

Gem Expression and Localization Study in the Brain Tissue of Adult Kunming Mice

Chang Cui

Haiyuan College of Kunming Medical University

Juanjuan Bai

Kunming Medical University

Xin Shu

Kunming Medical University

Li Long

Haiyuan College of Kunming Medical University

Zhixiang Fan

15926178865@163.com

Kunming Medical University

Article

Keywords: Gem, brain tissue, localization, immunohistochemistry, Kunming mice, Central nervous system

Posted Date: September 2nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10622237/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

To investigate the mRNA and protein expression of the Gem gene in different regions of the brain tissue of adult Kunming mice, thereby providing a foundation for exploring its functions in the central nervous system.

Methods

Adult healthy female Kunming mice were selected. Brain regions including the frontal lobe, parietal lobe, occipital lobe, temporal lobe, thalamus, cerebellum, and hippocampus were collected. RT-PCR and real-time RT-PCR were used to detect the expression of Gem mRNA in these regions. Immunohistochemistry was employed to examine the expression and localization of Gem protein in various brain regions.

Results

Gem mRNA was expressed in the frontal lobe, parietal lobe, occipital lobe, temporal lobe, thalamus, cerebellum and hippocampus of mouse brain, with no significant differences in expression levels among these regions ($P > 0.05$). Immunohistochemistry revealed strong Gem expression in hippocampal pyramidal and granule cells, cerebellar Purkinje cells, brainstem neurons, and choroid plexus epithelial cells of the ependyma. Expression was also detected in the large pyramidal cells of the cerebral cortex, as well as in the neurons and mitral/granule cells of the olfactory bulb.

Conclusion

Gem is widely expressed in the brain tissue of adult Kunming mice.

Background

Gem (GTP-binding protein overexpressed in skeletal muscle) is a member of the RGK subfamily of the Ras superfamily, which comprises Rad, Rem, Rem2, and Gem/Kir, and is highly expressed in muscle tissue^[1, 2]. The sequence of the Gem gene was first reported in 1994, cloned from mitogen-induced human peripheral blood T cells. Gem is a regulatory protein involved in plasma membrane receptor-mediated signal transduction, and its expression can be induced by mitogenic stimulation in several cell types, including primary lymphocytes, monocytes, endothelial cells, and human embryonic fibroblasts^[3, 4]. Gem possesses the characteristic structural domains of the RGK family: GTP/Mg²⁺-binding sites, Switch I ~ II, G1 ~ 5 BOX motifs, and a GDI domain. Among these, the GTP/Mg²⁺-binding sites are located at both ends of the amino acid sequence in most members of the small G-protein family; Switch I ~ II are

two highly flexible regions that undergo conformational changes upon GTP binding; the G1 ~ 5 BOXes are highly conserved motifs of GTP-binding proteins, involved in phosphate binding and promoting protein conformational changes during the GTP/GDP cycle; GDI (guanine nucleotide dissociation inhibitor) exerts a negative regulatory role by promoting the dissociation of small G-proteins from the plasma membrane [2, 5–7]. These proteins, characterized by several distinct features, are believed to exert two primary functions: regulating voltage-gated Ca^{2+} channels and reorganizing the actin cytoskeleton [8].

Studies have shown that Gem is expressed in multiple organs. It is widely expressed in various tissues and organs of goats, such as the spleen, lung, and large intestine [9], and is also detectable in the lungs of Tibetan chickens, as well as in the pectoral and leg muscles at different developmental stages [10]. High levels of Gem mRNA have been detected in the thymus, spleen, kidney, lung, and testis of humans and mice, whereas relatively low or undetectable levels of Gem mRNA are found in cardiac, brain, liver, and skeletal muscle cells. Initially, Gem was thought to be absent in neuronal tissues, including the mouse brain and the rat pheochromocytoma-derived PC12 cell line [3, 11]. Subsequent studies, however, revealed that Gem is more highly expressed in undifferentiated hippocampal glial cells than in differentiated ones. Moreover, Gem expression is higher in the renal tissue of adult mice compared to embryonic mice, which may be attributed to the species-specific nature of Gem gene expression patterns across tissues [1]. To date, however, systematic studies on the expression and localization of Gem in different regions of the nervous system remain lacking.

In this study, we employed RT-PCR, real-time RT-PCR and immunohistochemistry to analyze the expression and localization of Gem mRNA and protein in the central nervous system of adult Kunming mice, laying a foundation for further investigations into the physiological functions of Gem in the nervous system.

Materials and Methods

1. Materials

1.1 Laboratory animals

Adult female Kunming mice (6–8 weeks old), weighing 18–22 g, were obtained from the Laboratory Animal Center of Kunming Medical University. The mice were of the Clean (CL) grade. They were housed in a controlled environment with a temperature of 18–25°C and a relative humidity of 50%–60%. The animals were provided with food and water ad libitum. Cages were cleaned on a regular schedule. Prior to the experiments, the mice were acclimatized to the new environment for 24 hours under the conditions described above.

