TrkB-mediated activation of geranylgeranyltransferase I promotes dendritic morphogenesis

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Dendrite morphogenesis is regulated by neuronal activity or neurotrophins, which may function by activating intrinsic signaling proteins, including Rho family GTPases. Here we report that activity- and brain-derived neurotrophic factor (BDNF)–dependent dendritic morphogenesis requires activation of geranylgeranyltransferase I (GGT), a prenyltransferase that mediates lipid modification of Rho GTPases. Dendritic arborization in cultured hippocampal neurons was promoted by over-expression of GGT, and reduced by inhibition or down-regulation of GGT. Furthermore, GGT was activated by neuronal depolarization or BDNF, both of which promote dendritic arborization, in cultured hippocampal neurons. Moreover, exploration of a novel environment caused activation of GGT in the mice hippocampus, suggesting that neural activity activates GGT in vivo. Interestingly, GGT was physically associated with tropomyosin-related kinase B (TrkB), the receptor for BDNF, and this association was enhanced by depolarization. Disrupting the GGT-TrkB interaction or down-regulating GGT activity attenuated depolarization- or BDNF-induced dendrite development. Finally, the GGT effect on dendrite arborization was prevented by over-expressing Rac1 with the prenylation site deleted or mutated. Thus depolarization- or BDNF-dependent dendrite development may be mediated by GGT-induced prenylation of Rho GTPases.

BDNF | dendrite | neuronal activity | Rac | prenylation

Dendritic morphogenesis is a critical step for establishing neural circuits. The growth and branching of dendritic arbors are controlled by both external signals and intracellular pathways (1, 2). Neuronal activity and neurotrophins, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3, are known to regulate dendrite development through multiple signaling pathways, leading to cytoskeletal reorganization or gene expression required for dendritic growth (2–7). For example, members of the Rho family of small GTPases, including Rac1, Cdc42, and RhoA, are important for distinct aspects of dendrite development by modulating actin cytoskeleton (8, 9). Activation of Rho A attenuates dendrite growth and branching, whereas activation of Rac1 facilitates dendrite growth (8, 10, 11). Importantly, dendrite growth increased by visual activity requires Rho GTPases (12) and BDNF-dependent dendritic growth may be mediated by Rac1 (13). Thus, Rho GTPase may mediate the effects of BDNF or neuronal activity on dendrite development. However, the mechanism by which BDNF and activity regulate Rho GTPases remains largely unknown.

The Rho GTPase cycles between a GTP-bound active state and a GDP-bound inactive state, and this process is regulated by GTPase activating proteins and guanine nucleotide exchange factors (GEFs) (14, 15). Importantly, GTPases need to be translocated from the cytosol to the membrane for their activation (16, 17). This is achieved by prenylation, a reaction mainly catalyzed by farnesyltransferase (FT) or geranylgeranyltransferase I (GGT), which acts to covalently couple a lipid moiety to the cystine of C-terminal "CAAX" box (Cys-aliphatic-aliphatic-X) of the GTPase (18). Whether and how these prenyltransferases are regulated by cellular signaling pathways remains unclear. At the neuromuscular junction, we have previously shown that agrin activates GGT through the muscle-specific receptor tyrosine kinase MuSK, leading to acetylcholine receptor clustering at the postsynaptic membrane (19). Although GGT is extensively expressed in the brain (20, 21), whether it is also regulated through receptor tyrosine kinases during development of central neurons is unknown.

In this study, we investigated the role of GGT in dendrite development by focusing on its regulation by factors that promote dendritic development. We found that treatment of cultured hippocampal neurons with KCl or BDNF, both of which promote dendrite arborization, increased GGT activity and membrane association of Rac, the known substrate of GGT. In addition, GGT activity of mice hippocampus was increased after novelty exploration. Interestingly, GGT was found to be associated with tropomyosin-related kinase B (TrkB) and this association was increased by KCl-induced depolarization. The effect of depolarization or BDNF on dendrite development was attenuated by down-regulating GGT or disrupting the GGT-TrkB association. Together, these observations reveal the critical role of GGT in dendrite development, and the mechanism by which GGT is regulated in central neurons.

