultured

hippocampal neurons increased AMPARs in the postsynaptic surface and decreased AMPARs in the cytoplasm (Glebov et al., 2015). In terms of LTP and AMPAR insertion, several studies have documented the essential role of Rac1 signaling through investigations of several Rac1-GEFs [e.g., Kalirin-7, dedicator of cytokinesis protein 4 (DOCK4), and triple functional domain protein (Trio)] where increased AMPAR expression at the synapse was shown with increased Rac1 activation (Xie et al., 2007; Sadybekov et al., 2017; Guo et al., 2021). Like Rac1 and Cdc42, RhoA also plays a role in the regulation of AMPAR trafficking. The activation of RhoA via knocking down its negative regulator was reported to be associated with decreased surface AMPARs (Shen et al., 2020), and impaired LTP was observed in the RhoA-GAP, oligophrenin-1 (OPHN1) KO mice (Kasri et al., 2009). The loss of OPHN1 also impaired activity-dependent AMPAR endocytosis, which was reversed by pharmacological inhibition of RhoA-ROCK signaling (Khelfaoui et al., 2009).

The results obtained from electrophysiological studies support the roles of Rho GTPases in synaptic transmission and plasticity. For example, increased Rac1 activity, either through KO/knockdown of Rac1 upstream GAP proteins such as alpha-chimaerin and BCR/ABR, or the expression of constitutively active Rac1 mutants, led to unstable theta-burst stimulation (TBS)-induced LTP that gradually decayed to the baseline response at the CA1 synapse (Oh et al., 2010; Liu et al., 2016, 2018; Lv et al., 2019). On the other hand, decreased Rac1 activity, either through the expression of dominant negative Rac1 mutants or the upstream GAP protein alpha-chimaerin, or through genetic deletion of MAP1B, resulted in more stable LTP (Glebov et al., 2015; Liu et al., 2016, 2018; Lv et al., 2019). One study, however, did observe impaired hippocampal LTP in Rac1 KO mice (Haditsch et al., 2009) although in this study, high-frequency stimulation (HFS) rather than TBS was used to induce LTP. LTD is also affected by manipulations of Rac1 activity. In MAP1B KO mice, low-frequency stimulation (LFS)-induced LTD was impaired (Benoist et al., 2013), suggesting that Rac1 is necessary for LTD. However, increased Rac1 activity by knocking out/down Rac1 GAP proteins (e.g., alpha-chimaerin and BCR/ABR) or overexpressing Rac1 had no effects on LFS-induced LTD (Oh et al., 2010; Benoist et al., 2013; Valdez et al., 2016), although in KO mice lacking kinesin family member 21B (Kif21B), a microtubule-dependent molecular motor that regulates the engulfment of the Rac1-GEF, ELMO1/DOCK complex and enhances Rac1 activity, was found to have unstable LFS-induced LTD without alterations in HFS-induced LTP at CA1 synapses (Morikawa et al., 2018). Although no clear role has emerged for Cdc42 in LTD, its involvement appeared to be critical for LTP, since HFS-induced LTP was absent in the hippocampus of Cdc42 KO mice (Kim et al., 2014). The role of RhoA in LTP was shown in hippocampal slices using RhoA shRNA or ROCK inhibitors, which affected the maintenance, but not induction, of TBS-induced LTP (Rex et al., 2009; Briz et al., 2015). Although the expression of RhoA dominant negative mutants had no effect on LFS-induced LTD (Benoist et al., 2013), overactivation of RhoA in OPHN1 KO mice impaired LTD (Khelfaoui et al., 2009), suggesting a role for RhoA in LTD.

In summary, despite that Rac1, Cdc42, and Rac1 are all involved in both insertion and internalization of AMPARs at the synapses, they appear to play distinct roles in LTP and LTD (**Figure 2**). While Cdc42 is required for LTP induction, RhoA is more important for LTP maintenance. Rac1 participates in destabilizing LTP and is also required for LTD expression. Clearly more studies are necessary to link specific roles of Rho GTPases in LTP and LTD with AMPAR trafficking and spine plasticity.