1.2 Main reagents and instruments

The rabbit anti-mouse polyclonal antibody against Gem was purchased from Fitzgerald Industries in the U.S.; the mouse anti- β -actin monoclonal antibody was purchased from Beijing Kangwei Shiji Biotechnology Co., Ltd.; HRP-conjugated goat anti-rabbit IgG and goat anti-mouse IgG polyclonal antibodies were purchased from KPL in the U.S.; RNA extraction reagents were obtained from TianGen Biochemical Technology (Beijing) Co., Ltd.; the reverse transcription kit was purchased from Thermo Scientific in the U.S.; the primers for Gem and GAPDH were synthesized by BaoRi Medical Biotechnology (Beijing) Co., Ltd.; the high-efficiency RIPA tissue/cell lysis buffer was purchased from Beijing Solarbio Science & Technology Co., Ltd.; the protease inhibitor cocktail was purchased from Thermo Scientific in the U.S.; all other reagents were of analytical grade and domestically produced. The micro-volume nucleic acid and protein detector was purchased from Implen GmbH in Germany.

2. Method

2.1 Total RNA extraction and cDNA reverse transcription

Mice were euthanized by cervical dislocation, and brain tissues were dissected and washed with PBS (0.01 mol/L, pH 7.2–7.4). Total RNA was extracted using an Animal Tissue Total RNA Isolation Kit (DP431, Tiangen). RNA integrity was verified by agarose gel electrophoresis, and RNA concentration was measured before reverse transcription. Then, 1 μ g of total RNA was used for first-strand cDNA synthesis via reverse transcription using a reverse transcription kit (K1622, Thermo Fisher Scientific). The resulting cDNA was diluted 20-fold and served as a template for RT-PCR and Real-time PCR analyses.

2.2 RT-PCR testing

The target gene, Gem, was amplified using specific primers (TaKaRa). The upstream primer is 5'-AGTGCAACCATTATCCTCCTG, and the downstream primer is 5'-AATGCCCTCAAACAGTTCCTT. The reaction mixture consisted of 1 μ L of cDNA, 1 μ L of the upstream primer, 1 μ L of the downstream primer, 10 μ L of PCR Master Mix (TSE004, Beijing Qingke), and 7 μ L of sterile deionized water. The amplification conditions were as follows: 94°C for 5 min, followed by 94°C for 30 s, 60°C for 45 s, and 72°C for 1 min, repeated for 30 cycles, and finally held at 72°C for 5 min. The PCR product was analyzed by 2% agarose gel electrophoresis.

2.3 Real-time PCR detection

Real-time PCR was performed using Gem-specific primers (TaKaRa), with GAPDH serving as the internal control. The upstream primer for Gem was 5'-AGTGCAACCATTATCCTCCTG, and the downstream primer was 5'-AATGCCCTCAAACAGTTCCTT. The upstream primer for GAPDH was 5'-GGTGAAGGTCGGTGTGAACG, and the downstream primer was 5'-CTCGCTCCTGGAAGATGGTG. The reaction mixture consisted of 1 μ L of cDNA, 1 μ L of the upstream primer, 1 μ L of the downstream primer, 10 μ L of SYBR Green PCR Mix (catalog no. 04913914001, Roche), and 7 μ L nuclease-free water. The amplification conditions were as follows: 95°C for 10 min (initial denaturation); denaturation at 95°C for 10 s; annealing at 60°C for 1 min, repeated for 40 cycles; followed by a final extension step at 95°C for 15

s, 60°C for 1 min. Melting curve analysis was performed (95°C for 15 s, 60°C for 1 min, 95°C for 15 s). PCR products were verified by 2% agarose gel electrophoresis.

2.4 Immunohistochemistry

Normal mice were anesthetized by intraperitoneal injection of 1% sodium pentobarbital (80mg/kg). First, 50 ml of physiological saline was perfused through the left ventricle to remove blood; then, the brain tissue was thoroughly rinsed and fixed by thorough rinsing with 50 ml of 4% paraformaldehyde. The brain tissue was subsequently removed intact, placed in paraformaldehyde for fixation for 3 hours, dehydrated through a graded ethanol series, cleared in xylene, embedded in paraffin, and sectioned into 3- μ m-thick slices. The sections were dewaxed in xylene and sequentially rehydrated through a graded ethanol series. Antigen retrieval was performed using citrate buffer at high temperature (0.01 mol/L); the sections were rinsed three times for 5 minutes each with PBS. The primary antibody Gem (dilution: 1:200) was incubated overnight at 4°C. After washing three times for 5 minutes each with 0.1 mol/L PBS, the sections were incubated for 2 hours at room temperature with the secondary antibody (dilution: 1:3000). Following another round of washing with 0.1 mol/L PBS for 3 minutes each, the sections were incubated with DAB for 10 minutes, rinsed with PBS for 10 minutes, counterstained with hematoxylin for 1 minute, and finally washed with water. Next, the sections were immersed in acidified alcohol for 1 minute and then washed with water for 10 minutes. They were further dehydrated through a graded ethanol series (70%, 80%, 90% twice each, 95% twice, and 100% three times, each step lasting 5 minutes), followed by immersion in xylene, air-drying, and finally mounting on slides. The stained sections were observed and documented under a microscope (BX43 Olympus).