Results

Over-Expression of GGTβ Promotes Dendrite Growth and Branching. There are two subunits of GGT: an α subunit (GGTα) that is shared with FT and a distinct β subunit (GGTβ) (18). The expression of GGTα or GGTβ in cultured hippocampal neurons gradually increased and peaked at approximately 8 days in vitro (DIV) [supporting information (SI) Fig. S1 *A* and *B*], a pattern that coincides with the period of extensive dendrite growth. Next, we examined the role of GGT in dendrite development by over-expressing GGT in cultured hippocampal neurons. These cells were transfected with a vector encoding HA-GGTβ or FTβ together with an EGFP plasmid (to mark dendritic branches) at DIV5 and analyzed 48 h later. We found that over-expression of GGTβ, but not FTβ, resulted in the formation of more complex dendritic arbors (Fig. 1 *A–C*). Total dendritic branch tip number (TDBTN) or total dendritic length (TDL) of GGTβ-over-expressing cells was increased compared with cells transfected with EGFP alone (Fig. 1 *B* and *C*). The distribution of TDBTN showed that the complexity of dendritic branching increased over the entire population of these cultured

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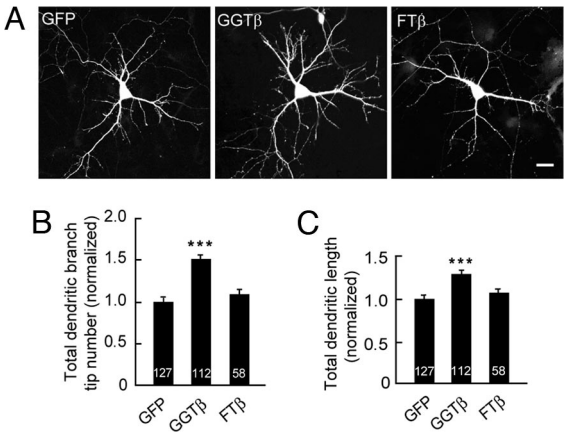

Fig. 1. GGTβ over-expression increases dendrite arborization. (*A*) Cultured rat hippocampal neurons at DIV5 were transfected with EGFP, either alone or together with GGTβ or FTβ at a ratio of 1:3. Shown are representative images of transfected neurons marked by GFP. (Scale bar, 20 μm.) Quantification of TDBTN (*B*) and TDL (*C*). The mean value taken from control neurons transfected with GFP alone was set as 1.0. The values of other groups were normalized to GFP-transfected neurons. Data are shown as mean ± SEM from four independent cultures (***, $P < 0.001$, ANOVA with Dunnett test). Neuron numbers are indicated in histograms.

neurons (Fig. S1*C*). Consistent effects of GGTβ were found in all experiments despite culture-to-culture variation, as shown by the un-normalized data (Fig. S1 *D* and *E*).

Activation of GGT by Neuronal Activity or BDNF. It is known that neuronal activity promotes dendritic arborization (2, 3). We thus examined whether GGT is regulated by neuronal activity. Cultured cortical neurons at DIV6 were treated with a high concentration of KCl (16 mM) or bicuculline (40 μM), a GABA-A channel inhibitor, for 45 min to elevate neuronal activity. Neurons were lysed and GGT activity was determined by using dansyl-GCVLL as the substrate (see SI *Text*). As shown in Fig. 2*A*, KCl- or bicuculline-treated cells showed GGT activity increased by 1.93 ± 0.18 and 1.88 ± 0.35 fold of that found for control cells, respectively. The effect of KCl treatment was specific, as co-treatment with the GGT inhibitor GGTi-2147 (2.5 μM) abolished the effect (Fig. 2*A*). In addition to neuronal activity, several secreted factors, including BDNF and insulin-like growth factor (IGF), are known to promote dendrite growth (4, 22). Although insulin can activate GGT in non-neuronal cells (23, 24), we found that neither insulin nor IGF-1 had any effect on GGT activity under our experimental conditions (Fig. 2*B*). In contrast, BDNF treatment caused a marked increase in the GGT activity, to a level similar to that found for bicuculline or KCl treatment (Fig. 2*B*), without altering the GGT protein level (Fig. S2*A*).

A potential substrate of GGT is Rac, a small GTPase critical for dendritic morphogenesis (12, 25, 26). Because geranylgeranylation of Rac at its C-terminal CAAX box is crucial for its association with the plasma membrane and its activation (16, 17), we reasoned that KCl-induced depolarization or BDNF might promote membrane targeting of Rac. Indeed, we found KCl treatment significantly increased the level of Rac in the membrane fraction, hereafter referred to as Rac(m) (Fig. 2*C*). This elevation depended on the GGT activity, as the presence of GGTi-2147 prevented the increase of the Rac(m) level induced by KCl (Fig. 2*C*). In addition, the basal level of membrane-associated Rac was also decreased in GGTi-2147 treated neurons (Fig. 2*C*), suggesting that the basal level of membrane-bound Rac also depends on the GGT activity. Next, Rac activity was determined by pull-down assay using GST-p21-binding domain of p21-activated kinase PAK1, which is used to isolate the active form of Rac (27). Like the changes in Rac(m), the level of active Rac was also increased by the KCl treatment (Fig. S2*B*), in agreement with the previous finding of Rac activation by neuronal activity (9). The effect of KCl on Rac activity was attenuated in the presence of the GGT inhibitor (Fig. S2*B*), suggesting the GGT activity is required for KCl-induced activation of Rac. Finally, in line with the finding that BDNF activates GGT (Fig. 2*B*), we found that BDNF treatment also caused an elevation of membrane-bound Rac in these cortical neurons, and this effect was attenuated in the presence of the GGT inhibitor (Fig. 2*D*). Given that GGTα is shared by GGT and FT, we examined the effects of depolarization or BDNF on the activity of FT by using dansyl-GCVLS as the substrate (see *SI Text*). We found that FT activity was not regulated in neurons by the treatment with KCl or BDNF (Fig. S2*C*). Consistently, these treatments had no effect on the level of membrane-associated Ras (Fig. S2*D*), a known substrate of FT (16, 18). Together, these results support the notion that neuronal activity or BDNF activates GGT, which in turn promotes membrane association and the activation of Rac.