RHO SIGNALING PATHWAYS

As discussed earlier, the activity of Rho GTPases is tightly regulated by various GEF and GAP proteins (Govek et al., 2005). Many GEFs and GAPs have been identified and most of these proteins are expressed in the brain with unique spatial and temporal expression patterns (Moon and Zheng, 2003; Rossman et al., 2005). These GEFs and GAPs relay signals from various surface receptors, including NMDARs. Since each Rho GTPase can be modulated by multiple GEFs and GAPs, the spatial pattern of GAPs and GEFs determines the diversity and specificity of signals received by Rho GTPases. The importance of these GEFs and GAPs in the brain is evident from alterations in single GEF or GAP protein that can affect neuronal structure and function. For example, GEF-H1, a GEF for RhoA, negatively affects spine density and spine length of hippocampal neurons (Xie et al., 2007). Deletion of Kalirin-7 in mice resulted in schizophrenia-like phenotypes (Cahill et al., 2009). Changes in GAPs such as OPHN1 and SYNGAP1 were also found to result in cognitive deficits and autistic-like phenotypes that were accompanied by disrupted spine structure and function in both human and animal models (Newey et al., 2005; Kasri et al., 2009; Clement et al., 2012; Berryer et al., 2013; Ba and Nadif Kasri, 2017).

Downstream of Rho-GTPases are a chain of effector proteins that are involved in the regulation of the actin cytoskeleton (**Figure 3**). P21-activated kinase (PAKs) and the Rho-associated coiled-coil kinase (ROCK) are the two well-studied families of protein kinases activated by Rac1/Cdc42 and RhoA, respectively (Edwards et al., 1999; Maekawa et al., 1999). Both PAKs and ROCKs are potent regulators of spine morphogenesis and synaptic plasticity. For example, KO mice for PAK1/3 and ROCK2 displayed altered spine morphology, impaired LTP and LTD (Meng et al., 2005; Asrar et al., 2009; Zhou et al., 2009; Huang et al., 2011). PAK2 heterozygous mice exhibited reduced spine density and impaired LTP (Wang et al., 2018). Further downstream of ROCKs and PAKs are LIM-domain kinases (LIMKs), which directly phosphorylate and inhibit the activity of the actin depolymerization factor cofilin (Arber et al., 1998; Meng et al., 2002, 2003, 2004; Ben Zablah et al., 2020). Cofilin is an actin-binding protein that primarily functions to sever and depolymerize actin filaments (Bamburg, 1999; Andrianantoandro and Pollard, 2006; Bamburg and Bernstein, 2008; Ben Zablah et al., 2020). Similar to PAK1/3 and ROCK2 KO mice, LIMK1 KO mice displayed reduced spine size and impaired LTP (Meng et al., 2002, 2004). PAKs and ROCKs can also regulate the activity of cofilin through phosphorylating and inactivating the cofilin phosphatase, Slingshot homolog 1

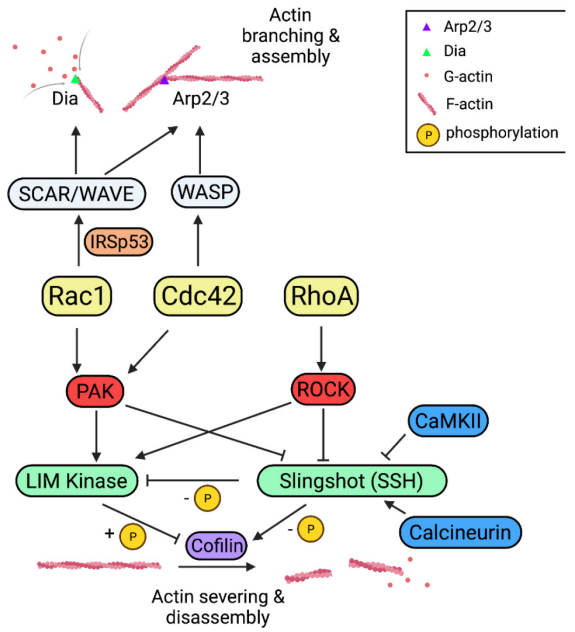

FIGURE 3 | Rho GTPase signaling pathways in actin dynamics and synaptic function. Rac1, Cdc42, and RhoA regulate actin dynamics either through cofilin or Arp2/3 complex pathways. Rac1/Cdc42 and RhoA activate PAKs and ROCKs, respectively, which phosphorylate and activate cofilin kinase LIMKs and inhibit cofilin phosphatase SSH, both leading to inactivation of cofilin thus reduced actin disassembly. Rac1 and Cdc42 promote actin assembly through the Arp2/3 complex and the formin-related protein (Dia) via WAVE and WASP, respectively.

