ANTAGONISTIC REGULATION OF NEURITE MORPHOLOGY THROUGH Gq/G11 AND G12/G13

Alexander Nürnberg1, Anja U. Bräuer2, Nina Wettschureck1, and Stefan Offermanns1,3

From Institute of Pharmacology1, University of Heidelberg, Im Neuenheimer Feld 366, D-69120 Heidelberg, Germany; and Institute for Cell Biology and Neurobiology2, Center for Anatomy, Charité-Universitätsmedizin Berlin, D-10115 Berlin, Germany and Department of Pharmacology3, Max-Planck-Institute for Heart and Lung Research, Parkstr. 1, 61231 Bad Nauheim, Germany

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Address correspondence to: Stefan Offermanns, Institute of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, D-69120 Heidelberg, Germany, Phone +49-6221-548246, Fax +49-6221-548549, Email stefan.offermanns@pharma.uni-heidelberg.de

The induction of neurite retraction and growth cone collapse via G-protein-coupled receptors is involved in developmental as well as in regenerative processes. The role of individual G-protein-mediated signalling processes in the regulation of neurite morphology is still incompletely understood. Using primary neurons from brains lacking Gαq/Gα11 or Gα12/Gα13 we show here that G12/G13-mediated signalling is absolutely required for neurite retraction and growth cone collapse induced by the blood-borne factors lysophosphatidic acid (LPA) and thrombin. Interestingly, the effects of LPA were mainly mediated by G13, while thrombin effects required G12. Surprisingly, lack of Gαq/Gα11 resulted in overshooting responses to both stimuli indicating that Gαq/G11-mediated signalling most likely via the activation of Rac antagonizes the effects of G12/G13.

INTRODUCTION

Chemorepellants which are able to induce neuronal growth cone collapse and neurite retraction play important roles as guidance cues during the development of the nervous system (1,2) and are also believed to prevent regeneration in the nervous system after injury (3-5). The regulation of growth cone morphology and neurite growth has been shown to be mediated by small GTPases of the Rho family. While Rac and Cdc42 activation as well as inactivation of RhoA promote neurite formation and extension of growth cones, RhoA activation induces growth cone collapse and neurite retraction (2,6-8).

The blood-borne factors thrombin and lysophosphatidic acid induce growth cone collapse and neurite retraction by activating specific G-protein-coupled receptors (9-11), and they have been involved in the inhibition of regenerative processes after nervous system injuries (12,13). In addition, LPA has been involved in various aspects of neural development (14,15). Both, thrombin and LPA receptors, are coupled to the heterotrimeric G-proteins Gq/G11, G12/G13, and Gi (16-18). Activation of Gq/G11 results in an increase in the enzymatic activity of β-isofoms of phospholipase C leading to the formation of inositol-1,4,5-trisphosphate (IP3) and diacylglycerol. This subsequently results in an increase in the intracellular Ca2+ concentration as well as an activation of various protein kinases including protein kinase C (19). In contrast, activation of G12/G13 leads to the stimulation of the Rho/Rho-kinase pathway via a subgroup of Rho guanine nucleotide exchange factors (20).

The relative roles of the G12/G13- and the Gq/G11-mediated signalling pathways in the acute effects of LPA and thrombin on neuronal morphology are rather unclear. While there is good evidence that G12/G13-mediated RhoA activation via increased actomyosin contractility plays an important role in LPA- and thrombin-induced neurite retraction and growth cone collapse (9,11,21,22), evidence has also been provided that Gq/G11-mediated signalling can mediate neurite retraction and growth cone collapse (21,23). However, studies analyzing the role of G-proteins in the regulation of neurite morphology via G-protein-coupled receptors have primarily been based on the use of constitutively active mutants of G-protein α-subunits. In addition, regulation of neuronal morphology via G-protein-coupled receptors has mainly been studied in neuronal cell lines, while the role of these regulatory processes in primary neurons as well as in the developing nervous system is unclear.
We therefore analyzed the role of $G_q/G_{11}$- and $G_{12}/G_{13}$-mediated signalling pathways in the regulation of neurite morphology by thrombin and LPA in primary hippocampal neurons derived from mice lacking the $\alpha$-subunits of $G_q/G_{11}$ or $G_{12}/G_{13}$ selectively in neuronal cells. We found that $G_{13}$ mediates neurite retraction and growth cone collapse induced by LPA, whereas $G_{12}$ mediates the effects of thrombin on neuronal morphology. Surprisingly, neurons lacking $G_{12}/G_{13}$ showed a strongly increased response to LPA and thrombin indicating that $G_{12}/G_{13}$- and $G_q/G_{11}$-mediated signalling have opposing effects on neurite morphology.