We next examined whether GGT was activated by neural activity stimulated by exploration of a new environment (28–31). As shown in Fig. 2*E*, exposure to novelty enriched environments for 30 min resulted in an increase in the GGT activity of mice hippocampus, whereas it had no effect on GGT protein levels (data not shown). In agreement with this finding, novelty exploration caused an increase in the level of membrane-bound Rac without altering the level of total Rac (Fig. 2*F*). These results suggest that GGT is activated during the natural mode of neural activation.

Increased Dendrite Arborization by High K^+ and BDNF Requires GGT Activity. Given the finding described in regard to the effect of neuronal activity and BDNF on GGT activity, we further examined whether GGT loss of function affects depolarization- or BDNF-induced dendrite development. Consistent with previous studies (7, 32–35), both KCl and BDNF treatments caused an increase in dendritic branches and length in cultured hippocampal neurons at DIV7 (Fig. 3 *C* and *D*). However, these effects of KCl and BDNF were abolished in the presence of GGTi-2147 (Fig. 3 *C* and *D*). To further examine the role of GGT, we suppressed the GGT expression in these neurons by using an RNA interference approach. Several siRNA sequences that were targeted to GGTβ were screened for their efficacy on suppressing GGTβ expression in human embryonic kidney (HEK) 293 cells co-transfected with HA-GGTβ. We found that siRNA-2 effectively suppressed the expression of GGTβ (Fig. 3*A* and Fig. S3 *A* and *C*). However, siRNA-1 and -3, or scrambled sequence for siRNA-2, had no effect on GGTβ expression (Fig. 3*A* and Fig. S3*A*). The effect of siRNA was further confirmed in cultured cortical neurons, in which the level of endogenous GGTβ mRNA was shown to be reduced by siRNA-2, but not by the scrambled or other siRNAs (Fig. 3*B* and Fig. S3*B*). The expression levels of GGT/FTα, FTβ, or TrkB were not affected by siRNA-2, indicting the specificity of the knockdown effect (Fig. 3 *A* and *B*). After transfection into cultured hippocampal neuron, siRNA-2 caused a decrease in dendrite arborization (Fig. 3 *C* and *D* and Fig. S3 *D–F*). This effect could be rescued by a siRNA-resistant mutant of GGTβ (GGTβ^{Res}; Fig. S3 *C-F*). Similar to the effect of GGTi-2147, down-regulation of GGTβ expression by siRNA-2 also abolished depolarization- or BDNF-induced effects on dendritic arborization (Fig. 3 *C* and *D*). Thus, GGT is crucial for depolarization- or BDNF-dependent dendrite development.

GGT-TrkB Association Is Essential for the Effect of High K^+ and BDNF. To elucidate the mechanism by which high K^+ and BDNF activate GGT, we examined the possibility that GGT may