(SSH1), which dephosphorylates and enhances cofilin function (Niwa et al., 2002; Soosairajah et al., 2005; Quassollo et al., 2015). SSH1 can directly interact with, dephosphorylate and inhibit LIMK1 (Soosairajah et al., 2005). Therefore, cofilin is an important converging point by which Rho GTPase regulate actin dynamics. Consistent with this, cofilin KO mice showed altered spine morphology and synaptic plasticity (Rust et al., 2010; Rust, 2015). In addition to affecting cofilin, both Rac1 and Cdc42 can control actin polymerization by regulating the Arp2/3 complex. The Arp2/3 complex is an actin nucleation factor to mediate the formation of branched actin network (Goley and Welch, 2006), which is thought to be essential for spine remodeling during spine growth (Hotulainen et al., 2009). The recruitment of the Arp2/3 complex to the actin filament is through the association with Wiskott-Aldrich syndrome protein (WASP) and WASP-family verprolin-homologous protein (WAVE) (Takenawa and Suetsugu, 2007). The neuronal (N)-WASP is activated by interaction with Cdc42, which relieves N-WASP from its intramolecular autoinhibition (Kim et al., 2000). Downregulation of N-WASP or deletion of its Arp2/3 binding region in hippocampal neurons resulted in decreased spine and synapse density (Wegner et al., 2008). Similar to WASP, loss of WAVE protein or manipulations of its phosphorylation status by cyclin-dependent kinase 5 (Cdk5) also caused deficits in spine formation and maintenance in mice (Soderling et al., 2003, 2007; Kim et al., 2006). WAVE is functionally similar to N-WASP, except that it lacks the binding domain for Rho-GTPases (Miki et al., 1998). Unlike Cdc42, Rac1 binds and activates WAVE through an adaptor protein, IRSp53, a substrate for the insulin receptor. The binding of WAVE protein to the SH3 domain of IRSp53 leads to the release of intramolecular inhibition of IRSp53 (Miki et al., 2000; Miki and Takenawa, 2002), and this results in the formation of a trimeric complex. Although Rac1/WAVE and Cdc42/N-WASP pathways converge on the Arp2/3 complex to modulate actin dynamics, they may play distinct roles in regulating memory. In a recent study on active forgetting of cold shock-sensitive or anesthesia-sensitive memory (ASM) and cold shock-insensitive or anesthesia-resistant memory (ARM) in *Drosophila*, it was found that the Arp2/3 complex was only required for Cdc42/WASP mediated ARM forgetting but not for Rac1/WAVE mediated ASM forgetting, and instead, Rac1/WAVE might function through another protein called Dia (Gao et al., 2019).

RHO GTPases IN MEMORY

Ample studies have indicated that Rho GTPases are involved in learning and memory. O'Kane et al. (2003) first reported that stimulation of the hippocampal CA1 region resulted in a noticeable increase in the activity of Rho GTPases. In a subsequent study using the protein toxin CNF-1 to increase the activity of Rho proteins, it was found that CNF-1 treated mice had an elevated level of actin filaments and enhanced freezing during a fear memory test and better performance in the Morris water maze test (Diana et al., 2007). However, this observed memory improvement may not be entirely attributable to the increased Rho GTPase activity because CNF-1 is not specific to the activation of the Rho proteins. For example, CNF-1 was reported to induce inflammatory responses and produce chemokines such as interleukin 8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein 3 (MIP-3) (Munro et al., 2004). Similar to the effects on synaptic plasticity, different Rho GTPases play differential roles in memory. For example, RhoA signaling has been shown to be required for memory formation. Perturbations of the RhoA-ROCK signaling by intracerebral infusion of the ROCK inhibitor, Y27632, impaired conditioned place aversion (CPA) memory in rats, whereas the infusion of the Rac1 inhibitor, NSC23766, had no effect (Wang et al., 2013). Similar results were obtained in a conditioned place preference (CPP) memory test (Fakira et al., 2016) where it was found that RhoA signaling cascade was enhanced during morphine-induced CPP and that bilateral intracranial infusion of the ROCK inhibitor, H1152, completely prevented the formation of CPP. The suppression of RhoA activity through manipulations of p27 protein in cyclin-dependent kinase (CK) KO mice was also reported to be associated with deficits in hippocampus-dependent learning and memory (Kukalev et al., 2017). Although increased RhoA activity was shown to be crucial for synaptic plasticity and memory formation in adult animals, its role in early development seems to be different. It was reported that the exposure to the anesthetic drug, sevoflurane, in the early development could lead