**EXPERIMENTAL PROCEDURES**

**Mice**

Mice with nervous system specific $G_{12}/G_{13}$ and $G_q/G_{11}$ deficiency have been described (24,25).

**Primary cultures from mouse hippocampus and cortex**

Cultures of hippocampal and cortical neurons were prepared as described previously (24). Hippocampi and cerebral cortices from E17.5 mouse embryos were dissected in ice-cold PBS containing 30 mM HEPES and 33 mM glucose, pH 7.38, washed once in PBS, incubated with 0.05% trypsin (Invitrogen) for 15 min at 37°C and triturated with fire-polished Pasteur pipettes. Cells were seeded in MEM supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate (all from Invitrogen), 25 mM glucose (Sigma) and 25 $\mu$M glutamic acid (Sigma) on poly-L-lysine (Sigma)-coated plastic dishes (Nunc; Falcon) at a density of 2×10⁴ cells/well ($\sim$1×10⁴ cells/cm², for live-cell imaging) or 2×10⁶ cells/well (2×10⁵ cells/cm², for G-LISA assays), respectively, and maintained at 37°C in 5% CO₂. After 4 h, the medium was replaced by neurobasal medium containing B-27 supplement and 0.5 mM L-glutamine (all from Invitrogen), and the cells were cultured for 12–18 h without changing the medium. To inactivate $G_{12}$ pertussis-toxin (100 ng/ml, Calbiochem) was added to the medium 8–10 h before experiments.

**Live cell imaging**

Growth cone collapse and neurite retraction assay was performed on a Leica DM IRE2 microscope equipped with 37°C 5% CO₂ environmental control chamber. Live cell images were recorded using Leica DC 350 FX camera and Leica FW4000 software (Leica Microsystems, Wetzlar). Images from randomly selected positions were acquired once before and 2, 5 or 20 min after bath application of LPA (Biomol), thrombin (Sigma) or ephrin-A5-Fc (R&D Systems). Alternatively, image sequences were recorded at a rate of 6 images/min 1 min before and 5 to 6 min after addition of agonists. Before use, ephrin-A5-Fc was preclustered with anti-human immunoglobulin G-Fc (Sigma) as described previously (26). Acquired images were analysed using NIH ImageJ program (27) and percentage of growth cones and average length of neurites before and after stimulation were calculated for 15 to 20 cells per experiment. Retraction was defined as neurite shortening expressed in percent of initial length. Results are presented as means of 3 to 11 independent experiments ± S.E. Dose-response to LPA was approximated with following equations: $y=-1.35+22.19/(1+10^{-6.55-x})$, $R^2=0.97$ and $y=-1.35+60.54/(1+10^{-7.107-x})$, $R^2=0.98$ for wild-type and $G_{12}/G_{13}$-deficient neurons respectively. Statistical significance was determined by the two-tailed t-test; ANOVA and Bonferroni’s correction were used for multiple comparisons.