2.5 Statistical Analysis

Statistical comparisons of the data were performed using SPSS 17.0 statistical software. Quantitative data are presented as mean $\pm$ standard deviation (SD). Comparisons among multiple groups were performed using one-way ANOVA, followed by Tukey's post hoc test. A P value < 0.05 was considered statistically significant.

Results

1. RT-PCR detection of Gem mRNA expression in different brain regions

RT-PCR was used to detect the mRNA expression of Gem in different regions of mouse brain tissue. The results showed that after reverse transcription, all cDNA templates derived from RNA extracted from the frontal lobe, parietal lobe, occipital lobe, temporal lobe, thalamus, cerebellum, and hippocampus could amplify the target product (Fig. 1), confirming the presence of Gem mRNA expression in the frontal lobe, parietal lobe, occipital lobe, temporal lobe, thalamus, cerebellum, and hippocampus of the mouse brain.

2. Real-time RT-PCR quantitative analysis of Gem mRNA expression in different brain regions

To determine whether there are differences in the expression levels of Gem mRNA among different regions of brain tissue, this study employed Real-time RT-PCR for detection. GAPDH was used as an internal reference, and the $2^{-\Delta\Delta C_t}$ method was applied, with the frontal lobe serving as the reference for data analysis. The results showed that, compared to the frontal lobe, the expression levels of Gem mRNA in the parietal lobe, occipital lobe, temporal lobe, thalamus, cerebellum, and hippocampus did not differ significantly ($P > 0.05$) (Fig. 2).

in different regions of brain

3. Immunohistochemical assay Gem Expression and localization of proteins in different brain regions

Immunohistochemical analysis was further performed to examine the expression and localization of Gem protein in brain tissues. The results are shown in Figs. 3 and 4. In the pyramidal layer of hippocampal CA1, CA2, and CA3 regions (Figs. 3A–C), pyramidal cells were densely arranged. These cells exhibited light staining in the cytoplasm, where positive Gem expression was detected. In the hilus of the dentate gyrus (Fig. 3D), large pyramidal cells were observed to be loosely and irregularly arranged. Positive Gem expression was present in their lightly stained cytoplasm. In the granular layer of the dentate gyrus (Fig. 3D), densely packed granule cells exhibited moderate nuclear staining, with positive Gem immunoreactivity localized to the nuclei. In the cerebral cortex, large pyramidal cells with a pyramidal shape were visible (Fig. 4B), characterized by deeply stained nuclei and moderately stained cytoplasm. Positive Gem expression was found both in the nuclei and cytoplasm of these large pyramidal cells. In the Purkinje cell layer of the cerebellar cortex (Fig. 4B), pear-shaped Purkinje cells were regularly arranged in a single layer, with strong Gem expression in both the nuclei and cytoplasm. In the brainstem (Fig. 4C), neurons exhibited deeply stained nuclei and cytoplasm, with strong Gem expression present in both the nuclei and cytoplasm of these neurons. In the ependyma (Fig. 4D), columnar epithelial cells of the choroid plexus were seen arranged in rows, with deeply stained cytoplasm; strong Gem expression was observed within the cytoplasm of these choroid plexus epithelial cells. In the glomerular layer of the olfactory bulb (Fig. 4E), interneurons distributed around the glomeruli were visible, with deeply stained cytoplasm; strong Gem expression was detected within the cytoplasm of these interneurons. In the mitral cell layer of the olfactory bulb (Fig. 4F), the cytoplasm of mitral cells was deeply stained, indicating positive Gem expression. In the granule cell layer of the olfactory bulb, round granule cells were observed, with deeply stained nuclei and cytoplasm; strong Gem expression was detected both in the nuclei and cytoplasm of these granule cells, indicating that Gem is widely expressed in various regions and cell types of the normal brain.

Discussion

The expression of Gem is highly regulated. Gem has been identified as an immediate-early gene in primary lymphocytes, monocytes, endothelial cells, and human embryonal carcinoma cells^[3, 4]. Gem mRNA has been detected in several distinct human and murine tissues, including the thymus, spleen, kidney, lung, and testis^[3]. In contrast to primary cells, Gem expression was undetectable in a variety of human hematopoietic tumor-derived cell lines (including T-cell, B-cell, myeloid, and erythroid lineages), either before or after treatment^[3]. Gem is known to participate in diverse physiological and pathological activities across various tissues and organs. Two pivotal biological effects of Gem depend on its site of expression: the inhibition of voltage-gated calcium channel activity and the suppression of Rho kinase-mediated cytoskeletal rearrangement^[12]. Currently, research on the Gem gene primarily focuses on voltage-dependent calcium channels, cytoskeletal regulation, and protein structure in mice and cell lines. Gem can modulate voltage-dependent calcium channel (VDCC) signaling and cytoskeletal rearrangement by inhibiting Rho kinase (ROK) regulators.

Furthermore, Gem is expressed in 3T3-L1 adipocytes, where it may promote lipid accumulation and adipogenesis^[9, 13]. Highly expressed Gem plays a crucial role in ECM-receptor interaction and natural killer cell-mediated cytotoxicity, whereas low Gem expression enhances the innate immune response of neutrophils by increasing NADPH oxidase assembly and the production of ROS and NETs^[14, 15]. Gem can regulate cell elongation, growth, proliferation, and motility; related findings suggest that Gem may be involved in tumor-related processes^[16–19]. As a novel regulator, Gem can also mediate chondrogenic differentiation and cartilage matrix formation via the Wnt/ β -catenin signaling pathway^[20].