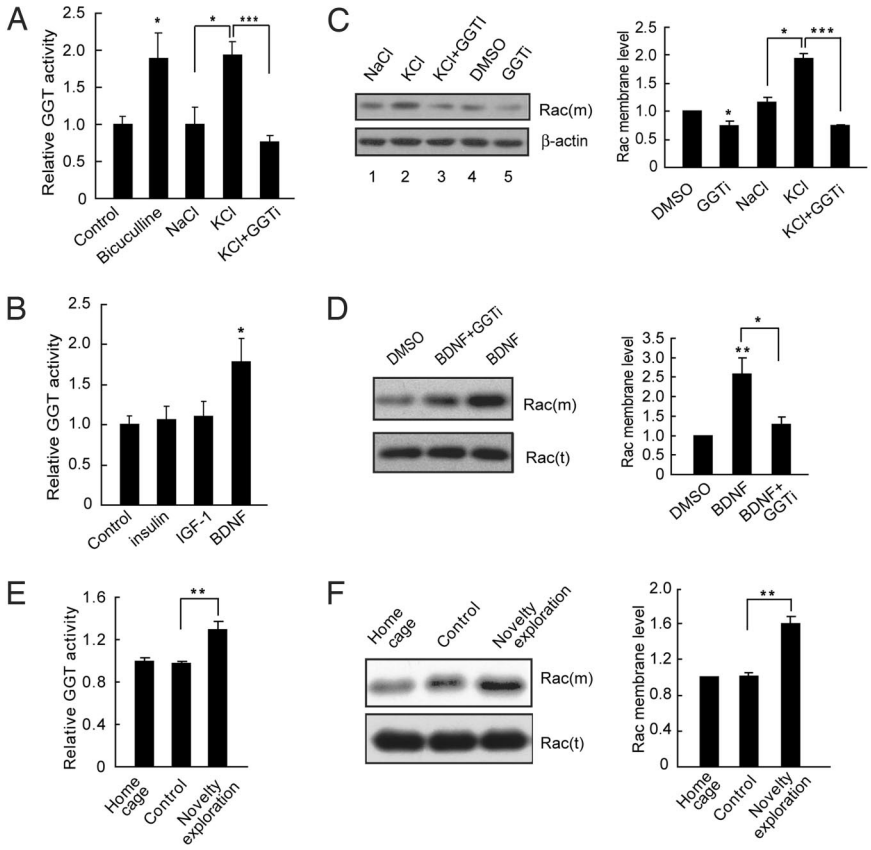

Fig. 2. Activation of GGT by neuronal activity or BDNF. (*A* and *B*) Neurons at DIV6 were treated with indicated reagents or vehicle (i.e., control), respectively, for 45 min. Under some conditions, before treatment with KCl, neurons were pretreated with GGTi-2147, a specific GGT inhibitor, or DMSO for 45 min. GGT activity was assayed as described in the *SI Text*. Data shown are mean ± SEM of five independent experiments, with each performed in triplicate. All values were normalized to control neurons. *, $P < 0.05$; ***, $P < 0.001$; ANOVA with Tukey (*A*) or Dunnett test (*B*). (*C* and *D*) Primary neurons were treated with NaCl, KCl, or BDNF for 45 min, without or with pretreatment with GGTi-2147 (45 min). Membrane proteins were separated and subjected to IB with antibody against Rac or β-actin. Histograms show relative levels of Rac associated with plasma membrane. Data shown are mean ± SEM from three experiments (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ANOVA with Tukey test). (*E*) Mice were divided evenly into three groups (two mice in each group) and subjected to the following treatments: remained in the home cage, moved to a cage with a novel environment (i.e., novelty exploration), or moved to another cage that was the same as the home cage (i.e., control). Thirty minutes after handling, the GGT activity of hippocampal lysates was determined. The mean value of home-caged mice from five independent experiments was set as 1.0. Values shown are mean ± SEM (**, $P < 0.01$; ANOVA with Tukey test). (*F*) After the aforementioned treatments, membrane fractions of mice hippocampus were separated and probed with anti-Rac antibody (*Left*). The histogram (*Right*) shows the relative amount of membrane Rac (normalized by total Rac). In each experiment, the value from home-caged mice was set as 1.0. Data are shown as mean ± SEM from five experiments (**, $P < 0.01$, ANOVA with Tukey test).

interact with the BDNF receptor TrkB. We found that GGTα and TrkB co-immunoprecipitated in the rat brain lysate (Fig. 4*A*). In addition, immunoprecipitation (IP) of GGTα caused co-IP of TrkB in the lysate of cultured cortical neurons (Fig. 4*B*). By contrast, control antibody (i.e., IgG) caused no positive bands (Fig. 4 *A* and *B*), suggesting the specificity of the interaction. Interestingly, the association of GGTα with TrkB was enhanced when the neurons were treated with KCl (Fig. 4*B*), suggesting that neuronal activity regulates GGT-TrkB interaction. To investigate whether these associations are mediated by direct interaction, we performed a pull-down assay. We found that GST-TrkB-intra (the intracellular region of TrkB fused with GST), as well as GST-GGTβ, binds to Myc-GGTα expressed in HEK293 cells (Fig. S4*A*) or endogenous GGTα in the brain (Fig. S4*B*), whereas GST alone had no binding. These results indicate direct interaction between TrkB and GGTα. Because GGTα is shared by GGT and FT, we investigated whether FTβ was present in the TrkB complex. We found that GGTβ, but not FTβ, was associated with TrkB in cultured cortical neurons (Fig. S4 *C* and *D*). This result is consistent with the finding that treatment with high K^+ or BDNF caused activation of GGT, but not FT (Fig. 2 *A* and *B* and Fig. S2*C*).