**RT-PCR**

mRNA was prepared from hippocampal neurons using a RNeasy mini kit (Qiagen) and reverse transcription was performed according to standard procedures. LPA receptor expression was detected by the TaqMan assay using a TaqMan® Universal PCR Master Mix kit. Following gene expression assays (all from Applied Biosystems) were employed: LPA-1, Assay ID: Mm00439145_m1; LPA-2, Assay ID: Mm00469562_m1; LPA-3, Assay ID: Mm01312593_m1; LPA-4, Assay ID: Mm01228533_m1; LPA-5, Assay ID: Mm01190818_m1. GAPDH (glyceraldehyde-3-phosphate dehydrogenase, Assay ID: 4352932E) and HPRT (hypoxanthine guanine phosphoribosyl transferase, Assay ID: Rn01527838_g1) were used as reference genes. Reactions were performed in a 96-well Optical Reaction Plate from Applied Biosystems and detected using an ABI PRISM™ 7700 Sequence Detection System equipped with 7500 fast System software (Applied Biosystems). $G_{12}$ (Gna12), $G_{13}$ (Gna13), RhoA and $\beta$-actin were amplified using iQ SYBR Green Supermix Kit (Bio-Rad) and following primers: Gna12, 5’-TGCTTGCACGGCATCAC-3’ and 5’-
GAAGTGCTTCTAATGCTCAAC-3′; Gna13, 5′-TGCTGGTAGATGCGGAGA-3′ and 5′-CATCGTATCGCAATCT-3′; RhoA, 5′-CACCTGATATCGCCACCA-3′; β-actin, 5′-TGACGGTGACATCCGTAAAGAC-3′ and 5′-TGCTAGGACGCCAGCAGTA-3′. Reactions were detected on a Chromo4 RT-PCR detector equipped with Opticon Monitor 3.1 Software (Bio-Rad). Relative expression ratios normalized by HPRT (LPA receptors) or β-actin (Gna12, Gna13 and RhoA) and statistical significance were calculated using the relative expression software tool (REST®, (28)). The data from 3–4 samples are presented as log2 of the ratio of gene expression in Gαq/α11-deficient neurons compared to wild-type ± S.E.

**RESULTS**

To analyze the role of particular signalling pathways which mediate the regulation of neurite morphology via G-protein-coupled receptors, we prepared hippocampal neurons at embryonic day 17.5 from mice with nervous system-specific Gαq/Gα11 or Gα12/Gα13 deficiency (Nes-Cre;Gnaq/α11lox/lox, Gna11lox/lox or Nes-Cre;Gna12/α13lox/lox, respectively) (24,25). Embryonic hippocampal neurons lacking Gαq/α11 or Gα12/Gα13 were basically indistinguishable from wild-type neurons. Wild-type and mutant neurons had the same number and average length of neurites at stage 2/3 (Fig. 1A and B), and there was no difference in the differentiation of neurons in vitro (Fig. 1C). However, neurons lacking Gαq and Gα11 showed a slightly reduced number of growth cone-positive neurites when compared to wild-type or Gα12/Gα13-deficient neurons (Fig. 1D).

Murine wild-type hippocampal neurons at stage 2/3 rapidly respond to LPA and thrombin with growth cone collapse and the retraction of their neurites (Fig. 2A and B; Fig. 3A, B, D and E; Supplementary movie 1). In contrast, neurons lacking Gα12/Gα13 were completely unresponsive showing no neurite retraction and growth cone collapse when exposed to 10 µM LPA (Fig. 2A; Fig. 3A and D; Supplementary movie 2) or 1 U/ml thrombin (Fig. 2B; Fig. 3B and E). Gα12/Gα13-deficient neurons were not generally unresponsive as indicated by the effect of ephrin A5 which acts independently of G-protein-mediated signalling pathways and induced growth cone collapse as well as neurite retraction both in wild-type and Gα12/Gα13-deficient neurons (Fig. 2C and 3C and F).