Gem has been implicated in various neurological disorders. Studies have shown that Gem promotes neuroprotection and neuroplasticity in both injured and healthy brains via Npas4 expression^[21]. Overexpression of Gem in neuroblastoma cells promotes neurite outgrowth and cell flattening by inhibiting the Rho-ROCK pathway^[22, 23]. Following acute spinal cord injury in adult rats, findings suggest that Gem may be associated with the proliferation of microglia and astrocytes, but not neurons^[24]. These results indicate that Gem may be involved in neuroprotection and neuroplasticity, and its expression varies across different cell types. However, the expression and function of Gem in different tissues and cell types within the nervous system remain unclear and warrant further investigation. This will provide new avenues for exploring the specific functions of Gem in central nervous system injury and repair.

In this study, we performed localization and quantification of Gem distribution at both the gene and protein levels in various normal mouse brain tissues, including the frontal, parietal, occipital, and temporal lobes, thalamus, cerebellum, hippocampus, brainstem, ependyma, and olfactory bulb. This lays a foundation for subsequent exploration of the physiological functions of Gem in different brain regions and cell types, and facilitates further research on the physiological roles of Gem in the nervous system. RT-PCR and real-time RT-PCR detection of Gem mRNA revealed its expression in the frontal lobe, parietal lobe, occipital lobe, temporal lobe, thalamus, cerebellum, and hippocampus of normal mice. Statistical analysis showed no significant differences in Gem mRNA expression among these regions. The

discrepancy between mRNA and protein levels suggests post-transcriptional regulation, implying that Gem may exert region-specific functions through differential protein expression. The high expression of Gem protein in the cerebellum suggests that it may play a vital role in maintaining body balance. Immunohistochemistry results demonstrated that Gem expression was predominantly observed in the pyramidal cells of the stratum pyramidale in the hippocampal CA1, CA2, and CA3 regions; the large pyramidal cells in the polymorphic layer (hilus) of the dentate gyrus; and the granule cells of the granular layer of the dentate gyrus. High expression of Gem was also detected in the Purkinje cells of the cerebellar cortex, neurons of the brainstem, and the choroid plexus epithelial cells of the ependyma. Additionally, Gem was expressed in the large pyramidal cells of the cerebral cortex, as well as in the neurons, mitral cells, and granule cells of the olfactory bulb. Furthermore, Gem expression was primarily localized in the cytoplasm, although it was also detected in the nuclei of certain cells. This cell-type-specific expression pattern strongly implies a correlation between Gem function and cell type.

Nevertheless, this study has certain limitations. First, the Western blot experiments were not fully validated with repeated trials. Second, the specific cell types expressing Gem protein in different brain regions have not been systematically identified. Third, the precise physiological functions of Gem in the nervous system and the underlying mechanisms remain to be elucidated. In our future work, we plan to perform additional Western blot analyses to verify Gem expression across brain regions, and employ immunofluorescence colocalization to precisely identify Gem-expressing cell types in each region. Furthermore, we will conduct mechanism-based physiological studies to clarify the functional roles of Gem in the central nervous system, thereby establishing a solid foundation for deeper exploration of its physiological significance.

Abbreviations

ANOVA

Analysis of Variance

bp

base pair

cDNA

complementary DNA

CpG

Cytosine-phosphate-Guanine

DAB

Diaminobenzidine

DNA

Deoxyribonucleic Acid

EtOH

Ethanol

GAP

GTPase-Activating Protein

GDI
Guanine Nucleotide Dissociation Inhibitor
GDP
Guanosine Diphosphate
Gem
GTP-binding protein overexpressed in muscle
GTP
Guanosine Triphosphate
H&E
Hematoxylin and Eosin
IgG
Immunoglobulin G
IEG
Immediate-Early Gene
KIF9
Kinesin-like protein 9
MAPK
Mitogen-Activated Protein Kinase
NOX
NADPH Oxidase
NFAT
Nuclear Factor of Activated T-cells
NETs
Neutrophil Extracellular Traps
NSCLC
Non-Small Cell Lung Cancer
PBS
Phosphate Buffered Saline
PCR
Polymerase Chain Reaction
RGK
Rad, Rem, Rem2, and Kir/Gem
RNA
Ribonucleic Acid
ROCK
Rho-Associated Kinase
RT
Reverse Transcription
Rho
Ras Homolog Gene Family

SD
Standard Deviation
siRNA
Small Interfering RNA
SDS
Sodium Dodecyl Sulfate
TBE
Tris-Borate-EDTA
TE
Tris-EDTA
Tris
Tris-Hydroxymethyl Aminomethane
VDCC
Voltage-Dependent Calcium Channel
Xyl
Xylene

Declarations

Ethics approval and consent to participate

All animal experiments were performed in accordance with the guidelines for the Care and Use of Laboratory Animals of Kunming Medical University. The study protocol was approved by the Animal Experimental Ethical Committee of Kunming Medical University (approval number: kmmu2021075, application number: 2021-075, application date: February 16, 2021). The animal facility license number is SYXK (滇) K2020-0006, and all experimental procedures were conducted in the SPF-grade animal housing and biosafety level 2 laboratory of Kunming Medical University. All personnel involved in animal experiments had received professional training (certificate number: LA2020237) and were competent in rodent handling, anesthesia, surgical procedures, and humane euthanasia.

