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Systemic or Forebrain Neuron Specific Deficiency of Geranylgeranyltransferase-1 Impairs Synaptic Plasticity and Reduces Dendritic Spine Density

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Abstract

Isoprenoids and prenylated proteins regulate a variety of cellular functions, including neurite growth and synaptic plasticity. Importantly, they are implicated in the pathogenesis of several diseases, including Alzheimer disease (AD). Recently, we have shown that two protein prenyltransferases, farnesyltransferase (FT) and geranylgeranyltransferase-1 (GGT), have differential effects in a mouse model of AD. Haplodeficiency of either FT or GGT attenuates amyloid-β deposition and neuroinflammation but only reduction of FT rescues cognitive function. The current study aimed to elucidate the potential mechanisms that may account for the lack of cognitive benefit in GGT-haplodeficient mice, despite attenuated neuropathology. The results showed that the magnitude of long-term potentiation (LTP) was markedly suppressed in hippocampal slices from GGT-haplodeficient mice. Consistent with the synaptic dysfunction, there

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AUTHOR CONTRIBUTIONS

DH performed electrophysiological experiments, conducted Golgi staining and measured dendritic spine density in GGT haplodeficient mice, analyzed the data, and wrote the draft of the manuscript. SC established the Golgi staining protocol and performed behavioral assessments and immunoblot analyses. AG genotyped/maintained the mice and collected tissues for analysis. KL conducted Golgi staining and measured dendritic spine density in forebrain neuron specific GGT-deficient mice. LY supervised electrophysiological experiments and contributed to the interpretation of the data. LL conceived and designed the experiments, interpreted the data, and revised/finalized the manuscript.

CONFLICT OF INTEREST

The authors declare that there are no competing interests.

was a significant decrease in cortical spine density and cognitive function in GGT-haplodeficient mice. To further study the neuronal specific effects of GGT deficiency, we generated conditional forebrain neuron-specific GGT-knockout ($GGT^{f/f}Cre+$) mice using a Cre/LoxP system under the CAMKIIα promoter. We found that both the magnitude of hippocampal LTP and the dendritic spine density of cortical neurons were decreased in $GGT^{f/f}Cre+$ mice compared with $GGT^{f/f}Cre-$ mice. Immunoblot analyses of cerebral lysate showed a significant reduction of cell membrane-associated (geranylgeranylated) Rac1 and RhoA but not (farnesylated) H-Ras, in $GGT^{f/f}Cre+$ mice, suggesting that insufficient geranylgeranylation of the Rho family of small GTPases may underlie the detrimental effects of GGT deficiency. These findings reinforce the critical role of GGT in maintaining spine structure and synaptic/cognitive function in development and in the mature brain.

Keywords

Protein prenylation; geranylgeranyltransferase; knockout mouse models; small GTPases; synaptic plasticity; dendritic spine density

INTRODUCTION

Proteins can undergo several different types of posttranslational modifications which result in proper tertiary structure, function, and subcellular location (Krishna and Wold, 1993). One important posttranslational modification is prenylation (Lane and Beese, 2006), which is the process of adding short-chain lipid molecules (isoprenoids) to target proteins via an irreversible covalent bond. Isoprenoids are intermediates in the mevalonate/cholesterol biosynthesis pathway (Goldstein and Brown, 1990) (Fig. 1). The two major isoprenoids are the 15-carbon farnesyl pyrophosphate (FPP) and the 20-carbon geranylgeranyl pyrophosphate (GGPP). FPP and GGPP are substrates of farnesyltransferase (FT) and geranylgeranyltranfersase-1 (GGT), which respectively catalyze the attachment of farnesyl or geranylgeranyl group to target proteins with the CAAX motif (McTaggart, 2006). A third prenyltransferase, geranylgeranyltranfersase-2 (RabGGT), also uses GGPP as its substrate to prenylate target proteins. Over 100 proteins are known to undergo prenylation, including heterotrimeric G-protein subunits and nuclear lamins (McTaggart, 2006, Berndt et al., 2011). Notably, the largest and most well studied group of prenylated proteins is the Ras superfamily of small GTPases such as Ras, Rho, and Rab proteins. These small GTPases serve as molecular switches and regulate a plethora of cellular processes and functions, including dendritic spine morphogenesis and synaptic plasticity (Hottman and Li, 2014). The importance of protein prenylation is further underscored by the findings that germline deletion of FT or GGT is embryonically lethal (Mijimolle et al., 2005, Sjogren et al., 2007), and dysregulation of prenylated proteins causes cancers and a number of other diseases including cardiovascular and cerebrovascular diseases, bone diseases, progeria, and potentially, neurodegenerative diseases such as Alzheimer's disease (AD) (McTaggart, 2006, Li et al., 2012, Wang and Casey, 2016).

We previously demonstrated that haplodeficiency of either GGT or FT reduced amyloid-β accumulation in a transgenic mouse model of AD (Cheng et al., 2013). However, only FT

haplodeficiency rescued cognitive function in these animals. GGT haplodeficiency similarly reduced amyloid plaques and neuroinflammation, but was not sufficient to rescue memory function of the animals. As geranylgeranylated Rho family proteins are crucial in synapse/spine formation and remodeling (Newey et al., 2005, Tolias et al., 2011), we hypothesized that GGT deficiency might have detrimental effects that could neutralize the benefits of attenuated AD-related neuropathology. The current study was undertaken to address the impact of GGT deficiency on dendritic spine density and synaptic plasticity, the cellular basis of learning and memory formation (Bliss and Collingridge, 1993, McGaugh, 2000). Our results showed that either germline/systemic GGT haplodeficiency or forebrain neuron-specific GGT deficiency reduced the magnitude of hippocampal long-term potentiation (LTP) and decreased the dendritic spine density of cortical neurons in mice. These findings corroborate the pivotal role of GGT in the development and maintenance of neurophysiological function of the brain.

EXPERIMENTAL PROCEDURES

Animals

Germline/systemic GGT-haplodeficient (GGT+/−) mice have been described previously (Liu et al., 2010, Cheng et al., 2013). The forebrain neuron-specific GGT deficient mice were generated by breeding the GGT floxed ($GGT^{f/f}$) mice (Sjogren et al., 2007) with a CaMKIIα promoter-driven Cre recombinase (Cre+) mice (Tsien et al., 1996). Further interbreeding of resulting siblings with genotypes of $GGT^{f/+}$Cre+ and $GGT^{f/+}$Cre− produced $GGT^{f/f}$Cre+ and $GGT^{f/f}$Cre− (wild type, WT) mice, which were used in this study. All genotypes were determined using DNA extracted from tail biopsies and amplified via PCR using gene-specific primers. The average mouse age was 8–12 months and both male and female were used. Littermate controls were used whenever possible. Investigators were blinded to genotypes of the mice during the experiments. All animal procedures in this study were prospectively reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota.

Electrophysiology

Mice were anesthetized using isoflurane and the depth of anesthesia was confirmed with a foot pinch, followed by decapitation as previously described (Parent et al., 2014). Briefly, brains were collected and cooled in a “cutting solution” containing (mM): 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 7 Dextrose, and 240 sucrose (Sigma). Transverse hippocampal slices (400μm) were prepared using a vibratome (Leica) while immersed in the ice-cold cutting solution. The slices were allowed to recover for at least 1 h (up to 4 h) in an artificial cerebrospinal fluid (aCSF) containing (mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, and 25 dextrose (pH 7.4) with constant bubbling of 95% O_2/5% CO_2. After recovery, slices were placed into the recording chamber (Automate Scientific, Berkeley, CA) with aCSF flowing at approximately 1.5 ml/min at 28–30 °C. Using a bipolar tungsten electrode (FHC) driven by a constant-current stimulus isolator (World Precision Instruments, Sarasota, FL), electrical stimulations were delivered to the CA3/CA1 boundary of the hippocampus targeting axon bundles of CA3 pyramidal neurons. Stimulation intensity was adjusted up to a maximum of 95μA. Recording

electrodes were carefully placed in the CA1 region of the hippocampus. Evoked field excitatory post-synaptic potentials (fEPSPs) were recorded with pulled borosilicate glass micropipettes filled with aCSF using a two channel 700B multiclamp amplifier, 1440A Digidata DA/AD converter, and pClamp data acquisition software (Molecular Devices, Sunnyvale, CA). The stimulation intensity was adjusted to elicit a fEPSP response of 0.6–0.8 mV for 20 minutes to produce a stable baseline. Once a sufficiently stable baseline was established, long-term potentiation (LTP) was induced and monitored for at least 40 min post induction. The induction protocol consists of two trains of theta burst stimulations with a 20-s interval. The fEPSP slopes from the 35–40 minute post induction were normalized to the baseline fEPSP slopes and compared between groups. External noise was reduced via a faraday cage, aluminum shielding, with a 50/60 Hz noise canceling Humbug hardware (Quest Scientific, North Vancouver, BC, Canada). Basal paired-pulse facilitation (PPF) was analyzed during the baseline recordings of LTP experiments. Two stimulations were given in a quick 30-ms succession, the amplitudes for each fEPSP were recorded, and a ratio of these amplitudes yielded the basal PPF ratio, which was compared between groups. Input/output (I/O) curves were generated from similarly prepared slices. Slices were subjected to 5 stimulations per intensity ranging from 0μA up to 150μA in 10μA intervals. The fEPSP magnitudes were averaged for each stimulation intensity and then compared across groups using the repeated measures ANOVA.