Unexpectedly, deficiency of Gαq/Gα11 resulted in an overshooting response to LPA (Fig. 2A and Supplementary movie 3) and thrombin (Fig. 2B). While both stimuli reduced the percentage of growth cone-positive neurites in wild-type neurons to 10-30%, no growth cones were detectable after exposure of Gαq/Gα11-deficient cells to LPA and thrombin (Fig. 3A and B). Similarly, the effect of LPA and thrombin on neurite retraction was largely increased in the absence of Gαq/Gα11. While LPA and thrombin reduced wild-type neurite length by about 20 and 10%, respectively, the extent of neurite retraction was increased 2-3-fold in the absence of Gαq/Gα11 (Fig. 3D and E). The overshooting response of neurons lacking Gαq/Gα11 was not due to a general overresponsiveness of these cells as demonstrated by the normal effect of ephrin A5 on growth cone and neurite morphology (Fig. 2C and 3C and F). Uncoupling of receptors from Gq-type G-proteins by pretreatment of cells with pertussis toxin had no significant effect on LPA-induced neurite retraction or growth cone collapse in wild-type neurons or Gαq/Gα11-deficient neurons (Fig. 3A and D). Thus, G12/G13- and Gq/G11-mediated signalling pathways obviously play opposing roles in the regulation of neurite morphology via G-protein-coupled receptors. While G12/G13 are critically involved in the induction of growth cone collapse and neurite retraction, Gq/G11 appear to...
counteract the activity of G12/G13-mediated signalling.

To test whether individual G-proteins are responsible for the observed effects of Gaq/Gai13 and Gaq/Gai11 deficiency on the regulation of growth cone and neurite morphology, we analyzed the effect of LPA and thrombin in neurons lacking only Ga12 or Ga13. Ga12-deficient neurons behaved similar to Ga12/Ga13-deficient cells and showed almost no response to LPA whereas neurons lacking Ga12 behaved like wild-type cells (Fig. 4A and C). Interestingly, thrombin effects were strongly reduced in the absence of Ga12 but not in the absence of Ga13 (Fig. 4B and D). This indicates that LPA-induced regulation of neurite morphology involves primarily Ga13 while thrombin effects are mediated preferentially by Ga12. Neurons lacking only Ga13 showed slightly overshooting responses to both LPA and thrombin (Fig. 4). However, the phenotype was not as excessive as that of neurons lacking both, Gaq and Gai11, indicating that both G-protein α-subunits are involved in the effects of LPA and thrombin.

To further analyze the overshooting response of Gaq/Gai11-deficient neurons to agonists of G-protein-coupled receptors we determined the dose-response relationship of LPA-induced neurite retraction in wild-type and Gaq/Gai11-deficient cells (Fig. 5A). In both cell types LPA induced maximal neurite retraction at concentrations of 3-10 µM while being about three times more efficacious in neurons lacking Gaq/Gai11. However, also the potency of LPA to induce neurite retraction appeared to be slightly increased in the absence of Gaq/Gai11. While LPA induced neurite retraction in wild-type cells with an EC50 of 280 nM, the EC50 of LPA effects in Gaq/Gai11-deficient neurons was about 80 nM.

The massively enhanced effect of LPA in the absence of Gaq/Gai11 could be due to the fact that activation of the Gaq/Gai11-mediated signalling pathway exerted a stimulatory effect on neurite and growth cone extension, which under wild-type conditions counteracts the effect of G12/G13 activation on neurite retraction and growth cone collapse. Alternatively, excessive neurite retraction and growth cone collapse in the absence of Gaq/Gai11 could simply result from an overactivation of the G12/G13-mediated signalling pathway due to altered expression levels of the receptors or an increased coupling efficiency of receptors to G12/G13 in the absence of Gaq/Gai11. Quantitative PCR showed that the expression levels of LPA receptors 1, 2 and 4 as well as of Gaq12, Gaq13 and RhoA were unchanged in Gaq12/Gai11-deficient neurons (Fig. 5B). For LPA3 and LPA5 receptors, no corresponding mRNA was detected.

To test whether coupling efficiency of receptors to G12/G13 was increased in the absence of Gaq/Gai11, we determined the activity of RhoA, the main effector of G12/G13, in untreated and LPA-treated wild-type, Gaq12/Gai13-deficient and Gaq/Gai11-deficient neurons. The increase in RhoA activity was comparable in wild-type cells and cells lacking Gaq/Gai11, while no RhoA activation could be observed in Gaq12/Gai13-deficient neurons (Fig. 4C). This indicates that Gaq/Gai11 deficiency did not result in overactivation of G12/G13-mediated signalling in response to LPA.