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by the Yunnan Provincial Department of Science and Technology Program (202401AY070001-208), and the Kunming Medical University Joint Special Project of Yunnan Basic Research Program (2019FE001-021 & 2018FE001-188).

Author Contribution

The division of labor among the authors: Chang Cui: Conducted the experimental operations, image processing, and data analysis, and drafted the manuscript. Juanjuan Bai: Conducted the experimental operations, image processing, and data analysis. Xin Shu: Conducted the experimental operations, image processing, and data analysis. Li Long: Conceived, organized, and supervised the study, and edited and revised the manuscript. Zhixiang Fan: Conceived, organized, and supervised the study, and edited and revised the manuscript.

Acknowledgement

We sincerely thank the Department of Basic Medical Sciences, Haiyuan College, Kunming Medical University, and the Department of Cell Biology and Medical Genetics, School of Basic Medical Sciences, Kunming Medical University, for their unwavering support. We also extend our heartfelt gratitude to the Laboratory Animal Center for providing specific pathogen-free (SPF) Kunming mice and standardized husbandry management, which ensured animal welfare and the smooth progress of our research.

Data Availability

Data Availability Statement The raw data supporting the findings of this study, including RT-PCR and real-time RT-PCR quantification data (Ct values and relative expression calculations) as well as immunohistochemical staining images, are not publicly available due to the large volume of image data and the lack of a dedicated repository for this study. However, all quantitative data supporting the results are fully presented in the figures and tables within the paper. The original RT-PCR gel images have been included in the Supplementary Information. The real-time RT-PCR raw data and immunohistochemical photomicrographs are available from the corresponding author upon reasonable request.

References

1. Piddini, E. et al. The Ras-like GTPase Gem is involved in cell shape remodelling and interacts with the novel kinesin-like protein KIF9 [J]. *EMBO J.* **20** (15), 4076–4087 (2001).
2. Yang, T. & Colecraft, H. M. Regulation of voltage-dependent calcium channels by RGK proteins. *Biochim. Biophys. Acta.* **1828** (7), 1644–1654 (2013).
3. Maguire, J. et al. Gem: an induced, immediate early protein belonging to the Ras family. *Science* **265** (5169), 241–244 (1994).
4. Vanhove, B. et al. *Gem, a GTP-binding protein from mitogen - stimulated T cells, is induced in endothelial cells upon activation by inflammatory cytokines* Vol. 5, 51–61 (Endothelium, 1997).
5. Shi et al. *J. Anhui Agricultural Sci. (in Chinese)*, **40**(5): 2566–2569. (2012).

6. Correll, R. N. et al. The RGK family of GTP-binding proteins: regulators of voltage-dependent calcium channels and cytoskeleton remodeling. *Cell. Signal.* **20** (2), 292–300 (2008).
7. Kahn, R. A., Der, C. J. & Bokoch, G. M. The ras superfamily of GTP-binding proteins: guidelines on nomenclature. *FASEB J.* **6** (8), 2512–2513 (1992).
8. Hatzoglou, A. et al. Gem associates with Ezrin and acts via the Rho-GAP protein Gmip to down-regulate the Rho pathway. *Mol. Biol. Cell.* **18**, 1242–1252 (2007).
9. Xu, Q. et al. Molecular characterization of GTP binding protein overexpressed in skeletal muscle (Gem) and its role in promoting adipogenesis in goat intramuscular preadipocytes. *Animal Biotechnol.* **31**, 1–8 (2018).
10. Xu Qing, L. et al. Cloning and Spatiotemporal Expression Analysis of GEM Gene in Tibetan Chicken[J]. *Acta Agriculturae Boreali-Sinica (in Chinese)*. **33** (02), 42–48 (2018).
11. Beguin, P. et al. Regulation of Ca²⁺ channel expression at the cell surface by the small G-protein kir/Gem. *Nature* **411**, 701–706 (2001).
12. Ward, Y. et al. Phosphorylation of critical serine residues in Gem separates cytoskeletal reorganization from down-regulation of calcium channel activity. *Molecular and Cellular Biology* 2004, 24:651–661. (2004).
13. Gong Haixia, G. et al. Study on gene expression profiles of adipose tissue in obese rats[J]. *Chin. J. Med. Genet. (in Chinese)*. **20** (3), 268–271 (2003).
14. Zhang, D. & S. Z Expression profile analysis to predict potential biomarkers for glaucoma: BMP1, DMD and Gem. *PeerJ* **8**, e9462 (2020).
15. Johnson¹, J. L. et al. The atypical small GTPase Gem/Kir is a negative regulator of the NADPH oxidase and NETs production through macroautophagy. *J. Leukoc. Biol.* **110** (4), 629–649 (2021).
16. Tseng, Y. H. et al. Regulation of growth and tumorigenicity of breast cancer cells by the low molecular weight GTPase Rad and nm23. *Cancer Res.* **61**, 2071–2079 (2001).
17. Fischer, R., Wei, Y., Anagli, J. & Berchtold, M. W. Calmodulin binds to and inhibits GTP binding of the ras-like GTPase Kir/Gem. *J. Biol. Chem.* **271**, 25067–25070 (1996).
18. Makale, M. Cellular mechanobiology and cancer metastasis. *Birth Defects Res. C: Embryo Today Rev.* **81**, 329–343 (2007).
19. Hatzoglou, A. et al. Gem associates with Ezrin and acts via the Rho-GAP protein Gmip to down-regulate the Rho pathway. *Mol. Biol. Cell.* **18**, 1242–1252 (2007).
20. Gan, L. et al. Decreased expression of Gem in osteoarthritis cartilage regulates chondrogenic differentiation via Wnt/ β -catenin signaling. *J. Orthop. Surg, Res.* **18**(1). (2023).
21. Hiroo Takahashi, R. et al. Ras-like Gem GTPase induced by Npas4 promotes activity-dependent neuronal tolerance for ischemic stroke. *Proc Natl Acad Sci U S A* 118(32). (2021).
22. Opatowsky, Y. et al. Structure–function studies of the G-domain from human Gem, a novel small G-protein. *FEBS Lett.* **580**, 5959–5964 (2006).