Tissue collection and preparation

Tissue collection protocols were followed as previously described (Cheng et al., 2013). Briefly, mice were deeply anesthetized and blood was collected by cardiac puncture with heparinized needles. Following perfusion with ice-cold phosphate buffered saline, brain hemispheres were removed and fixed in 4% paraformaldehyde or snap frozen in liquid nitrogen and stored at −80°C until further analyses.

Preparation of membrane and cytosolic fractions

Cortical samples were homogenized in an ice cold, low osmotic lysis buffer containing 5 mM Tris-HCL (pH 7.4), 2 mM EDTA, 1x protease inhibitors (Roche Applied Science, Indianapolis, IN), and 1x phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Homogenates were centrifuged at 1,500 × *g* for 15 min at 4°C to remove tissue debris. The clear supernatant was transferred to a new tube and further centrifuged at 100,000 × *g* for 60 min at 4°C in a Beckman Coulter Optima™ MAX-XP Tabletop Ultracentrifuge. The resulting supernatant was collected as the cytosolic fraction without disturbing the pellet. The pellet was washed twice with lysis buffer by centrifugation at 100,000 × g for 15 min at 4°C. Finally, the pellet was resuspended in lysis buffer of original volume and this fraction was used as the membrane fraction.

Immunoblot analysis

For immunoblot analysis, equal aliquots of membrane and cytosolic fractions were separated by SDS-PAGE and transferred to PVDF membranes. The membranes were incubated with primary antibodies (all from Santa Cruz Biotechnology, Dallas, TX) against the following: RhoA (26C4, sc-418), Rac1 (C-14, sc-217), H-RAS (C-20, sc-520), and calnexin (H-70, sc-11397). Overnight primary antibody incubation was followed by incubation with HRP-

conjugated secondary antibodies. Signal was detected by the ECL Plus Western Blotting System (GE Healthcare) and quantified using ImageJ software. For confirmation of membrane and cytosolic fractionation, the blots were stripped and re-probed with antibodies against a membrane protein, calnexin (sc-11397), and a cytosolic protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (V-18, sc-20357), respectively.

Golgi staining and analysis of dendritic spine density

Based on the procedures described previously (Dumanis et al., 2009, Adlard et al., 2011), mouse brains were freshly dissected and processed for Golgi staining using the FD Rapid GolgiStain™ kit (Cat. #: PK401A, FD NeuroTechnologies, Inc.). Golgi staining followed all manufactures' instructions. Bright-field microscopy images (100x) were taken of pyramidal neurons in cortical layers II/III. Spine linear density was measured using Image Pro Plus software. Fifteen neurons were randomly selected from each animal and five apical oblique (AO) and five basal shaft (BS) dendrites were chosen from tertiary dendritic segments of each neuron. Spine densities were quantified and compared across groups.

Behavioral assessment

The Morris water maze test was used to assess the spatial learning and memory abilities of mice as described previously (Lewis et al., 2010, Cheng et al., 2013). Briefly, the test was conducted with a round basin filled with water in a room containing extra-maze visual cues for orientation. The acquisition of the spatial task consisted of placing the mice into the basin successively in north (N), east (E), south (S), and west (W) positions, with the escape platform hidden 1 cm beneath water level in the middle of the NE quadrant. In each trial, the mouse was allowed to swim until it found the hidden platform, or until 60 sec had elapsed, at which point the mouse was placed on the platform where it remained for 10 sec before being returned to a cage containing paper towels to dry. The escape latency and swim path length were recorded by the ANYMAZE system (SD instruments, San Diego, CA) for four trials daily for 5 days. The day after the acquisition phase, a probe trial for memory retention was conducted by removing the platform and placing the mouse into the basin at the N position. The time spent in the target quadrant and crosses over the previous platform position were recorded in a single 60 sec trial. Two hours later, the visible platform version of the test was performed with the escape platform lifted 1 cm above water level and moved to the SE quadrant. A 15-ml conical tube filled with black ink was placed upside down on the top of the escape platform as a viewing aid, by which the visual acuity of the mice was evaluated.

Statistical analysis

All data are expressed as means ± standard errors of the means (SE). Comparison of different genotype groups was performed by Student's t test or repeated measures analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

Hippocampal synaptic plasticity is impaired in GGT haplodeficient mice

Our previous work demonstrated that GGT haplodeficient (GGT+/−) mice express ~50% GGT protein content compared with WT mice (Cheng et al., 2013). GGT activity and prenylation of Rho GTPases has a major effect on synaptic turnover and stability (Newey et al., 2005, Tolias et al., 2011). To assess the impact of GGT haplodeficiency on neuroplasticity, we performed a series of electrophysiological experiments at the hippocampal Schaffer collateral-CA1 synapses in acute brain slices.

First, the basal synaptic transmission was assessed by compilation of input-output (I/O) curves (Fig. 2A), which shows the field excitatory post-synaptic potential (fEPSP) amplitude responding to increasing stimulus intensities. There was a trend towards decreased fEPSP amplitudes for a given stimulation intensity in GGT+/− mice compared with their WT littermates; however, it did not reach statistical significance by repeated measures ANOVA. This result indicates that there is no significant change in basal synaptic transmission in the hippocampus of GGT+/− mice.

Next, the short-term presynaptic plasticity was evaluated by examining the paired-pulse facilitation (PPF) ratios, which tests the ability of two successive stimuli to elicit an increased post-synaptic response. The mechanisms underlying PPF are exclusively pre-synaptic (Regehr, 2012). PPF is a function of presynaptic release probability due to increased presynaptic Ca^{2+} concentration, leading to a greater release of the excitatory neurotransmitter, glutamate, to the post-synaptic cell. The results showed that the basal PPF at a 30ms inter-stimulus interval was significantly reduced in GGT+/− mice compared with WT littermates (Fig. 2B). This finding suggests that GGT haplodeficiency may interfere with synaptic vesicle release probability from the pre-synaptic terminals.

Finally, the extent of long-term synaptic plasticity was measured. LTP in the hippocampus is thought to be the cellular mechanism underlying learning and memory and is defined as the long-lasting increase in signal transmission between two neurons (Bliss and Collingridge, 1993, McGaugh, 2000). The results showed that the magnitude of LTP was severely reduced in GGT+/− mice compared with their wild-type littermates (Fig. 2C and 2D). Taken together, these electrophysiological data demonstrate that germline/systemic GGT haplodeficiency impairs both presynaptic and postsynaptic plasticity.

Dendritic spine density of cortical neurons is markedly reduced in GGT haplodeficient mice

Following the electrophysiological experiments, we attempted to explore the cellular and structural basis for the reduced synaptic plasticity observed in GGT+/− mice. Small GTPases that are geranylgeranylated by GGT play critical roles in regulating dendritic spine formation/pruning (Newey et al., 2005, Tolias et al., 2011). Therefore, we quantified dendritic spine density in cortical neurons using the Golgi staining method. The results showed that there was a substantial decrease in both apical and basal dendritic spine counts in GGT+/− mice compared with WT littermate controls (Fig. 3), indicating impaired spine genesis and/or reduced basal synaptic stability (enhanced elimination) in GGT+/− mice.