to persistent impairment of learning and memory in rodents (Lee et al., 2014), and that this effect was related to the activation of RhoA signaling pathway as the sevoflurane induced shortening of the dendritic protrusion length was blocked by the treatment of the ROCK inhibitor, Y27632 (Zimering et al., 2016). Bisphenol A (BPA), a chemical substance shown to impair the growth and development of the nervous system, was also reported to elevate the RhoA activity in hippocampal neurons and this was accompanied by decreased spike amplitudes and synaptic strength (Wang et al., 2020). Cdc42 is also involved in memory. For example, although neuron-specific Cdc42 conditional KO mice did not show alterations in short-term or long-term memory, they exhibited impairments in remote memory in both fear and spatial memory tests (Kim et al., 2014). In mouse nucleus accumbens (NAc), Cdc42 was activated by methamphetamine (METH) and viral expression of dominant negative and constitutively active Cdc42 impaired and enhanced METH-induced CPP memory, respectively (Tu et al., 2019). In *Drosophila* olfactory memory mediated by the mushroom bodies (MBs), ASM, and ARM were affected by different Rho GTPases (Shuai et al., 2010; Zhang et al., 2016; Gao et al., 2019). Expression of dominant negative Cdc42 in the MBs caused slower decay of ARM, whereas expression of constitutively active Cdc42 led to faster ARM decay (Zhang et al., 2016). The role of Rac1 in memory has been studied more extensively compared to Rho A and Cdc42. In Rac1 conditional KO mice where Rac1 was deleted in mature neurons, working memory in the delayed matching-to-place (DMP) water maze test was impaired (Haditsch et al., 2009), implicating Rac1 in memory acquisition but not in memory consolidation. However, subsequent studies using conditional expression of mutant Rac1 suggested that Rac1 is associated with memory forgetting instead of formation. This effect of Rac1 was initially demonstrated in *Drosophila* (Shuai et al., 2010) where controlled expression of dominant negative and constitutively active Rac1 mutants in the MBs using the Gal/UAS binary system delayed and facilitated aversive olfactory memory decay, respectively, without affecting memory acquisition (Shuai et al., 2010). Other than the passive memory decay, Rac1 was also investigated in the active memory forgetting using retroactive interference (RI) and reversal learning and shown to have similar effects as in the passive memory decay (Shuai et al., 2010). The effects of Rac1 in *Drosophila* memory forgetting are mediated by scribble scaffold protein (Cervantes-Sandoval et al., 2016). In mice, expression of constitutively active Rac1 hastened the memory forgetting in a novel object recognition (NOR) test whereas the expression of dominant negative Rac1 delayed forgetting (Liu et al., 2016). A similar role of Rac1 was shown in social memory, where increased Rac1 activity in the hippocampus by expressing constitutively active Rac1 accelerated the decay of social memory whereas inhibition of Rac1 activity delayed the social memory decay (Liu et al., 2018). In addition, it was found that Rac1 activity was elevated by social isolation and that socially isolated mice displayed faster social memory decay (Liu et al., 2018). Manipulations of Rac1 activity in the hippocampus using Rac1 inhibitor (NSC23766) and activator (CN04-A) were also reported to alter the maintenance of contextual fear memory in a similar fashion (Gan et al., 2016; Jiang et al., 2016). The role of Rac1 in forgetting is also supported by studies where Rac1 upstream regulators were manipulated. For example, KO mice lacking the Rac1 GAP protein, BCR/ABR, showed impaired spatial, and NOR memory by facilitating their decay (Oh et al., 2010). In KO mice deficient of another Rac1 GAP protein, ArhGAP15, the hyperactive Rac1 led to longer escaping latency during the reversal phases in the water maze test without affecting the learning process, as well as less freezing in both contextual and cued fear memory test (Zamboni et al., 2016). Recent studies have used optogenetic techniques that allow a more precise temporal and spatial control of Rac1 activity. For example, optical activation of photo-activable (PA) Rac1 in the amygdala was found to facilitate the cue-based long-term fear memory decay that was observed 24 h after training (Das et al., 2017). It was found that Rac1 activity increased after fear conditioning training and this increased Rac1 activity remained until fear memory disappeared, and that interfering Rac1 activity specifically during this period of time by optical stimulation of PA-Rac1 controlled the speed of memory decay (Lv et al., 2019).