23. Ward, Y. et al. The GTP binding proteins Gem and Rad are negative regulators of the Rho–Rho kinase pathway. *J. Cell. Biol.* **157**, 291–302 (2002).
24. Hai Wena, J. C. et al. b, and Feng Zhanga, (n) Spatiotemporal patterns of Gem expression after rat spinal cord injury. *Brain Res* 1516: 11–19. (2013).

Figures

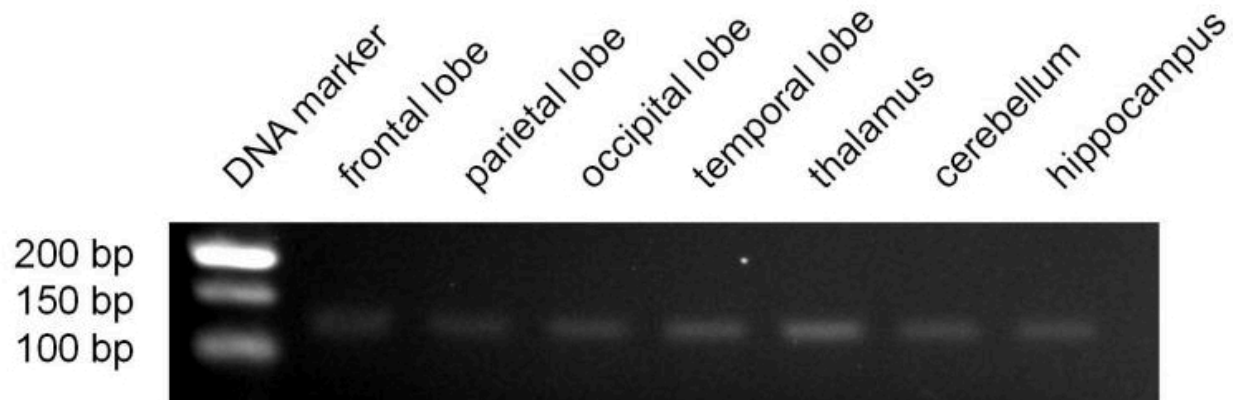