Because spine formation/density correlates with long-term synaptic plasticity and memory (Segal, 2005, 2017), our results strongly suggest that the reductions in synaptic plasticity in GGT+/− mice are a result of decreases in dendritic spine genesis or stability.

Learning and memory function is impaired in GGT haplodeficient mice

Apparent reduction in synaptic plasticity and dendritic spine density raised the question whether learning and memory function was affected in the GGT+/− mice. To address this question, GGT+/− and WT littermates were subjected to the Morris water maze test. As shown in Fig. 4A, GGT+/− mice clearly exhibited learning impairment in the acquisition phase of the test, compared with the WT controls. Consistently, in the probe trial, GGT+/− mice made significantly fewer crosses over the previous platform position than the WT mice, showing that GGT+/− mice had deficits in memory retention (Fig. 4B). In the visible platform test, both GGT+/− and WT mice were able to locate the escape platform in less than 10 sec on average, indicating no visual acuity problems in these mice. In addition, to exclude the possibility that GGT+/− and WT mice might have different swimming abilities, the path length was recorded during the Morris water maze test and the swimming speed was calculated. The results show that there was no difference between GGT+/− and WT mice in swimming speed (21.1 ± 1.9 vs. 22.3 ± 1.1 cm/sec). These results show that learning and memory impairment in GGT+/− mice was not caused by changes in non-cognitive parameters such as visual acuity or swimming ability.

The magnitude of hippocampal LTP is modestly reduced in forebrain neuron-specific GGT deficient mice

To avoid any potential influence of germline GGT haplodeficiency during embryonic development and to determine the specific role of neuronal GGT in adult brain, we generated forebrain neuron-specific GGT knockout ($GGT^{f/f}$Cre+) mice and their WT littermates ($GGT^{f/f}$Cre−) by crossbreeding $GGT^{f/f}$ mice (Sjogren et al., 2007) with CaMKIIα-Cre mice (Tsien et al., 1996). Since CaMKIIα-Cre is only expressed in neurons of the adult forebrain, the essential function of GGT during embryonic development is preserved in these mice. $GGT^{f/f}$Cre+ mice were subjected to the same sets of electrophysiological experiments as in GGT+/− mice.

The results from the I/O curves showed that there was no difference in the average fEPSP amplitudes for a given stimulation intensity in the brain slices of $GGT^{f/f}$Cre+ mice compared with their $GGT^{f/f}$Cre− littermates (Fig. 5A). Similar I/O responses observed in $GGT^{f/f}$Cre+ and $GGT^{f/f}$Cre− indicate that forebrain neuron-specific GGT deficiency does not affect basal transmission.

Similarly, comparison of PPF ratios showed no significant differences between $GGT^{f/f}$Cre+ mice and $GGT^{f/f}$Cre− littermates (Fig. 5B). These results suggest that $GGT^{f/f}$Cre+ mice do not exhibit a decrease in the probability of synaptic vesicle release from the pre-synaptic terminal. Therefore, forebrain neuron-specific GGT deficiency does not significantly affect presynaptic plasticity, in contrast with the results in GGT+/− mice, in which presynaptic plasticity was compromised.

In the experiments for long-term synaptic plasticity, we found that the magnitude of LTP was reduced in GGT$^{f/f}$Cre+ mice compared with GGT$^{f/f}$Cre− littermates (Fig. 5C and 5D). Interestingly, the reduction in the magnitude of LTP in GGT$^{f/f}$Cre+ mice was less severe than that in GGT+/− mice, relative to their respective WT littermate controls. These results suggest that the normal level of GGT in both neurons and non-neuronal cells is important in maintaining long-term synaptic plasticity.

The dendritic spine density in cortical neurons is reduced in forebrain neuron-specific GGT deficient mice

To address whether the changes in dendritic spine densities underlie the reduced synaptic plasticity in GGT$^{f/f}$Cre+ mice, we quantified tertiary apical and basal dendrites from cortical neurons. We found that both apical and basal dendritic spine counts were decreased in cortical neurons from GGT$^{f/f}$Cre+ mice compared with GGT$^{f/f}$Cre− littermates, indicating the importance of neuronal GGT for maintaining normal dendritic spine densities (Fig 6). Consistent with the reduction in the magnitude of LTP, the reduction of dendritic spine densities in GGT$^{f/f}$Cre+ mice was to a lesser degree than that in GGT+/− mice, relative to their respective WT littermate controls. These results further suggest that the normal level of GGT in both neurons and non-neuronal cells is crucial to maintain dendritic spine density and synaptic plasticity.

Membrane association of geranylgeranylation-targeted small GTPases is reduced in forebrain neuron-specific GGT deficient mice

The well-known targets of GGT are the Rho family of small GTPases, including Rac1 and RhoA, which play pivotal roles in dendritic morphogenesis and synaptic plasticity (Newey et al., 2005, Tolias et al., 2011). These proteins are extensively geranylgeranylated by GGT under normal physiological conditions and this lipid modification is required for their proper membrane association and function. To determine the impact of neuron-specific GGT deletion on cellular location of small GTPases in the brain of GGT$^{f/f}$Cre+ mice, the tissue lysate of cerebral cortex was subjected to subcellular fractionation by ultracentrifugation, followed by immunoblot analysis of Rac1, RhoA, and H-Ras. The results showed that the levels of membrane-associated (geranylgeranylated) Rac1 and RhoA were significantly reduced, while their levels in cytosolic (un-geranylgeranylated) fractions increased in GGT$^{f/f}$Cre+ mice compared with GGT$^{f/f}$Cre− littermates (Fig. 7). Importantly, the cellular distribution of H-Ras, an exclusively farnesylated small GTPase[40], was unaffected (Fig. 7), indicating that the specific deletion of GGT only reduced geranylgeranylation of target proteins but not farnesylation of other proteins. In addition, these findings validated that the reduction of protein geranylgeranylation could be readily detected in the tissue lysate of the cerebral cortex even though only neuronal GGT was deleted in GGT$^{f/f}$Cre+ mice. As geranylgeranylation plays a critical role in the cellular trafficking and function of Rac1 and RhoA, reduced geranylgeranylation of these small GTPases most likely underlies the reduction of dendritic spine density and the impairment of synaptic plasticity in GGT-deficient mice.

DISCUSSION

The role of GGT in the nervous system has been explored previously. Gao et al summarized recent findings on the role of GGT-mediated prenylation in the brain (Gao et al., 2016). However, the majority of the previous studies used pharmacological approaches to manipulate the activity of GGT. While statins and GGT inhibitors are valuable pharmacological tools to probe prenylation pathways, these approaches have limitations. Statins inhibit the activity of HMG-CoA reductase and thus decease the production of isoprenoid substrates instead of directly inhibiting the activity of a specific prenyltransferase (Goldstein and Brown, 1990, Endo, 1992, Eckert et al., 2009). Statin-induced limitation of isoprenoids (FPP and GGPP) could affect both farnesylation and geranylgeranylation by all prenyltransferases rather than GGT alone (Winter-Vann and Casey, 2005). In addition, prenylation-independent effects of isoprenoids and cholesterol further complicate the impact of treatment with statins (Forman et al., 1995, Hanley et al., 2000, Gan et al., 2001, Holstein et al., 2002, Kukar et al., 2005, Murthy et al., 2005). Although synthetic inhibitors targeting GGT have facilitated some of the experiments, potential side effects and the poor blood-brain barrier permeability limit the application of these inhibitors for long-term studies *in vivo* (Lobell et al., 2001, Noker et al., 2008, Khan et al., 2011, Yuan et al., 2015). Here we took the genetic approach and generated conditional forebrain neuron-specific GGT deficient mice, as well as using germline/systemic GGT haplodeficient mice, to better understand the role of GGT in synaptic function.