In summary, while Cdc42 and RhoA seem to be involved the formation of memory, Rac1 is more important in memory forgetting. However, given that Rac1 KO in the excitatory neuron can also impair memory formation (Haditsch et al., 2009; Gao et al., 2015), the endogenous Rac1 level could be normally maintained at an intermediate level, which can then be up- or down- regulated to facilitate or slow down memory decay. Because the RI effect on memory and Rac1 activity was only evident 22 h (but not 8 h) after the initial training, there may exist a time window in which the Rac1 activity level is sensitive to endogenous modulation (Liu et al., 2016). This is consistent with the observations that activation of Rac1 during training but not after training impaired long-term memory, and that Rac1 inhibition during LTP induction, not after induction, impaired LTP (Martinez and Tejada-Simon, 2011; Das et al., 2017). Defining the molecular events during this critical window will be important to understand how memory is stored and consolidated.

RHO GTPases IN BRAIN DISEASES

Considering the critical roles of Rho GTPases in spine formation and morphology, synaptic plasticity, and memory, it is not surprising that dysfunctions in these proteins are linked to various brain diseases, some of which will be briefly discussed below.

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive loss of memory. The pathological hallmark of AD is the accumulation of extracellular amyloid plaques formed by amyloid beta (Ab) peptides and the aggregation of intracellular neurofibrillary tangles containing hyperphosphorylated Tau proteins (Hardy and Selkoe, 2002). Aβ peptides have been shown to induce abnormal assembly

of actin bundles and the formation of cofilin-actin rods in neurons accompanied by neurite dystrophy and neuronal loss (Maloney and Bamburg, 2007), suggesting the involvement of Rho proteins. Evidence supporting the association between the elevated Rac1 activity and AD comes from both human and animal studies. Elevated Rac1 activity was observed in the hippocampus of post-mortem brains of AD patients as well as in APP/PS1 transgenic mice across different ages (3–9 months) and a fruit fly AD model (Wu et al., 2019). Suppression of Rac1 activity either by intragastric application of Rac1 inhibitor, Ehop-016, or by expression of dominant negative Rac1 rescued memory loss of APP/PS1 mice in water maze test, suggesting that increased Rac1 activity was responsible for the memory loss (Wu et al., 2019). Abnormalities in Rac1 were also reported in another AD mouse model in which elevated Rac1 activity was observed in the hippocampus of 6-week old 3xTg-AD mice, while the total Rac1 protein level was reduced in 7-month old 3xTg-AD mice (Borin et al., 2018). Contrary to the results obtained from the APP/PS1 mouse model, the intranasal treatment using constitutively active Rac1 rescued spine deficits in in 6.5-month-old 3xTg-AD mice (Borin et al., 2018). These results suggest that alterations in Rac1 may be age-dependent and affected by animal models. The effect of Rac1 in AD is likely mediated by changes in the actin cytoskeleton as Rac1 activation induced by Aβ peptides was associated with increased colocalization with actin filaments in hippocampal neurons, and the inhibition of Rac1 abolished the increased actin filaments (Mendoza-Naranjo et al., 2007). Increased Rac1 activity may also exacerbate AD symptoms through free radicals in astrocytes as Rac1 was required for Aβ-induced production of reactive oxygen species in these cells (Lee et al., 2002). The relationship between Aβ and Rac1 activity is not unidirectional; while Aβ treatment was shown to increase Rac1 activity, the expression of constitutively active Rac1 was able to increase the production of Aβ via increasing gamma-secretase-mediated cleavage of amyloid precursor protein (APP) (Gianni et al., 2003). Rac1 was also shown to enhance the transcription of APP via acting on the −233 to −41 bp region in the APP gene promoter, and inhibition of Rac1 activity by the Rac1 inhibitor, NSC23766, dominant negative Rac1, or siRNA knockdown, all reduced APP mRNA and protein level in the HEK293 cells (Wang et al., 2009).