To further evaluate the role of GGT-TrkB interaction in dendrite development, we used the approach of dominant-negative suppression of GGT activity. Previous studies have shown that mutation of lysine 164 (K164), tyrosine 166 (Y166), or tyrosine 200 (Y200) inhibited the prenylation activity by preventing the catalytic activity of prenyltransferase or the formation of the enzyme-substrate complex (36, 37). Thus, over-expression of the mutated GGTα that interacts with TrkB may compete with the endogenous GGT for TrkB and inhibit TrkB-mediated GGT activation. After co-transfection with GFP-TrkB into HEK293 cells, K164A (i.e., lysine to alanine, $GGT\alpha^{K164A}$) or Y200F (i.e., tyrosine to phenylalanine, $GGT\alpha^{Y200F}$) was found to be associated with TrkB to a similar extent as the WT GGTα, whereas GGTα with Y166A (i.e., tyrosine to alanine) mutation ($GGT\alpha^{Y166A}$) showed lower association with TrkB (Fig. 4*C*). We transfected hippocampal neurons with empty vector or the vector encoding WT or mutated forms of GGTα and treated the neurons with KCl or BDNF. We found that the effect of high K^+ or BDNF on dendrite arborization was prevented by over-expressing $GGT\alpha^{K164A}$ or $GGT\alpha^{Y200F}$, but not WT GGT ($GGT\alpha^{WT}$) or GGTα with Y166A mutation (Fig. 4 *D–F*). Because FTβ was not found in the TrkB complex (Fig. S4*D*), the dominant-negative role of $GGT\alpha^{K164A}$ or $GGT\alpha^{Y200F}$ was presumably a result

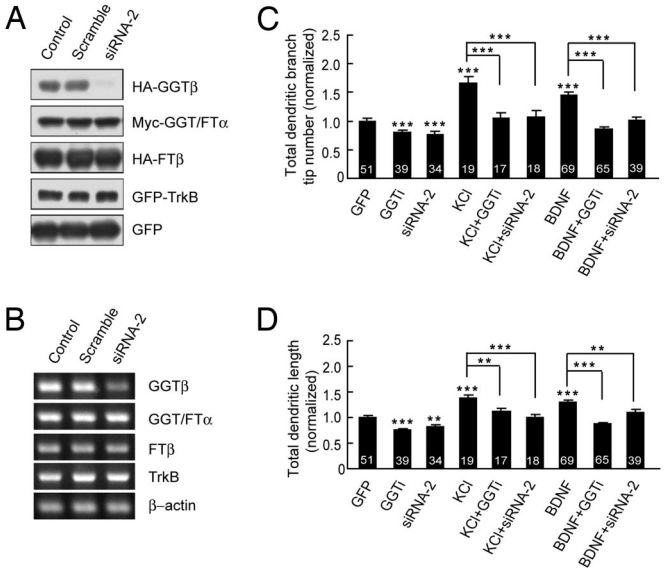

Fig. 3. Impairment of KCl- or BDNF-induced dendrite growth by inhibiting or down-regulating GGT. (*A*) HEK293 cells were co-transfected with indicated plasmids and GGT-siRNA-2, or scrambled siRNA. Cell lysates were subjected to IB with antibodies against HA, Myc, or GFP, respectively. (*B*) Primary neurons were transfected with the described siRNAs and cultured for 24 h. RT-PCR was used to determine the effect of siRNAs on the expression of indicated genes. (*C* and *D*) Hippocampal neurons at DIV5 were transfected with EGFP, or together with GGTβ-siRNA-2. After 24 h, transfected neurons were treated for 30 h with indicated reagents (GGTi-2147, KCl, BDNF, KCl plus GGTi-2147, or BDNF plus GGTi-2147). Histograms show normalized TDBTN (*C*) or TDL (*D*). Cell numbers are indicated in the histograms. Data are presented as mean ± SEM from three experiments (**, $P < 0.01$; ***, $P < 0.001$; ANOVA with Tukey test).

of blockade of interaction between TrkB and endogenous GGT, rather than affecting endogenous FT activity. In line with this finding, KCl depolarization caused an increase of Rac(m) in neurons transfected with empty vector (control) or WT of GGTα, but not in those expressing DN-GGTα, GGTα^{K164A}, or GGTα^{Y200F} (Fig. S5). These results suggest that GGT acts as a mediator for TrkB signaling.