We found that there were no significant changes in basal synaptic transmission in either GGT+/− or $GGT^{f/f}$Cre+ mice, evidenced by similar I/O curves from different genotypes. These results indicate that these mice similar post-synaptic responses compared with WT mice. In the experiments measuring the presynaptic short-term plasticity, we found that hippocampal PPF ratios were reduced in GGT+/− mice, but not in $GGT^{f/f}$Cre+ mice. Changes in PPF ratio indicate a reduced ability for presynaptic neurons to release neurotransmitter to the post-synaptic cell. PPF changes are exclusively pre-synaptic and can be caused by several mechanisms (Regehr, 2012), such as increased Ca^{2+} sequestration, misaligned cytoskeletal features that reduce the efficiency of vesicle release, or increased inhibitor signaling from GABAergic neurons. It is not clear which mechanisms were responsible for the reduced PPF ratios in GGT+/− mice. Interestingly, such deficits in PPF did not occur in $GGT^{f/f}$Cre+ mice, suggesting that the changes in short-term plasticity might be a result of altered neuronal development caused by a germline reduction of GGT in GGT +/− mice. This notion is supported by the importance of GGT during early development, as a complete germline deletion of GGT is embryonically lethal (Sjogren et al., 2007, Liu et al., 2010).

The induction and maintenance of LTP in the hippocampus is widely considered as the cellular/synaptic correlate of learning and memory (Bliss and Collingridge, 1993, McGaugh, 2000). We found that the magnitude of LTP was reduced in both GGT+/− and $GGT^{f/f}$Cre+ mice, indicating the importance of both systemic and neuronal GGT in long-term synaptic plasticity. Intriguingly, the deficit in LTP was more severe in GGT+/− mice than that in $GGT^{f/f}$Cre+ mice compared with their respective WT littermates. This observation further suggests the critical role of normal GGT levels during early development and for synaptic

function in mature brain. Germline haplodeficiency of GGT led to a significant reduction in both short-term and long-term synaptic plasticity. Our results corroborate some of the previous findings using GGT inhibitors. It has been shown that while acute treatment with a GGT inhibitor in hippocampal slices from WT mice does not affect the induction and maintenance of LTP (Kotti et al., 2008, Mans et al., 2012), long-term treatment with a GGT inhibitor reduces the magnitude of LTP (Kotti et al., 2008).

Modifiable by activity and experience, dendritic spines have long been thought to provide a morphological basis for LTP and memory (Segal, 2005, 2017). Our study showed that dendritic spine densities in cortical neurons were significantly reduced in both GGT+/− and $GGT^{f/f}$Cre+ mice. Consistent with our results from LTP experiments, the reduction of dendritic spine density was more evident in GGT+/− mice than in $GGT^{f/f}$Cre+ mice compared with their respective WT littermate controls. It is worth noting that dendritic spines undergo a dynamic process. The dendritic spine density is regulated by the rate of spine formation and elimination during development and in the mature brain (Alvarez and Sabatini, 2007, Berry and Nedivi, 2017). Some spines only exist for minutes to hours whereas other spines persist for days to years (Berry and Nedivi, 2017). In the present study, the steady-state counts (“snapshots”) of dendritic spines were assessed and the net decrease in dendritic spines in GGT-deficient mice could result from either attenuated formation of new spines or enhanced elimination of old spines. Future studies using *in vivo* imaging techniques will be required to differentiate the process of spine formation and elimination in these animals. In addition, the morphologies of the dendritic spines are diverse. Based on their shapes, the dendritic spines can be categorized into four different types: mushroom, thin, and stubby spines and filopodia (Berry and Nedivi, 2017). Mushroom spines are defined as the spines that have a large bulbous head and a short, narrow neck. These spines are considered to be more mature and contain the largest excitatory synapses. Thin spines refer to those smaller spines that lack the bulbous head and the thin neck. Compared with the mushroom-type spines, the thin spines carry smaller or immature synapses but they are more dynamic and may have higher capacity for synaptic plasticity (Harris et al., 1992). The stubby spines are shorter than thin spines, without a clear head and neck structure. These spines are considered immature, as they exist primarily during early development and rarely in the mature brain (Harris et al., 1992). The last type is called filopodia, which are smallest, hairlike structures that often lack synapse and are considered most immature and dynamic (Ziv and Smith, 1996, Knott et al., 2006). Notably, it has been recognized that the resolution of light microscopy for distinguishing small morphological differences is limited and many researchers count any resolvable dendritic protrusion as a spine (Berry and Nedivi, 2017). In the present study, we adopted this approach and counted all dendritic protrusions as spines. We observed that there were different shapes of spines protruding from the dendrites of cortical neurons. However, due to the resolution limitation of the images, we were only able to differentiate the mushroom-shaped spines from other types. Based on the estimation, there was an approximately 32% decrease in the mature mushroom-shaped spines in GGT+/− and $GGT^{f/f}$Cre+ mice, compared with their respective WT controls. These results suggest that immaturity of the spines as well as reduced spine density might contribute to the impaired synaptic plasticity in these mice, although the limited resolution of light microscopy precluded the accurate quantification of each type of dendritic spines. As the importance of

GGT in synaptic function becomes evident, further investigations on the role of GGT in regulating spine/synapse structure and dynamics using high resolution imaging techniques are warranted.

Our results on dendritic spine densities in GGT-deficient mice are in line with previous findings from studies using pharmacological and transgenic approaches. Using an inhibitor of GGT *in vitro* and transgenic expression of a dominant-negative mutant GGT *in vivo*, Luo et al reported that GGT might be a signaling molecule itself and GGT-mediated protein prenylation could play an important role in regulating neuromuscular synapse formation and/or maintenance (Luo et al., 2003). Subsequent studies showed that activation of GGT is required for activity- and brain-derived neurotrophic factor (BDNF)–dependent dendritic morphogenesis and synaptogenesis in cultured hippocampal neurons and cerebellar Purkinje cells (Zhou et al., 2008, Wu et al., 2010, Li et al., 2013). Further, inhibition of GGT activity by lateral ventricular injection of a GGT inhibitor was found to decrease dendritic spine density in the hippocampus of treated mice (Yuan et al., 2015). In addition, it was reported that GGT inhibitor-treated mice showed a decrease in learning and memory ability in the Morris water maze test (Yuan et al., 2015). In agreement with the results using the pharmacological treatment, our study showed that GGT-haplodeficient mice exhibited impairment in both learning and memory function compared with their WT littermate controls. These findings demonstrate that GGT deficiency leads to cognitive impairment, most likely due to dendritic spine/synaptic dysfunction at the cellular level. Others have also identified GGT as a significant player in the regulation of neurite/dendritic outgrowth and synaptic markers (Samuel et al., 2014, Li et al., 2016, Moutinho et al., 2016). However, not all results are consistent. For example, Samuel et al have shown that inhibition of geranylgeranylation increases neurite branching in cultured neurons (Samuel et al., 2014). Li et al recently reported that protein prenylation acts as an endogenous brake on axonal growth (Li et al., 2016). Through high-throughput drug screening, they identified statins as the most effective molecules to enhance neurite outgrowth of different types of neurons in culture and demonstrated that inhibition of protein prenylation accounted for the statin-induced increase in axonal growth. They further showed that the expression level of GGT, but not FT, was elevated in motor neurons of patients with early-onset amyotrophic lateral sclerosis (ALS), suggesting that protein prenylation inhibitors might have therapeutic potential to accelerate neuronal regeneration. It is worth noting that this elevation of GGT expression only occurs under the pathological condition of early-onset ALS and specifically in motor neurons (Li et al., 2016). Thus, inhibition of GGT in motor neurons of early-onset cases of ALS to normal level of GGT might be beneficial. However, here we show that under physiological conditions, germline/systemic or forebrain neuron-specific reduction of GGT results in detrimental effects on synaptic plasticity and dendritic spine density. These findings indicate that a proper level of GGT expression/activity is required for normal structure and function of neurons during development and in mature brain.