Figure 1

Expression of Gem mRNA in different parts of brain by RT-PCR

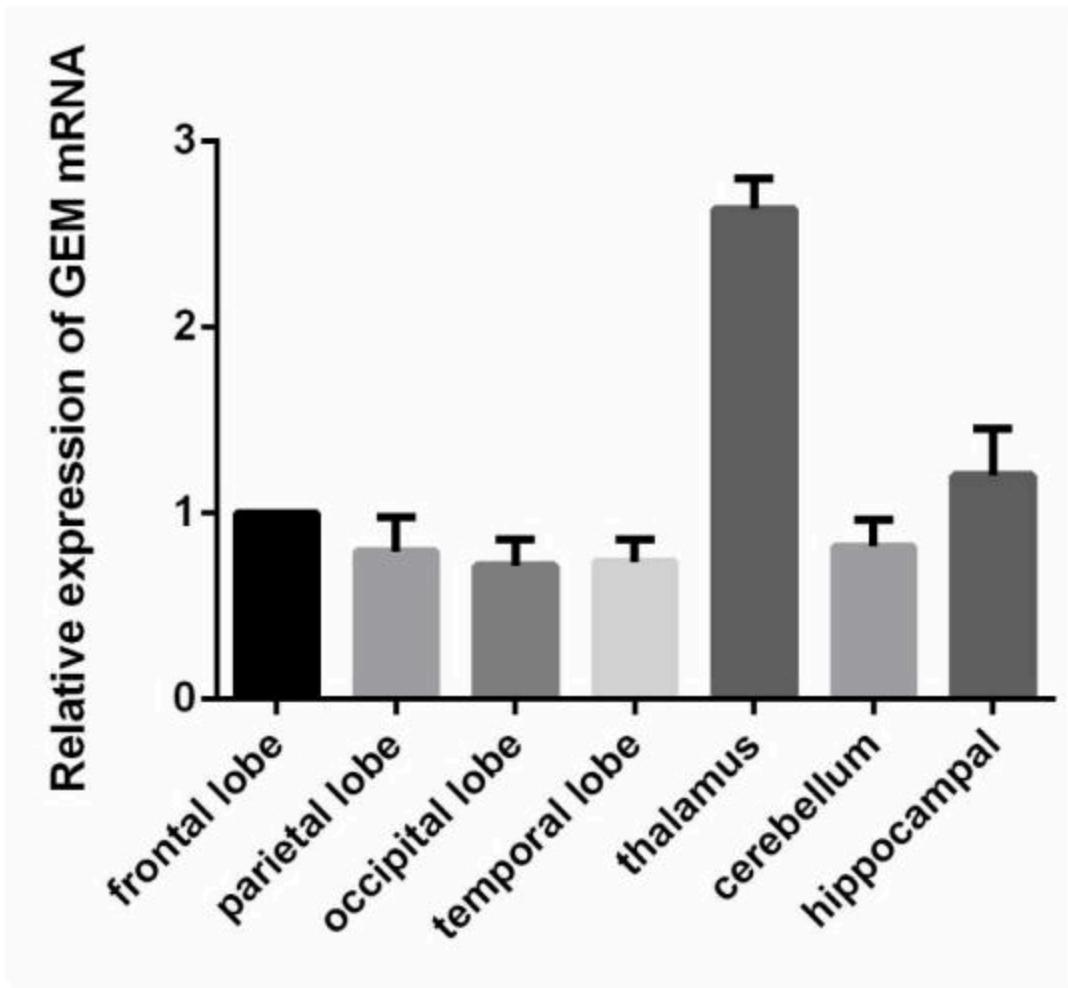
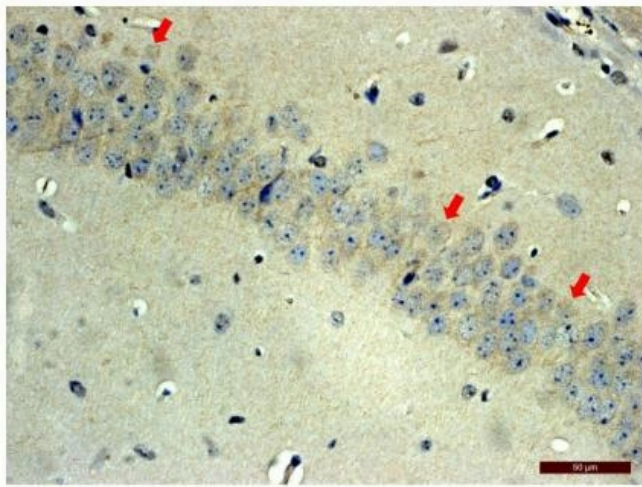
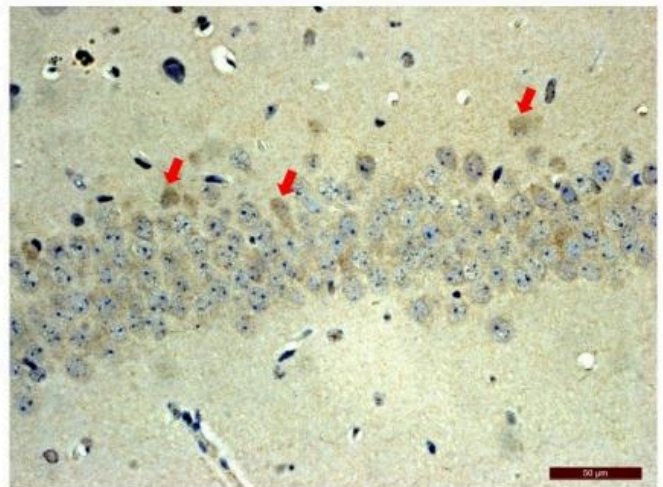


Figure 2

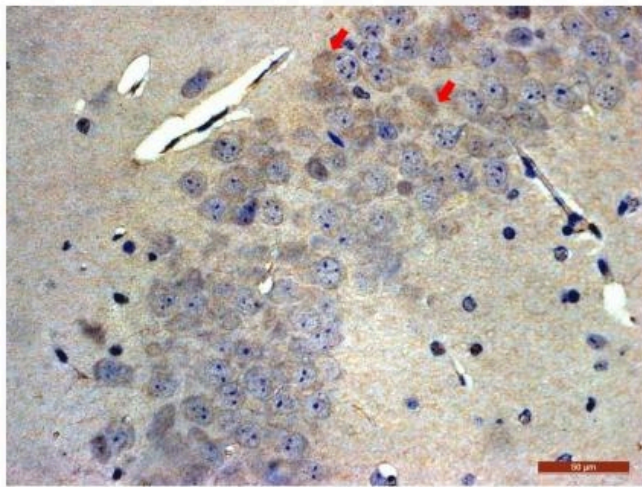
Relative expression of the Gem mRNA detected by real time RT-PCR in different regions of brain