Abnormal upregulation of Ccd42 was also shown in the brain of AD patients (Zhu et al., 2000). In addition, Aβ treatment of hippocampal neuronal culture induced the activation of Cdc42, along with Rac1, in a time- and dose-dependent manner (Mendoza-Naranjo et al., 2007). Similar to Rac1, the increased Cdc42 activity was colocalized with actin filaments and the inhibition of Cdc42 resulted in decreased actin filaments, supporting the involvement of actin changes in AD pathogenesis (Mendoza-Naranjo et al., 2007). In cultured hippocampal neurons from rats, Aβ-induced cofilin-actin rods were suppressed by dominant negative Cdc42 and enhanced by constitutively active Cdc42 (Davis et al., 2009). Although elevated Cdc42 level was found to be associated with AD, its downregulation in non-neuronal cells may also potentially contribute to AD. For example, KO mice of triggering receptor expressed on myeloid cells 2 (TREM2), a receptor predominantly expressed in the microglia and whose mutations were associated with increased risk of AD, showed alterations in Cdc42, and Rac1 signaling and their activator partially ameliorated impaired microglial migration in response to Aβ treatment (Rong et al., 2020).

In contrast to increased Rac1 and Cdc42 activity, decreased RhoA activity was found in the hippocampus of AD brains (Huesa et al., 2010). In addition, RhoA protein level was reduced in neuropil but increased in neurons that have neurofibrillary tangles. In the Tg2576 AD mouse model, altered distribution of RhoA was observed with a higher expression found in the dystrophic neurites but a lower level at the synapses (Huesa et al., 2010). In cultured PC12 cells, Aβ treatment caused activation of RhoA within 2 h of treatment and inhibition of RhoA by C3 transferase or dominant negative RhoA prevented Aβ-induced morphological alterations and neuronal death in cultured hippocampal neurons (Chacon et al., 2011). RhoA/ROCK signaling pathway is also involved in the production of Aβ peptides. In SH-SY5Y cells transfected with APP and in PDAPP AD mouse model, the amount of Aβ was decreased by the application of Y-27632 (Zhou et al., 2003). The role of RhoA in Aβ production could be mediated by Rac1 and Cdc42 through affecting the gamma-secretase cleavage of APP (Zhou et al., 2003). Unlike Rac1 and Cdc42 that can be palmitoylated on their C-terminus to facilitate their recruitment to the lipid raft on the plasma membrane where gamma-secretase was located, RhoA was found mainly in the non-raft fraction (Kumanogoh et al., 2001; Levental et al., 2010; Albanesi et al., 2020). Therefore, the effect of RhoA/ROCK pathway on APP processing and Aβ production is likely indirect. RhoA has also been shown to contribute to Aβ-mediated neurotoxicity by interfering with microtubule stability (Pianu et al., 2014). In non-neuronal cells such as cerebral endothelial cells and platelets, Aβ was found to activate RhoA/ROCK signaling and lead to the disruption of blood-brain barrier and activation of platelets, both of which are associated with AD (Sonkar et al., 2014; Park et al., 2017).

AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social interaction and communication, and repetitive interests and behaviors. The pathogenesis of ASD is associated with genetic and environmental factors. To date, many genes have been identified that are linked to increased risk of developing ASD, and among these genes, 20 of them encode proteins involved in Rho signaling pathways (Guo et al., 2020), underscoring the importance of Rho proteins in the pathogenetic process of ASD. In addition, alterations in the activity of Rho GTPases are associated with many other genes linked to ASD. For example, duplication or deletion of the p11.2 region on chromosome 16 (16p11.2) is linked to ASD, and among the genes located in this region, potassium channel tetramerization domain containing 13 (kctd13) appeared to be important. kctd13 deficiency decreased synaptic transmission in the hippocampus and this was accompanied with increased RhoA activation. Inhibition