It is well known that Rho GTPases are major regulators of synaptic plasticity, dendritic growth, and spine morphogenesis (Newey et al., 2005, Tolias et al., 2011). These small GTPases primarily undergo geranylgeranylation, which is critical for their proper cellular localization and interactions with their downstream effector proteins. In particular, the role of major Rho proteins, such as Rac1 and RhoA, in neuronal structure and function has been

extensively studied (Newey et al., 2005, Tolias et al., 2011). Activation of Rac1 promotes dendritic arborization, remodeling, and synapse formation, whereas activation of RhoA exhibits opposite effects, reducing dendritic complexity and spine density. Thus, Rac1 and RhoA have a crucial regulatory role to ensure the plasticity of dendritic structure and function. Our study provides evidence that GGT deficiency in neurons caused a significant reduction in the membrane association (geranylgeranylation) of Rac1 and RhoA in the brain of $GGT^{f/f}$Cre+ mice. Such mislocalization of Rac1 and RhoA caused by inadequate geranylgeranylation is expected to impair their interactions with effector proteins and disrupt the balance of their functions, leading to dendritic spine destabilization. Indeed, the critical role of prenylated Rac1 in dendritic morphogenesis has been shown previously; deletion or mutation of the geranylgeranylation site in Rac1 fails to mediate the beneficial effects of GGT overexpression on dendritic arborization in cultured neurons (Zhou et al., 2008). Using a similar approach, another study reported that expression of a non-prenylated Rac1 led to abnormal cell morphology and neurite initiation because of aberrant activation of cytosolic signaling pathways (Reddy et al., 2015). Therefore, reduction of dendritic spine density and synaptic plasticity in GGT-deficient mice most likely result from inadequate geranylgeranylation and dysfunction of these Rho proteins. Interestingly, a recent study found that GGT activity and protein/gene expression levels were significantly decreased in the brains of aged mice compared with those of young mice (Afshordel et al., 2014). Consistently, membrane-associated (geranylgeranylated) RhoA and Rac1 levels were reduced in the aged mouse brains. These findings suggest that GGT/protein geranylgeranylation may play an important role in brain aging and that GGT-deficient mice may serve as a model of accelerated brain aging. Certainly, GGT target proteins are not limited to Rac1 and RhoA. To fully assess the scope of geranylgeranylated proteins affected by GGT deficiency, an unbiased prenylomic analysis will be required. Currently such a prenylomic approach has been applied for studies *in vitro* and is actively pursued for studies *in vivo* (Palsuledesai et al., 2016).

The present study demonstrates that germline/systemic or forebrain neuron specific deficiency of GGT reduces dendritic spine density and impairs synaptic plasticity in the brains of young adult mice, concurrently with reduced geranylgeranylation of Rho proteins. These results closely resemble changes found in the brains of aged mice, suggesting an important role of GGT and its target proteins in normal brain aging. While inhibition of GGT could be beneficial under pathological conditions with overactivation or upregulation of GGT, our findings caution potential adverse effects on synaptic/cognitive function from the chronic use of GGT inhibitors.

Acknowledgments

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ABBREVIATIONS

ALS	amyotrophic lateral sclerosis
aCSF	artificial cerebrospinal fluid
AD	Alzheimer's disease
ANOVA	analysis of variance
AO	apical oblique
BS	basal shaft
CaMKII α	Ca2+/calmodulin-dependent protein kinase
CNS	central nervous system
Cre+	Cre recombinase transgene positive
FEPSPs	field excitatory post-synaptic potentials
FPP	farnesyl pyrophosphate
FT	farnesyltransferase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GGPP	geranylgeranyl pyrophosphate
GGT	geranylgeranyltransferase-1
GGT+/−	GGT-haplodeficient
$GGT^{f/f}$	GGT gene floxed
GGTf/fCre+	conditional forebrain neuron-specific GGT knockout
GTP	guanosine triphosphate
I/O	input/output
LTP	long-term potentiation
PPF	paired pulse facilitation
WT	wild type

References

Adlard PA, Bica L, White AR, Nurjono M, Filiz G, Crouch PJ, Donnelly PS, Cappai R, Finkelstein DI, Bush AI. Metal ionophore treatment restores dendritic spine density and synaptic protein levels in a mouse model of Alzheimer's disease. PLoS One. 2011; 6:e17669. [PubMed: 21412423]

Afshordel S, Wood WG, Igbavboa U, Muller WE, Eckert GP. Impaired geranylgeranyltransferase-I regulation reduces membrane-associated Rho protein levels in aged mouse brain. J Neurochem. 2014; 129:732–742. [PubMed: 24428713]

Alvarez VA, Sabatini BL. Anatomical and physiological plasticity of dendritic spines. Annu Rev Neurosci. 2007; 30:79–97. [PubMed: 17280523]

Berndt N, Hamilton AD, Sebti SM. Targeting protein prenylation for cancer therapy. Nat Rev Cancer. 2011; 11:775–791. [PubMed: 22020205]

Berry KP, Nedivi E. Spine Dynamics: Are They All the Same? Neuron. 2017; 96:43–55. [PubMed: 28957675]

Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993; 361:31–39. [PubMed: 8421494]

Cheng S, Cao D, Hottman DA, Yuan L, Bergo MO, Li L. Farnesyltransferase haplodeficiency reduces neuropathology and rescues cognitive function in a mouse model of Alzheimer disease. J Biol Chem. 2013; 288:35952–35960. [PubMed: 24136196]

Dumanis SB, Tesoriero JA, Babus LW, Nguyen MT, Trotter JH, Ladu MJ, Weeber EJ, Turner RS, Xu B, Rebeck GW, Hoe HS. ApoE4 decreases spine density and dendritic complexity in cortical neurons in vivo. J Neurosci. 2009; 29:15317–15322. [PubMed: 19955384]

Eckert GP, Hooff GP, Strandjord DM, Igbavboa U, Volmer DA, Muller WE, Wood WG. Regulation of the brain isoprenoids farnesyl- and geranylgeranylpyrophosphate is altered in male Alzheimer patients. Neurobiol Dis. 2009; 35:251–257. [PubMed: 19464372]

Endo A. The Discovery and Development of Hmg-Coa Reductase Inhibitors. Journal of Lipid Research. 1992; 33:1569–1582. [PubMed: 1464741]

Forman BM, Goode E, Chen J, Oro AE, Bradley DJ, Perlmann T, Noonan DJ, Burka LT, McMorris T, Lamph WW, Evans RM, Weinberger C. Identification of a nuclear receptor that is activated by farnesol metabolites. Cell. 1995; 81:687–693. [PubMed: 7774010]

Gan X, Kaplan R, Menke JG, MacNaul K, Chen Y, Sparrow CP, Zhou G, Wright SD, Cai TQ. Dual mechanisms of ABCA1 regulation by geranylgeranyl pyrophosphate. J Biol Chem. 2001; 276:48702–48708. [PubMed: 11641412]

Gao S, Yu R, Zhou X. The Role of Geranylgeranyltransferase I-Mediated Protein Prenylation in the Brain. Mol Neurobiol. 2016; 53:6925–6937. [PubMed: 26666664]

Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature. 1990; 343:425–430. [PubMed: 1967820]

Hanley K, Komuves LG, Ng DC, Schoonjans K, He SS, Lau P, Bikle DD, Williams ML, Elias PM, Auwerx J, Feingold KR. Farnesol stimulates differentiation in epidermal keratinocytes via PPARalpha. J Biol Chem. 2000; 275:11484–11491. [PubMed: 10753967]

Harris KM, Jensen FE, Tsao B. Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. J Neurosci. 1992; 12:2685–2705. [PubMed: 1613552]

Holstein SA, Wohlford-Lenane CL, Hohl RJ. Isoprenoids influence expression of Ras and Ras-related proteins. Biochemistry. 2002; 41:13698–13704. [PubMed: 12427032]

Hottman DA, Li L. Protein prenylation and synaptic plasticity: implications for Alzheimer's disease. Mol Neurobiol. 2014; 50:177–185. [PubMed: 24390573]

Khan OM, Ibrahim MX, Jonsson IM, Karlsson C, Liu M, Sjogren AK, Olofsson FJ, Brisslert M, Andersson S, Ohlsson C, Hulten LM, Bokarewa M, Bergo MO. Geranylgeranyltransferase type I (GGTase-I) deficiency hyperactivates macrophages and induces erosive arthritis in mice. J Clin Invest. 2011; 121:628–639. [PubMed: 21266780]

Knott GW, Holtmaat A, Wilbrecht L, Welker E, Svoboda K. Spine growth precedes synapse formation in the adult neocortex in vivo. Nat Neurosci. 2006; 9:1117–1124. [PubMed: 16892056]

Kotti T, Head DD, McKenna CE, Russell DW. Biphasic requirement for geranylgeraniol in hippocampal long-term potentiation. Proc Natl Acad Sci U S A. 2008; 105:11394–11399. [PubMed: 18685105]