Since LPA and thrombin can activate Rac in parallel with RhoA (29,30) and since Rac can antagonize RhoA function (31-33), we determined LPA effects on Rac activity in wild-type and Gaq/Gai11-deficient neurons. Interestingly, the LPA-induced activation of the small GTPase Rac, which could be seen in wild-type neurons, was absent in neurons lacking Gaq11/Gai11 (Fig. 5D). This indicates that in wild-type neurons both RhoA and Rac are activated and that the Gi/Gi13-mediated Rac activation which promotes neurite and growth cone extension may counteract the effect of G12/G13-mediated RhoA activation on neurite retraction and growth cone collapse under wild-type conditions.

**DISCUSSION**

A variety of G-protein-coupled receptors have been shown to be able to mediate the induction of neurite retraction and growth cone collapse (9,11,34,35). Regulation of growth cone and neurite morphology is involved in developmental as well as in regenerative processes of the nervous system (12,13). Receptors which are able to stimulate neurite retraction and growth cone collapse couple to G12/G13, Gaq/Gai11 and Gi-type G-proteins. The fact that pretreatment of neurons with pertussis toxin did not affect the ability of LPA or thrombin to reduce neurite retraction and growth cone collapse (11,34,36,37) (see Fig. 3) strongly indicates that Gi-type G-proteins are not involved...
in these effects. It has been difficult to evaluate the relative roles of G12/G13 and Gq/G11 in the regulation of neurite morphology since there are no appropriate inhibitors available which specifically block either of the G-protein families. Based on the expression of constitutively active mutants of Ga12 and Ga13 in various cell culture lines, there is good evidence that these G-protein α-subunits can mediate growth cone collapse and neurite retraction (21,22). However, the role Gq/G11 plays in these processes has been controversial.

Data involving Gq/G11 in mediating neurite retraction and growth cone collapse in response to LPA and thrombin are based on the use of constitutively active mutants of Gaq which after expression in various cell lines have been shown to cause neurite retraction similar to Ga12/Ga13 (21,23). However, other groups have reported that expression of constitutively active Gaq causes massive cell death in neurons and other cells (22,38). Thus, it might be difficult to differentiate the toxic effects of activated mutants of Gaq from cellular responses specifically induced via physiological Gq activation. In addition, blockade of Gq/G11-mediated signalling in a neuronal cell line has been shown to be without any effect on LPA-induced neurite retraction (39,40).

In order to clarify the role of different G-protein-mediated signalling pathways in the regulation of neurite morphology, we took a genetic approach to study the regulation of neurite and growth cone morphology via endogenous receptors in primary neurons lacking either Gαq/Gα11 or Gα12/Gα4. Our data clearly show that G12/G13 are required for LPA- and thrombin-induced neurite retraction and growth cone collapse. Surprisingly, we observed overshooting responses in neurons lacking Gαq/Gα11 which strongly indicates that these G-proteins do not promote neurite retraction and growth cone collapse but are rather involved in a pathway which counterregulates the stimulation of neurite retraction and growth cone collapse via G12/G13. Interestingly, it has been shown in Drosophila that expression of an activated version of Drosophila Go4 results in the ectopic midline crossing of neurons (41). Thus, Gq/G11-mediated signalling may regulate axonal pathfinding by inhibiting repulsive effects and by promoting neurite and growth cone extension.