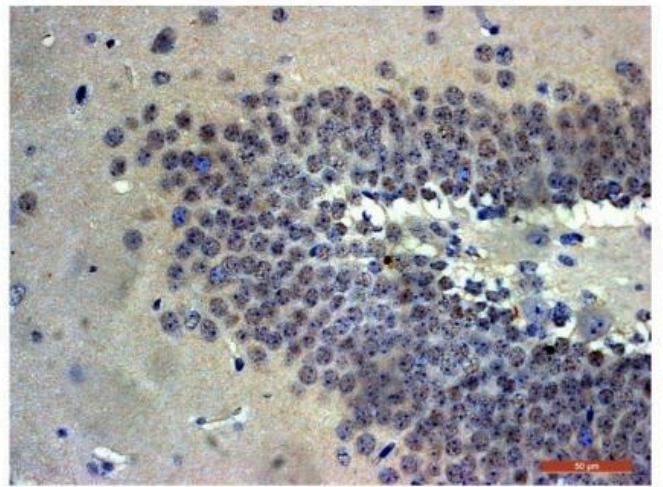
A



B



C



D

Figure 3

Immunohistochemical detection of Gem expression uses the immunoperoxidase method and hematoxylin counterstain in different regions of hippocampal tissues. Bar = 50 µm in

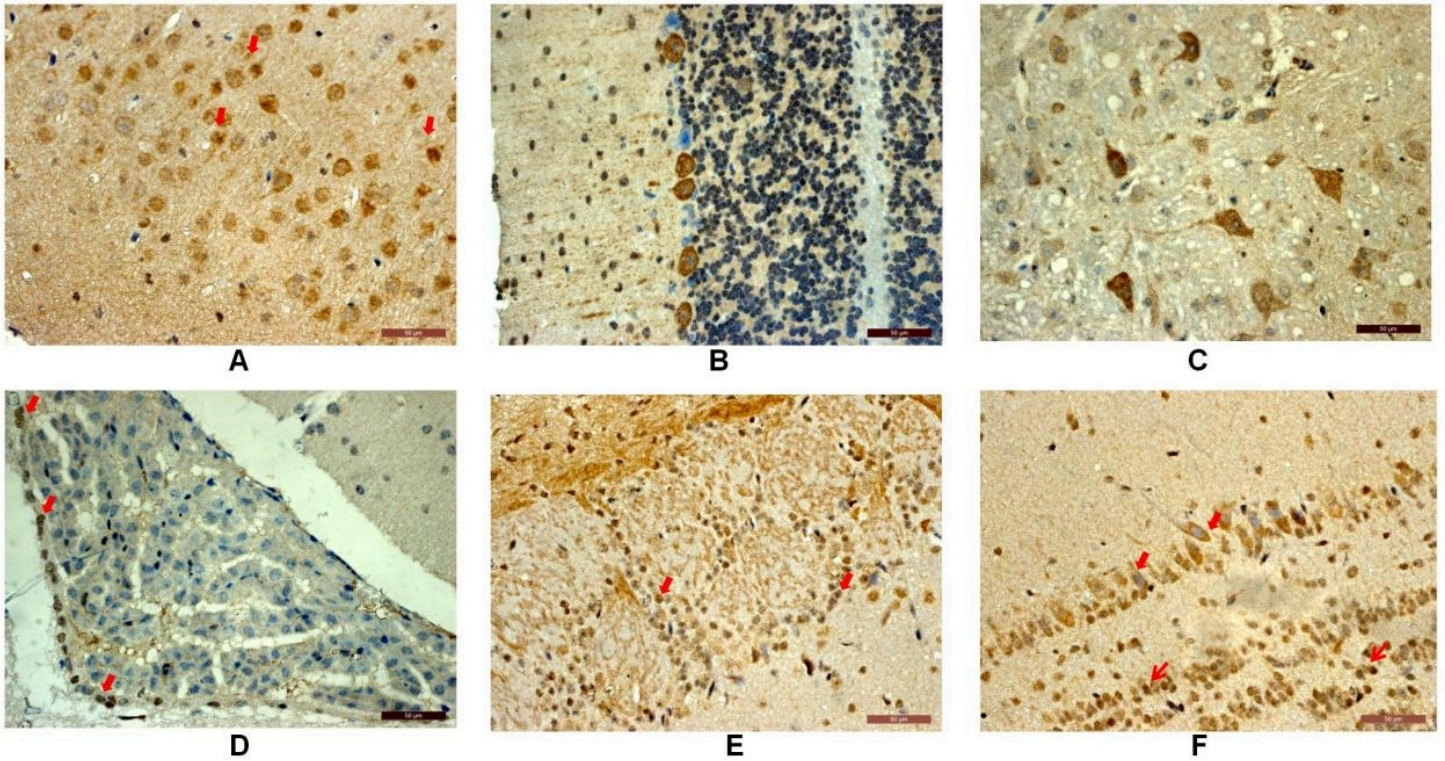


Figure 4

Immunohistochemical detection of Gem expression uses the immunoperoxidase method and hematoxylin counterstain in different regions of brain. Bar = 50 μ m in A-D.