Krishna RG, Wold F. Post-translational modification of proteins. Advances in enzymology and related areas of molecular biology. 1993; 67:265–298. [PubMed: 8322616]

Kukar T, Murphy MP, Eriksen JL, Sagi SA, Weggen S, Smith TE, Ladd T, Khan MA, Kache R, Beard J, Dodson M, Merit S, Ozols VV, Anastasiadis PZ, Das P, Fauq A, Koo EH, Golde TE. Diverse compounds mimic Alzheimer disease-causing mutations by augmenting Abeta42 production. Nat Med. 2005; 11:545–550. [PubMed: 15834426]

Lane KT, Beese LS. Thematic review series: lipid posttranslational modifications. Structural biology of protein farnesyltransferase and geranylgeranyltransferase type I. J Lipid Res. 2006; 47:681–699. [PubMed: 16477080]

Lewis TL, Cao D, Lu H, Mans RA, Su YR, Jungbauer L, Linton MF, Fazio S, LaDu MJ, Li L. Overexpression of human apolipoprotein A-I preserves cognitive function and attenuates neuroinflammation and cerebral amyloid angiopathy in a mouse model of Alzheimer disease. J Biol Chem. 2010; 285:36958–36968. [PubMed: 20847045]

Li H, Kuwajima T, Oakley D, Nikulina E, Hou J, Yang WS, Lowry ER, Lamas NJ, Amoroso MW, Croft GF, Hosur R, Wichterle H, Sebti S, Filbin MT, Stockwell B, Henderson CE. Protein Prenylation Constitutes an Endogenous Brake on Axonal Growth. Cell reports. 2016; 16:545–558. [PubMed: 27373155]

Li L, Zhang W, Cheng S, Cao D, Parent M. Isoprenoids and related pharmacological interventions: potential application in Alzheimer's disease. Mol Neurobiol. 2012; 46:64–77. [PubMed: 22418893]

Li Z, Sun C, Zhang T, Mo J, Shi Q, Zhang X, Yuan M, Chen L, Mao X, Yu R, Zhou X. Geranylgeranyltransferase I mediates BDNF-induced synaptogenesis. J Neurochem. 2013; 125:698–712. [PubMed: 23534605]

Liu M, Sjogren AK, Karlsson C, Ibrahim MX, Andersson KM, Olofsson FJ, Wahlstrom AM, Dalin M, Yu H, Chen Z, Yang SH, Young SG, Bergo MO. Targeting the protein prenyltransferases efficiently reduces tumor development in mice with K-RAS-induced lung cancer. Proc Natl Acad Sci U S A. 2010; 107:6471–6476. [PubMed: 20308544]

Lobell RB, Omer CA, Abrams MT, Bhimnathwala HG, Brucker MJ, Buser CA, Davide JP, deSolms SJ, Dinsmore CJ, Ellis-Hutchings MS, Kral AM, Liu D, Lumma WC, Machotka SV, Rands E, Williams TM, Graham SL, Hartman GD, Oliff AI, Heimbrook DC, Kohl NE. Evaluation of farnesyl:protein transferase and geranylgeranyl:protein transferase inhibitor combinations in preclinical models. Cancer Res. 2001; 61:8758–8768. [PubMed: 11751396]

Luo ZG, Je HS, Wang Q, Yang F, Dobbins GC, Yang ZH, Xiong WC, Lu B, Mei L. Implication of geranylgeranyltransferase I in synapse formation. Neuron. 2003; 40:703–717. [PubMed: 14622576]

Mans RA, McMahon LL, Li L. Simvastatin-mediated enhancement of long-term potentiation is driven by farnesyl-pyrophosphate depletion and inhibition of farnesylation. Neuroscience. 2012; 202:1–9. [PubMed: 22192838]

McGaugh JL. Memory--a century of consolidation. Science. 2000; 287:248–251. [PubMed: 10634773]

McTaggart SJ. Isoprenylated proteins. Cell Mol Life Sci. 2006; 63:255–267. [PubMed: 16378247]

Mijimolle N, Velasco J, Dubus P, Guerra C, Weinbaum CA, Casey PJ, Campuzano V, Barbacid M. Protein farnesyltransferase in embryogenesis, adult homeostasis, and tumor development. Cancer cell. 2005; 7:313–324. [PubMed: 15837621]

Moutinho M, Nunes MJ, Correia JC, Gama MJ, Castro-Caldas M, Cedazo-Minguez A, Rodrigues CM, Bjorkhem I, Ruas JL, Rodrigues E. Neuronal cholesterol metabolism increases dendritic outgrowth and synaptic markers via a concerted action of GGTase-I and Trk. Scientific reports. 2016; 6:30928. [PubMed: 27491694]

Murthy S, Tong H, Hohl RJ. Regulation of fatty acid synthesis by farnesyl pyrophosphate. J Biol Chem. 2005; 280:41793–41804. [PubMed: 16221687]

Newey SE, Velamoor V, Govek EE, Van Aelst L. Rho GTPases, dendritic structure, and mental retardation. J Neurobiol. 2005; 64:58–74. [PubMed: 15884002]

Noker P, Coward L, Gorman G, Jia L. Pharmacokinetics, tissue distribution, and excretion of GGTI-2418, an inhibitor of geranylgeranyl transferase I, in mice. Cancer Research. 2008; 68(Supplement)

Palsuledesai CC, Ochocki JD, Kuhns MM, Wang YC, Warmka JK, Chernick DS, Wattenberg EV, Li L, Arriaga EA, Distefano MD. Metabolic Labeling with an Alkyne-modified Isoprenoid Analog Facilitates Imaging and Quantification of the Prenylome in Cells. ACS chemical biology. 2016; 11:2820–2828. [PubMed: 27525511]

Parent MA, Hottman DA, Cheng S, Zhang W, McMahon LL, Yuan LL, Li L. Simvastatin Treatment Enhances NMDAR-Mediated Synaptic Transmission by Upregulating the Surface Distribution of the GluN2B Subunit. Cell Mol Neurobiol. 2014

Reddy JM, Samuel FG, McConnell JA, Reddy CP, Beck BW, Hynds DL. Non-prenylatable, cytosolic Rac1 alters neurite outgrowth while retaining the ability to be activated. Cellular signalling. 2015; 27:630–637. [PubMed: 25479592]

Regehr WG. Short-term presynaptic plasticity. Cold Spring Harbor perspectives in biology. 2012; 4:a005702. [PubMed: 22751149]

Samuel F, Reddy J, Kaimal R, Segovia V, Mo H, Hynds DL. Inhibiting geranylgeranylation increases neurite branching and differentially activates cofilin in cell bodies and growth cones. Mol Neurobiol. 2014; 50:49–59. [PubMed: 24515839]

Segal M. Dendritic spines and long-term plasticity. Nat Rev Neurosci. 2005; 6:277–284. [PubMed: 15803159]

Segal M. Dendritic spines: Morphological building blocks of memory. Neurobiol Learn Mem. 2017; 138:3–9. [PubMed: 27311757]

Sjogren AK, Andersson KM, Liu M, Cutts BA, Karlsson C, Wahlstrom AM, Dalin M, Weinbaum C, Casey PJ, Tarkowski A, Swolin B, Young SG, Bergo MO. GGTase-I deficiency reduces tumor formation and improves survival in mice with K-RAS-induced lung cancer. J Clin Invest. 2007; 117:1294–1304. [PubMed: 17476360]

Tolias KF, Duman JG, Um K. Control of synapse development and plasticity by Rho GTPase regulatory proteins. Prog Neurobiol. 2011; 94:133–148. [PubMed: 21530608]

Tsien JZ, Chen DF, Gerber D, Tom C, Mercer EH, Anderson DJ, Mayford M, Kandel ER, Tonegawa S. Subregion- and cell type-restricted gene knockout in mouse brain. Cell. 1996; 87:1317–1326. [PubMed: 8980237]

Wang M, Casey PJ. Protein prenylation: unique fats make their mark on biology. Nat Rev Mol Cell Biol. 2016; 17:110–122. [PubMed: 26790532]

Winter-Vann AM, Casey PJ. Post-prenylation-processing enzymes as new targets in oncogenesis. Nat Rev Cancer. 2005; 5:405–412. [PubMed: 15864282]