of RhoA activity reversed the deficits caused by kctd13 deletion (Escamilla et al., 2017). Altered RhoA activity was also reported in the loss-of-function mutations of another key gene in 16p11.2, TAO kinase 2 (TAOK2) (Richter et al., 2019). TAOK2 deficiency caused dosage-dependent impairments in cognitive processes as well as abnormalities in neuronal structures and functions (Richter et al., 2019) and these changes were accompanied by reduced RhoA activity, and importantly pharmacological enhancement of RhoA activity restored abnormalities in neuronal structures (Richter et al., 2019). Alterations in Rac1 activity were also affected by several autism-risk gene mutations, including fragile X mental retardation 1 (Fmr1), neurexin 1 (Nrx1), neuroligin 4 (Nlg4), and tuberous sclerosis 1 (Tsc1). The mutations of these genes in *Drosophila* impaired reversal learning by affecting Rac1-mediated forgetting, and this impairment was rescued by overexpressing constitutively active Rac1 (Dong et al., 2016). Abnormal actin cytoskeleton was observed in the brain of ASD patients with compromised Rho signaling, suggesting that dysregulated actin may underlie the effects of Rho proteins in ASD (Griesi-Oliveira et al., 2018). In SH3 and multiple ankyrin repeat domains 3 (Shank3) KO mice, a widely used mouse model with autistic-like social deficit and repetitive behavior, reduced cortical actin filaments and Rac1/PAK activity were observed, which were rescued by reactivating Rac1 or inhibiting cofilin activity (Duffney et al., 2015).

Fragile X Syndrome

Many Rho signaling proteins were reported to be linked to intellectual disability (Newey et al., 2005; van Galen and Ramakers, 2005). Fragile X syndrome (FXS) is the most commonly known single gene cause of ASD and intellectual disability. It is caused by a trinucleotide expansion within the Fmr1 gene on the X chromosome, resulting in an absence of FMR1 protein 1 (FMRP1) (Verkerk et al., 1991). Fmr1 KO mice displayed excessive immature spines, aberrant activation of Rac1, increased cofilin inactivation and actin polymerization (Pyronneau et al., 2017). Viral expression of constitutively active cofilin in the somatosensory cortex of Fmr1 KO mice or inhibition of PAK1, rescued cofilin changes and synaptic phenotypes in FXS mice (Pyronneau et al., 2017). Interestingly, although the overall baseline activity of Rac1 was increased in the brain of Fmr1 KO mice (Bongmba et al., 2011), the activation of Rac1 and PAK induced by TBS was impaired (Chen et al., 2010). Consistent with the impairment in activity-dependent Rac1 activation, inhibition of Rac1 rescued impaired LTP, elevated LTD and memory deficits in Fmr1 KO mice (Bongmba et al., 2011; Martinez and Tejada-Simon, 2018a,b).

SUMMARY

By using various approaches and techniques, including KO mouse models, pharmacological compounds and expression of mutant proteins, Rho GTPases and their effectors have been shown to play essential roles in the regulation of spine formation, spine morphology, receptor trafficking and synaptic plasticity, and learning and memory. It is important to note that the results obtained from these different approaches are not always consistent and this could be due to caveats associated with specific techniques used. For example, genetic KO mice may suffer from developmental compensation, whereas pharmacological reagents may have non-specific effects, both of which may complicate the interpretation of the data. Although members of Rho GTPases share many signaling molecules targeting the actin cytoskeleton, they often exert differential effects on synaptic structure and function. How these differential effects are achieved remains a challenging question to address. Downstream effector proteins targeting cellular processes other than actin dynamics may be important in this aspect. The temporal and spatial regulation of Rho GTPases also provides an important direction for future studies. In this regard, the use of photo-activatable Rac1 and cofilin may be particularly attractive (Wu et al., 2009; Das et al., 2017; Stone et al., 2019). Studies of Rho proteins in other brain cells, including astrocytes and microglia, are also of importance in understanding the role of Rho signaling in synaptic regulation. Given the extensive involvement of Rho signaling proteins in various brain diseases, understanding how these proteins are altered in the diseased brain continues to be a focus of future investigations. All these studies should aid the development of new strategies and molecular targets to treat related brain disorders.

AUTHOR CONTRIBUTIONS

HoZ, HiZ, and ZJ wrote and approved the manuscript. All authors contributed to the article and approved the submitted version.

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