Rac1 Prenylation Is Required for GGT, Depolarization, or BDNF-mediated Dendritic Growth. Rac1 is a known substrate of GGT and is implicated in dendrite development (8, 25). To explore the role of Rac1 in GGT-induced dendrite arborization, we generated two mutated forms of Rac1—Rac1$^{\Delta C}$ and Rac1^{C189S}—by deleting the C-terminal CAAX box or substituting the cystine with serine, respectively (Fig. 5*A*). These two mutants expressed to a similar extent as WT Rac1 (Fig. S6*A*). However, neither Rac1$^{\Delta C}$ nor Rac1^{C189S} associated with the plasma membrane of transfected HEK293 cells (Fig. S6*B*) or primary neurons (see insets in Fig. S6*C*); rather, a fraction of them translocated into the nucleus. In contrast, a large fraction of transfected WT Rac1 (Rac1^{WT}) was found at the plasma membrane (Fig. S6 *B* and *C*). After transfection into cultured hippocampal neurons, Rac1^{WT} promoted dendritic arborization, whereas Rac1$^{\Delta C}$ or Rac1^{C189S} had no effect compared with neurons transfected with GFP alone (Fig. 5*C* and Fig. S6*D*). Furthermore, the effect of GGTβ over-expression on dendrite arborization was attenuated when neurons were co-transfected with Rac1$^{\Delta C}$ or Rac1^{C189S} (Fig. 5 *B* and *C* and Fig. S6*D*). Thus, GGT may function in dendrite development at least in part through prenylation of Rac1.

Although Rac1$^{\Delta C}$ and Rac1^{C189S} were unable to associate with plasma membrane, they remained the ability to interact with Tiam1 (Fig. S7*A*), a specific Rac GEF that has been shown to mediate TrkB-mediated Rac activation (13). We reasoned that these mutated forms of Rac1 might act as dominant-negatives by competing with endogenous Rac for GEFs such as Tiam1. In line with this notion, over-expression of these Rac mutants caused inhibition of high K^+-induced activation of endogenous Rac in cultured neurons (Fig. S7*B*). Furthermore, expression of Rac1$^{\Delta C}$ or Rac1^{C189S} prevented KCl or BDNF from increasing dendritic growth and arborization (Fig. 5*D* and Fig. S6*E*). Therefore, the prenylation of Rac1 is required for depolarization- or BDNF-induced Rac activation and dendrite development.

Discussion

In this study, the prenyltransferase GGT is identified as a crucial regulator for depolarization or BDNF-induced dendritic arborization. Interestingly, GGT is activated by neuronal activity or BDNF, and this activation is probably achieved by the GGT interaction with TrkB.

Many cytoplasmic signaling proteins need to be associated with the plasma membrane to be activated efficiently. This process is achieved by posttranslational modifications, including palmitoylation, myristoylation, or prenylation (38–40). The Rho family GTPases are prenylated at the C-terminal CAAX box by GGT (18, 41). In addition to small GTPases, prenyltransferases can modify other signaling proteins (18). For example, prenylation of Ca^{2+}/calmodulin-dependent protein kinase CLICK-III/CaMKIγ is responsible for its association with lipid raft and its role in dendritogenesis (42). It has been shown recently that GGT activity can be strictly regulated by multiple factors in non-neuronal cells, such as breast cancer cells or skeletal muscle cells (19, 23), but whether and how protein prenylation is regulated in neurons remain largely unknown. Here we show that GGT activity is regulated by neuronal activity or BDNF in cultured neurons and in a novelty exploration test. After treatment with high K^+, bicuculline, or BDNF, the GGT activity was markedly increased in cultured neurons (Fig. 2 *A* and *B*). In line with this finding, these treatments increased the association of Rac with the plasma membrane (Fig. 2 *C* and *D*). The increased membrane association of Rac was most likely mediated by the GGT activation, as GGT inhibitor decreased Rac(m) level and Rac activity. We note that the membrane translocation and activation of Rac occurred within 45 min of the aforementioned treatments and the effect of GGT inhibitors was apparent within a similar period. Because the half-life of Rac1 is approximately 4 h (43) and transfection with GGT mutants did not change the basal level of Rac(m) but attenuated KCl-induced increase of Rac(m) (see Fig. S5), GGT-mediated Rac modification is probably regulated by a signaling mechanism rather than by metabolic turnover. Interestingly, GGT was associated with the BDNF receptor TrkB and this association was enhanced by depolarization. Disrupting the TrkB-GGT interaction impeded activity- or BDNF-induced dendrite development. Based on these findings, we propose that neuronal activity or BDNF acts to expand the "readily available pool" of signaling proteins, such as Rac, at the plasma membrane by regulating GGT, and this event is crucial for dendritic arborization.

The Rho family of GTPases are central in regulating neuronal morphology, including the development of dendrites, and the formation and maintenance of spines and synapses (44). Previous studies have shown that neuronal activity or BDNF regulates Rho GTPases through Rho-GEF Trio or Rac1-GEF Tiam1 (13, 45). Tiam1 has been shown to play a crucial role in coupling NMDA receptors to dendrite growth by regulating Rac1 activity (46). Interestingly, we found that the Rac1 mutants deficient for prenylation still bind to Tiam1, and act as dominant-negatives in activity- or BDNF-induced dendrite development, suggesting that both regulators, GGT and Tiam1, are required for Rac activation.