Wu KY, Zhou XP, Luo ZG. Geranylgeranyltransferase I is essential for dendritic development of cerebellar Purkinje cells. Molecular brain. 2010; 3:18. [PubMed: 20540740]

Yuan M, Gao S, Sun C, Chen L, Shi Q, Hu J, Yu R, Zhou X. Inhibiting geranylgeranyltransferase I activity decreases spine density in central nervous system. Hippocampus. 2015; 25:373–384. [PubMed: 25330763]

Zhou XP, Wu KY, Liang B, Fu XQ, Luo ZG. TrkB-mediated activation of geranylgeranyltransferase I promotes dendritic morphogenesis. Proc Natl Acad Sci U S A. 2008; 105:17181–17186. [PubMed: 18957540]

Ziv NE, Smith SJ. Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. Neuron. 1996; 17:91–102. [PubMed: 8755481]

Highlights

- Protein prenylation catalyzed by gerenylgeranyltransferase-1 (GGT) plays key roles in neurological functions.
- Genetic reduction of GGT systemically or in forebrain neurons decreases synaptic plasticity and dendritic spine density.
- Germline haplodeficiency of GGT leads to impaired learning and memory.
- Insufficient geranylgeranylation of Rho family of small GTPases mediates the adverse effects of GGT deficiency.
- Normal GGT is crucial for maintaining spine structure and synaptic/cognitive function in development and in mature brain.

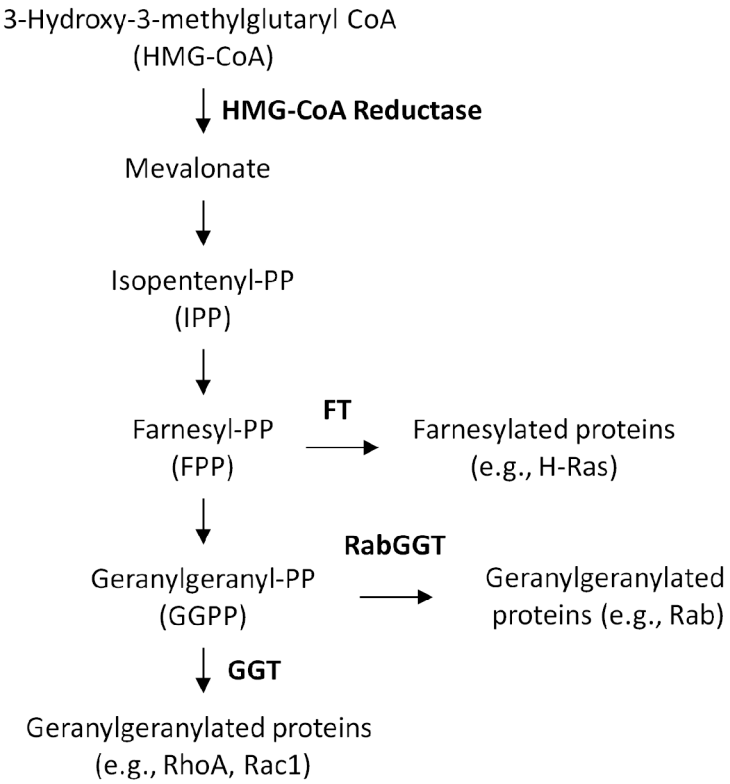

Fig. 1. Isoprenoid synthesis and protein prenylation pathways

PP pyrophosphate; FT, farnesyltransferase; GGT, geranylgeranyltransferease-1; RabGGT, geranylgeranyltransferase-2.

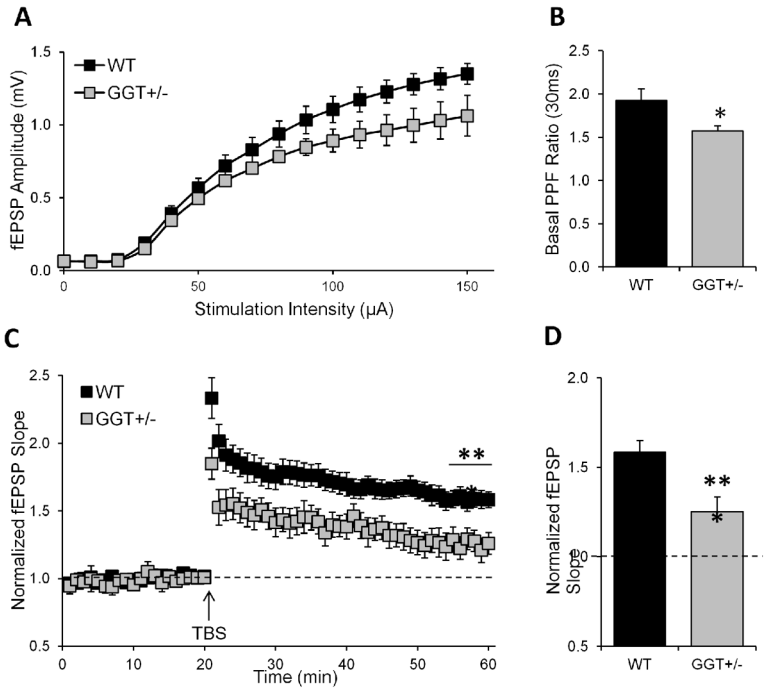

Fig. 2. Basal synaptic transmission, short- and long-term synaptic plasticity in GGT+/− and WT mice

A) Input/output (I/O) curves. The field excitatory post-synaptic potentials (fEPSPs) were recorded and averaged from 5 stimulations per intensity ranging from 0μA up to 150μA in 10μA intervals in hippocampal slices from GGT+/− and WT mice (n = 15–25 slices/5 mice per genotype). **B**) Paired-pulse facilitation (PPF) ratios. Basal PPF responses were quantified from baseline recordings during LTP experiments in hippocampal slices from GGT+/− and WT mice (n = 21 slices/5–7 mice per genotype). Two stimulations were performed in a quick 30ms succession. The amplitudes for each fEPSPs were recorded and the PPF ratio was expressed as the ratio of the responses from the stimulations. **C**) and **D**) Long-term potentiation (LTP). Following a 20-min stable baseline, the LTP was induced by a theta burst stimulation protocol with a 20-sec interval and the fEPSPs recorded. The magnitude of LTP was expressed as the slopes of the rising phase of the fEPSPs normalized to baseline in hippocampal slices from GGT+/− and WT mice (n = 24–32 slices/9–10 mice per genotype. The LTP magnitude of the last 5 minutes (35–40 minute post-induction) was averaged for each genotype of mice. *$P < 0.05$; *** $P < 0.001$.

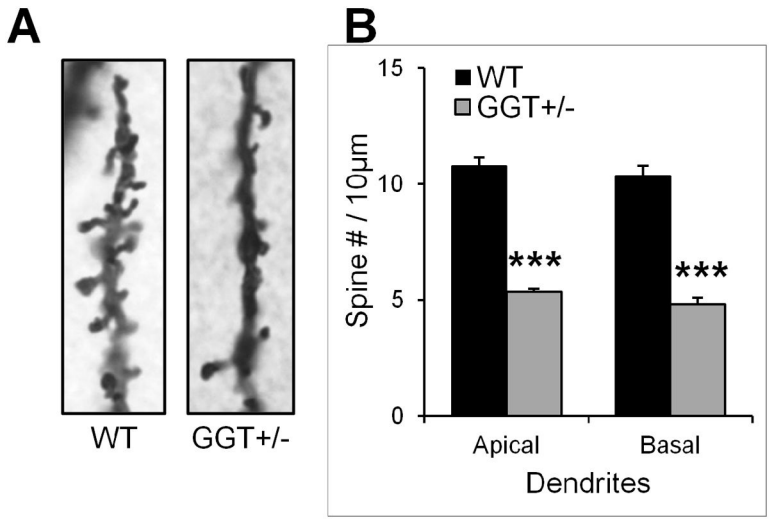

Fig. 3. Dendritic spine density in pyramidal neurons in cortical layers II/III of GGT+/− and WT mice

A) Representative images of dendrites in Golgi-stained brain sections from GGT+/− and WT mice (n=4 mice/genotype). **B**) Average spine densities on apical oblique and basal shaft dendrites of pyramidal neurons (n = 75 apical or basal dendritic segments/15 neurons per mouse). *** $P < 0.001$.