The excessive response of Gaq/Gα11-deficient neurons to LPA and thrombin was obviously not due to an enhanced activation of the G12/G13-mediated pathway as indicated by the unaltered RhoA activation in neurons lacking Gaq/Gα11. Since the effect of a G-protein-independent activator of neurite retraction and growth cone collapse like ephrin A5 were not affected by Gaq/Gα11 deficiency, unspecific effects can be ruled out. Thus, Gq/G11-mediated signalling per se has an unexpected inhibitory effect on Rho/Rho-kinase-mediated neurite retraction and growth cone collapse. Given the fact that Gq/G11 couple receptors to β isoforms of phospholipase C resulting in transient increases in [Ca2+]i, the promotion of neurite extension via Gq/G11-mediated signalling is likely to be mediated by Ca2+. Recent evidence indicates that the spatiotemporal pattern of intracellular Ca2+ signals can have profound effects on axonal growth cones. While small and large elevations in the intracellular Ca2+ concentration result in repulsion, moderate elevation promotes growth cone extension (42,43). Increases in [Ca2+]i have been shown to induce activation of Rac in nonneuronal and neuronal cells (44-47). Consistent with this hypothesis, we observed that the LPA-induced Rac activation which was seen in wild-type neurons, was absent in Gaq/Gα11-deficient neurons. Rac activation is well known to counteract RhoA activation, to inhibit neurite retraction and to promote growth cone extension. It is therefore most likely that the Gq/G11-mediated increase in [Ca2+]i, via activation of the small GTPase Rac inhibits neurite retraction and growth cone collapse.

When we tested the effect of LPA and thrombin on neurite and growth cone morphology in cells lacking only Gα12 or Gα13, we observed that Gα12 deficiency blocked thrombin-induced effects whereas lack of Gα13 resulted in a loss of LPA-induced effects. This is consistent with observations made in fibroblasts and human embryonic kidney cells in which LPA effects appear to be mediated by G13 while thrombin effects involve G12 (48,49). Evidence has been provided that this is due to the preferential coupling of LPA receptors to G13 and of thrombin-activated receptors to G12 in intact cells (49,50).

In the present manuscript, we show that G12/G13 are absolutely required for neurite retraction and growth cone collapse induced by the
blood-borne factors thrombin and lysophosphatidic acid. Interestingly, analysis of $\alpha_{12}$ and $\alpha_{13}$ single-deficient neurons showed that the effects of thrombin were primarily mediated by $\alpha_{12}$ whereas the LPA effects depended on $\alpha_{13}$. Surprisingly, the overshooting response of $\alpha_{q}/\alpha_{11}$-deficient neurons revealed a so far unknown role of the neuronal $\alpha_{q}/\alpha_{11}$-mediated signalling pathway in antagonizing the effects of $\alpha_{12}/\alpha_{13}$-mediated signalling. Inhibition of $\alpha_{12}/\alpha_{13}$-mediated activation of Rho/Rho-kinase signalling has been suggested as a strategy to improve neurite growth and sprouting after neural injury (3). Our data clearly support this concept and, in addition, indicate that an activation of $\alpha_{q}/\alpha_{11}$-mediated signalling would be of additional benefit.

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FOOTNOTES

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The abbreviations used are: LPA, lysophosphatidic acid; WT, wild-type; KO, knock-out; EphA5, EphrinA5; PTX, pertussis toxin.
FIGURE LEGENDS

**Fig. 1.** Basal morphology of wild-type (WT), Gα₁2/Gα₁₃- (12/13 KO) and Gα₉/Gα₁₁-deficient (q/11 KO) hippocampal neurons isolated at day E17.5 and cultured for 12–18 hours in vitro. (A) Average number of neurites per single cell. (B) Average length of a neurite. (C) Percent of the neurons which entered the stage 3. (D) Percent of growth cone-positive neurites. Data are from n=6 (wild-type neurons) and n=5 (mutant neurons) experiments, and at least 30 cells per experiment were analysed. n.s., not significant; *, p < 0.05.

**Fig. 2.** Effect of LPA, thrombin and ephrin A5 on morphology of neurites of wild-type (WT), Gα₁2/Gα₁₃- (12/13 KO) and Gα₉/Gα₁₁-deficient (q/11 KO) hippocampal neurons cultured for 12–18 hours in vitro. Shown are representative images of cells before (0 min) and after (2, 5 or 20 min) bath application of 10 µM LPA (A), 1 U/ml thrombin (B) or 2 µg/ml ephrinA5 (C). Scale bar 12.5 µm.