Because GGT has a number of substrates besides GTPases (18), it is possible that GGT may also regulate dendrite development by its modification of other proteins. It is known that zinc is required

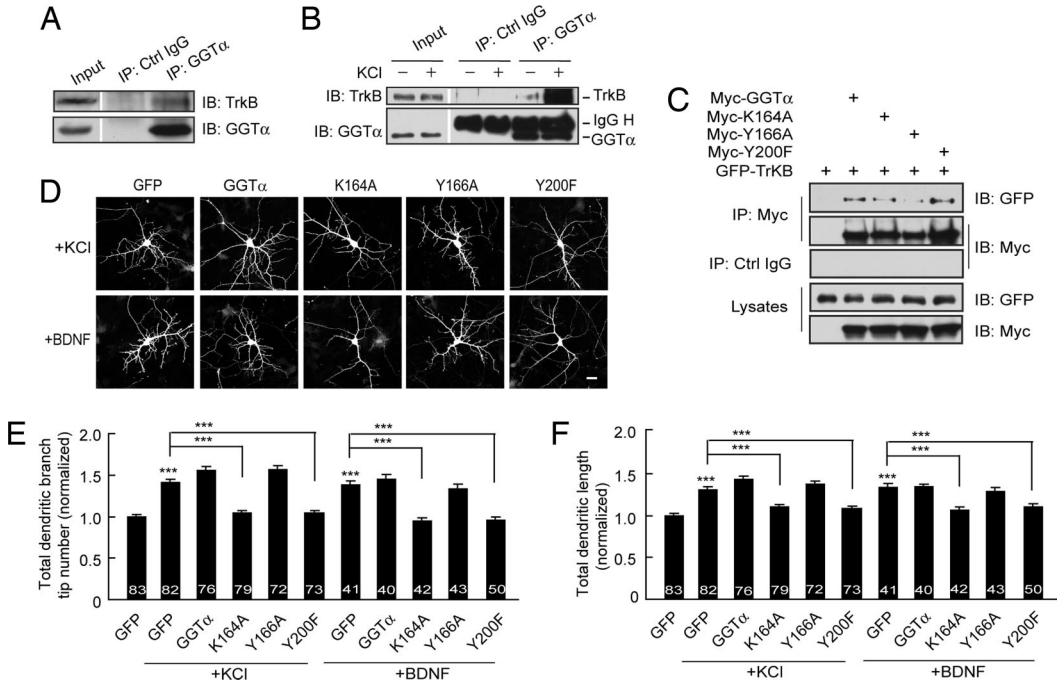

Fig. 4. GGT mutants K164A and Y200F impede KCl- or BDNF-induced dendrite growth. (*A*) Homogenates (500 μg protein) of P15 rat brains were subjected to IP with GGTα antibody. Normal rabbit IgG (i.e., control IgG) was used as control. Resulting immunocomplexes were probed with TrkB or GGTα antibody. Ten percent of lysates (50 μg protein) were loaded as input. (*B*) Cortical neurons at DIV6 were treated with or without KCl for 45 min. Immunoprecipitates with anti-GGTα antibody were subjected to IB with TrkB or GGTα antibody. Normal rabbit IgG (i.e., control IgG) was used as control. IgG H indicates IgG heavy chain. Five percent of lysates (50 μg protein) were loaded as input. (*C*) Association of WT or mutated forms of GGTα with TrkB. Lysates of HEK293 cells co-transfected with GFP-TrkB and different mutated forms of GGTα (tagged with Myc at the N terminus) were subjected to IP with anti-Myc antibody, followed by IB with GFP antibody. Normal mouse IgG (i.e., control IgG) was used as IP control. (*D–F*) Hippocampal neurons at DIV5 were transfected with GFP alone or together with different mutated forms of GGTα (1:3). Twenty-four hours later, transfected neurons were treated with KCl or BDNF for 30 h. (*D*) Representative images are shown. (Scale bar, 20 μm.) Histograms show normalized TDBTN (*E*) or TDL (*F*). The mean values from GFP-transfected neurons were set as 1.0. Data are shown as mean ± SEM from three experiments (***, $P < 0.001$; ANOVA with Tukey test). The numbers in the histograms indicate neuron numbers from three experiments.

for activation of prenyltransferases (18). A recent work shows that zinc activates TrkB by an activity-regulated mechanism independent of neurotrophins, and regulates synaptic plasticity (47). Thus, it shall be interesting to determine the role of prenyltransferases in regulating other neuronal functions, such as synaptic plasticity.