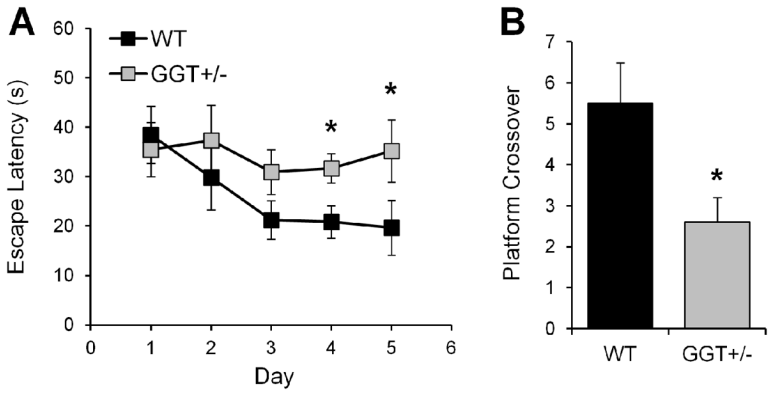

Fig. 4. Spatial learning and memory performance of GGT+/− and WT mice in the Morris water maze test

A) Escape latencies during the acquisition phase of the Morris water maze test (n=5–6 mice/genotype). **B**) Platform crossover in the target quadrant in the probe trial (n=5–6 mice/genotype). * $P < 0.05$.

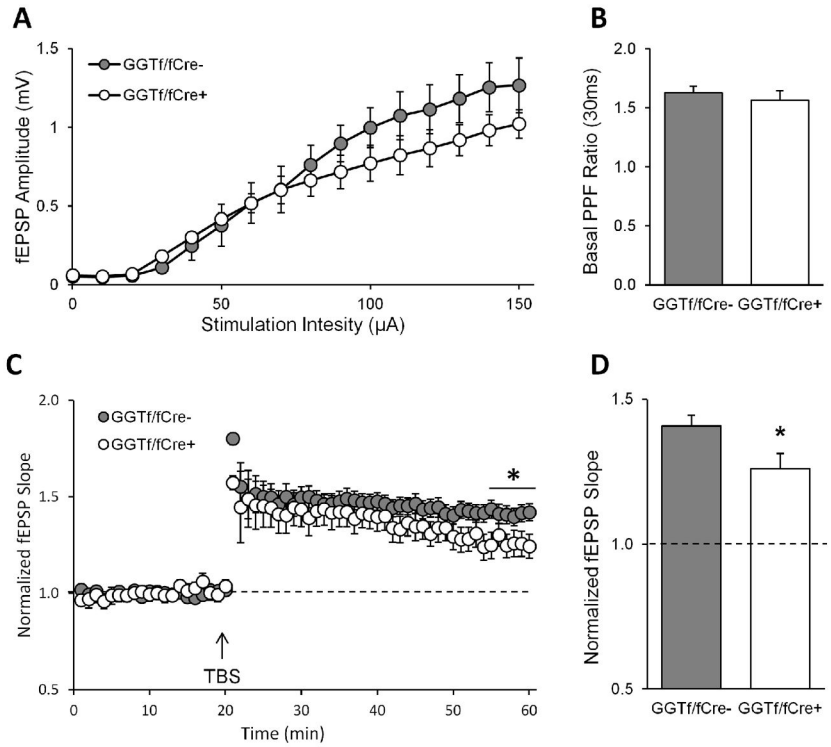

Fig. 5. Basal synaptic transmission, short- and long-term synaptic plasticity in GGTf/fCre+ and GGTf/fCre− mice

A) Input/output (I/O) curves. The field excitatory post-synaptic potentials (fEPSPs) were recorded and averaged from 5 stimulations per intensity ranging from 0μA up to 150μA in 10μA intervals in hippocampal slices from GGTf/fCre+ and GGTf/fCre− mice (n = 17–19 slices/4 mice per genotype). **B**) Paired-pulse facilitation (PPF) ratios. Basal PPF responses were quantified from baseline recordings during LTP experiments in hippocampal slices from GGTf/fCre+ and GGTf/fCre− mice (n = 17–20 slices/9 mice per genotype). Two stimulations were performed in a quick 30ms succession. The amplitudes for each fEPSPs were recorded and the PPF ratio was expressed as the ratio of the responses from the stimulations. **C**) and **D**) Long-term potentiation (LTP). Following a 20-min stable baseline, the LTP was induced by a theta burst stimulation protocol with a 20-sec interval and the fEPSPs recorded. The magnitude of LTP was expressed as the slopes of the rising phase of the fEPSPs normalized to baseline in hippocampal slices from GGTf/fCre+ and GGTf/fCre− mice (n = 18–24 slices/10 mice per genotype. The LTP magnitude of the last 5 minutes (35–40 minute post-induction) was averaged for each genotype of mice. $*P < 0.05$.

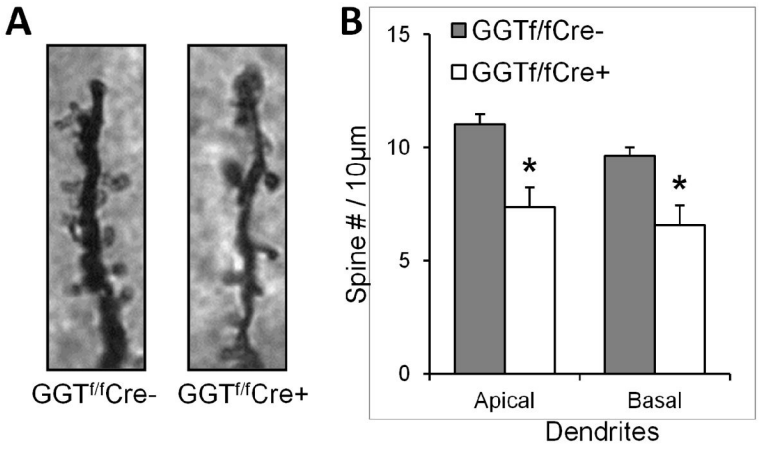

Fig. 6. Dendritic spine density in pyramidal neurons in cortical layers II/III of GGTf/fCre+ and GGTf/fCre− mice

A) Representative images of dendrites in Golgi-stained brain sections from GGTf/fCre+ and GGTf/fCre− mice (n=3–4 mice/genotype). **B**) Average spine densities on apical oblique and basal shaft dendrites of pyramidal neurons (n = 75 apical or basal dendritic segments/15 neurons per mouse). * $P < 0.05$.

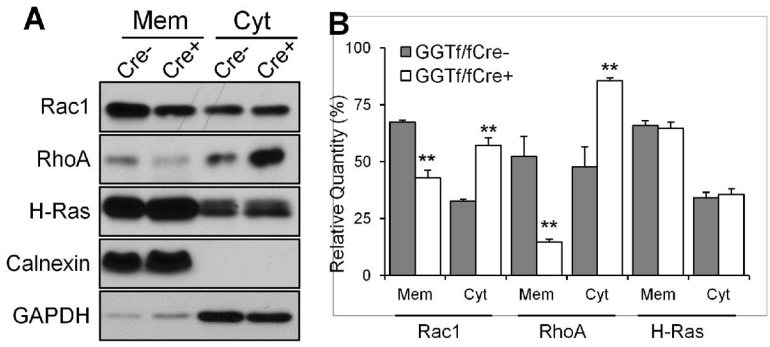

Fig. 7. Subcellular distribution of selected small GTPases in the brain of GGTf/fCre+ and GGTf/fCre− mice

Forebrain tissue homogenates from GGTf/fCre+ and GGTf/fCre− mice were subjected to ultracentrifugation for the preparation of membrane (Mem) and cytosolic (Cyt) fractions (n = 3 mice/genotype). **A**) Representative images from immunoblot analyses for Rac1, RhoA, and H-Ras. Calnexin and GAPDH were used as markers for membrane-associated and cytosolic proteins, respectively. **B**) Densitometric quantification of immunoblot analysis, showing relative distribution of each protein in the membrane (prenylated) and cytosolic (unprenylated) fraction. $^{**}P < 0.01$.