**Fig. 3.** The role of different G-protein-mediated signalling pathways in the regulation of neurite and growth cone morphology by LPA and thrombin. Wild-type (WT), Gα₁₂/Gα₁₃- (12/13 KO), Gα₉/Gα₁₁-deficient (q/11 KO), and pertussis toxin-pretreated (WT+PTX, q/11 KO+PTX) hippocampal neurons were analysed before and after addition of LPA (A, D), thrombin (B, E) and EphrinA5 (C, F). (A, B) Number of growth-cone positive neurites before (0 min) and 2 and 5 minutes after addition of 10 µM LPA (A) or 1 U/ml thrombin (B). (C) Number of growth-cone positive neurites before (-) and in 20 minutes after addition of 2 µg/ml Ephrin A5 (+). (D, E) Neurite retraction in response to 10 µM LPA (D) or 1 U/ml thrombin (E). (F) Neurite retraction in 20 minutes after addition of 2 µg/ml Ephrin A5. Data are from 3-11 independent experiments. n.s., not significant; *, p<0.001; **, p<0.01; ***, p<0.05 (paired t-test); #, p<0.001; ##, p<0.05 (vs. WT).

**Fig. 4.** Specific roles of G₁₂ and G₁₃ in LPA- and thrombin-induced growth cone collapse and neurite retraction. Number of growth-cone positive neurites (A, B) and neurite retraction (C, D) before (-) and after (+) stimulation with 10 µM LPA (A, C) or 1 U/ml thrombin (B, D) were analysed in wild-type (WT), Gα₁₂- (12 KO), Gα₁₃- (13 KO), Gα₁₂/Gα₁₃- (12/13 KO), Gα₁₁- (11 KO) and Gα₉/Gα₁₁-deficient (q/11 KO) hippocampal neurons. Data are from 3-11 independent experiments. n.s., not significant; *, p<0.001; **, p<0.05 (paired t-test); #, p<0.001; ##, p<0.05 (multiple comparisions).

**Fig. 5.** Characterization of G₉/G₁₁-mediated inhibition of neurite retraction and growth cone collapse. (A) Neurite retraction in wild-type (WT), Gα₁₂/Gα₁₃- (12/13 KO), Gα₉/Gα₁₁-deficient (q/11 KO) hippocampal neurons measured 5 min after exposition to different concentrations of LPA. (B) Ratio of LPA1, 2 and 4 receptor, Gα₁₂ (Gna12), Gα₁₃ (Gna13) and RhoA expression in Gα₉/Gα₁₁-deficient hippocampal neurons compared to wild-type neurons. The pair wise fixed reallocation randomisation test showed no significant difference between wild-type and Gα₉/Gα₁₁-deficient neurons (p: 0.34–0.96). (C,D) Effect of 10 µM LPA on RhoA (C) and Rac1 (D) activity in wild-type (WT), Gα₁₂/Gα₁₃- (12/13 KO) and Gα₉/Gα₁₁-deficient (q/11 KO) cortical neurons. Cells were incubated with LPA for 15–60 sec. (RhoA) or for 15 sec. (Rac1). n.s., not significant; *, p<0.05.
SUPPLEMENTARY MOVIES

Cultured hippocampal neurons isolated from wild-type (Movie 1), \( \Gamma_{\alpha_{12}}/ \Gamma_{\alpha_{13}} \) (Movie 2) and \( \Gamma_{\alpha_{q}}/\Gamma_{\alpha_{i1}} \)-deficient (Movie 3) embryonic mouse brains were imaged 30 to 50 seconds before \((-30 \text{ to } -50 \text{ to } 0 \text{ sec})\) and about 5 minutes after \((0 \text{ to } 330\text{–}360 \text{ sec})\) bath application of 10 \( \mu \text{M} \) LPA (added at \( t=0 \text{ sec} \)). Scale bar 20 \( \mu \text{m} \) (10 \( \mu \text{m} \) for the insert).
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Antagonistic regulation of neurite morphology through Gq/g11 and G12/G13
Alexander Nürnberg, Anja U. Bräuer, Nina Wettschureck and Stefan Offermanns

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