Materials and Methods

Reagents and Constructs. GGTi -2147, dansyl-GCVLL, and dansyl-GCVLS were from Calbiochem. GGPP and FPP were from Echelon Biosciences. Recombinant human BDNF was from Peprotech (for details of other reagents and constructs, see *SI Text*).

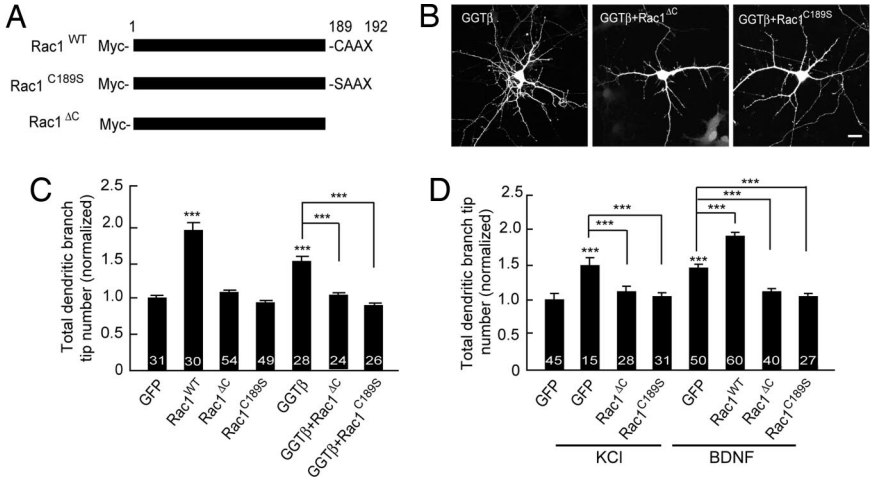

Fig. 5. Impairment of KCl- or BDNF-induced dendrite growth by mutated forms of Rac1. (*A*) Schematic structure of Rac1 constructs. Rac1^{WT}, WT Rac1; Rac1^{C189S}, a mutated Rac1 with the substitution of cystine at 189 with serine; and Rac1$^{\Delta C}$, a mutated Rac1 with the C terminus CAAX box deleted. (*B–D*) Hippocampal neurons at DIV5 were transfected with EGFP, either alone or together with indicated constructs. Twenty-four hours later, transfected neurons were treated with or without KCl or BDNF for 30 h. (*B*) Representative images are shown. Histograms show normalized TDBTN (*C* and *D*). The mean values from GFP-transfected neurons were set as 1.0. Data are shown as mean ± SEM from three experiments (***, $P < 0.001$, ANOVA with Tukey test). The numbers in the histograms indicate neuron numbers from three experiments.

Neuron Culture, Transfection, and Treatments. Hippocampal neurons were isolated from E18 Sprague-Dawley rats according to the protocol of Chen *et al.* (48). Briefly, hippocampi were dissected, dissociated with 0.125% trypsin, triturated, and plated at a density of 300,000 cells on a polyD-lysine-coated 35-mm-diameter dish (Corning). For morphological analysis, neurons at DIV5 were transfected with EGFP or together with testing constructs at a ratio of 1:3 by using a calcium phosphate precipitation method (49) and fixed for analysis 48 h later (see *SI Text*). For biochemical analysis, neurons were transfected with Lipofectamine 2000 (Invitrogen) according to manufacturer guidelines. Sometimes, neurons 24 h after transfection were treated with various reagents, e.g., KCl (16 mM), NaCl (16 mM), BDNF (25 ng/ml), insulin (100 nM), IGF1 (100 nM), Bicuculline (40 μM), or GGTi-2147 (2.5 μM), either alone or with combinations.

Biochemical Characterization and GGT/FT Activity Assay. IP, pull-down and immunoblotting were performed as described previously (19, 50). GGT or FT activity was measured in 96-well microtiter plates as previously described with minor modifications (51, 52), using dansyl-GCVLL or dansyl-GCVLS as the substrate, respectively (see *SI Text*).

Novelty Exploration. Adult ICR mice, 8–12 weeks old, were habituated to the laboratory overnight and transferred into a new, enriched cage (500 $\times$ 360 $\times$ 285 mm), moved to another cage that was the same as the home cage (325 $\times$ 210 $\times$ 185 mm), or kept in their home cages (325 $\times$ 210 $\times$ 185 mm). Thirty minutes after handling, the mice were killed and the homogenates (50 μg protein) of hippocampi were analyzed for GGT activity or subjected to membrane separation.

Image Processing and Quantification. Images were acquired on a Zeiss LSM 510 laser scanning confocal microscope and quantitatively analyzed using one-way ANOVA with a Tukey test or Dunnett test. Data are shown as mean $\pm$ SEM ($P < 0.05$ considered significant; see *SI Text*).

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