

Increased expression of galanin and its receptors in the dorsal hippocampus during epileptogenesis in a rat model of pilocarpine-induced temporal lobe epilepsy

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KEYWORDS

antiepileptic, epilepsy, galanin, hippocampus, pilocarpine

ABSTRACT

Background: The neuropeptide galanin has been shown to exhibit anticonvulsant effects in animal models. However, only a few studies have attempted to identify the galanin receptor subtype (s) involved in this effect. In the present study, the expression of galanin and that of its receptors in a rat model of temporal lobe epilepsy was studied to reveal the epileptogenesis-related alterations.

Methods: Pilocarpine-induced status epilepticus (SE) rat model was used for temporal lobe epilepsy. Galanin and galanin receptor mRNA was detected by Real-time polymerase chain reaction in dorsal hippocampus of rats at three different time points following SE: at the acute phase (24h), latent period (7 days), and chronic epileptic period (8 weeks). Using immunohistochemistry, galanin positive fibers and bodies were also observed.

Results: In the acute seizure phase, a significant up-regulation of the galanin mRNA was observed in the hippocampus. Meanwhile, galanin-positive cells appeared, while galanin-positive fibers disappeared in the hilus of the dentate gyrus, stratum radiatum of CA1, and stratum lucidum of CA3, indicating an increase in galanin synthesis and release during the acute seizure phase. The gene expression analysis also indicated a significant increase in the transcription of galanin receptors GalR1 and GalR3 24h after SE. The increased levels of GalR1 mRNA were also observed in the latent and chronic epileptic periods.

Conclusion: In general, these data suggest that adaptive changes occur in the galanin system during epileptogenesis that may affect its antiepileptic effects. Furthermore, these changes should be taken into account when attempting to pharmacologically modulate this system.

1 Introduction

Epilepsy, one of the most prevalent chronic neurological disorders, is characterized by a persistent susceptibility to generate epileptic seizures [1], which could be sensitized by stress [2]. Despite the continued progress in epilepsy research, about 30% of epileptic patients have “intractable seizures” that do not respond to even the best treatment method [3]. Consequently, a critical need for innovative therapeutic compounds to combat pharmacoresistance has been observed [4]. Recently, the potential anticonvulsant and neuroprotective effects of neuropeptides, the most diverse family of neurotransmitters in the brain, have been further investigated.

Galanin, a 29–30 amino acid neuropeptide, is among the most inducible neuropeptides in the brain and is widely distributed throughout the central nervous system (CNS) [5,6]. So far, three galanin receptors have been cloned, namely, GalR1, GalR2, and GalR3 [7]. It has been shown that galanin can exert anticonvulsant effects after intrahippocampal administration in the picrotoxin-kindled model [8]. Furthermore, studies have reported that the likelihood of developing SE is increased in galanin knock out mice (GalKO), while it is reduced in galanin-overexpressing (GALOE) mice [9–11]. These findings suggest that galanin plays a significant role in modulating the occurrence and severity of SE. However, to date, only a few studies have addressed the role of galanin receptors in epilepsy [12–16]. The aim of the present study is to investigate the alterations in the expression of galanin and its receptors in the dorsal hippocampus of a pilocarpine-induced rat model of temporal lobe epilepsy (TLE), providing new insights into their potential involvement in epileptogenesis. This work was designed so as to determine the epileptogenesis-related alterations in the expression of galanin and

that of its receptors in the dorsal hippocampus of a pilocarpine-induced rat model of TLE. To this end, we performed a comparative real-time polymerase chain reaction (RT-PCR) analysis in order to detect the quantitative differences in the galanin receptors' gene expression at different time points after a pilocarpine-induced SE and to provide evidence linking galanin receptors to epilepsy.

2 Methods

2.1 Animals and housing

The experiments were performed using 5-week-old male Sprague–Dawley rats. The animals were kept at $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and exposed to a 12h artificial light/dark (8 am/20 pm) cycle, with access to a standard diet and tap water. All experiments were started at the same time of the day in order to minimize the possible effects of circadian variation. The study approval was obtained from the Animal Care Committee of the Capital Medical University (animal ethics number: AEEI-2016-015).

2.2 Induction of SE

In order to induce SE, the animals were injected intraperitoneally with lithium chloride (LiCl) at 3 mEq/kg (127 mg/kg), followed (18–20 h later) by intraperitoneal injections of pilocarpine (10 mg/kg) every 30 min [17]. All pilocarpine-induced behavioral alterations were recorded, while the appearance of motor seizure manifestations was considered to be the beginning of SE according to Racine's scale [18]: Stage 1, freezing, mouth, or facial movements; Stage 2, head nodding or isolated twitches; Stage 3, unilateral or bilateral forelimb clonus; Stage 4, rearing; and Stage 5, rearing and falling. After 45–60 min of SE, diazepam was administered

as a single injection of 20 mg/kg in order to limit the SE. The pilocarpine-treated rats that had experienced SE (i.e., all that had reached Stage 4) were sacrificed 24 h, 7 days, and 8 weeks after SE. The control animals were manipulated as described above, with the exception that the pilocarpine solution was replaced with saline.

2.3 Real-time polymerase chain reaction

The RNA samples from the control and pilocarpine-treated rats were processed in parallel under the same conditions. The tissues were lysed and homogenized, and the total RNA was isolated from the rat dorsal hippocampus (bregma -2 to -3 mm) and the ventral hippocampus (bregma -6 to -7 mm) using the TRIZOL reagent (Sigma-Aldrich, St. Louis, MO, USA). The total RNA (200 ng) was reverse transcribed using the SuperScript™ III RT reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. RT-PCR reactions were performed in Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and in an ABI 7300 Sequence Detector (Applied Biosystems, Foster City, CA, USA) under the following cycling conditions: 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and 1 min at 60°C. The samples were run in triplicate. The following specific primers were used: rat galanin (sense 5'-CACATGCCATTGA CAACCAC-3' and antisense 5'-AACTCCATT ATAGTGCGGACG-3'); GalR1 (sense 5'-TCGGG ACAGCAACCAAAC-3' and antisense 5'-TGCA GATGATTGAGAACCTTGG-3'); GalR2 (sense 5'-GCCGCCATCGGGCTCATCTG-3' and antisense 5'-GTGAGGTGCGCTCCATGCT-3'); and GalR3 (sense 5'-ACAGATCTCTTCATCCTCAACTT-3' and antisense 5'-GTGAGGTAGATGAGCAGATG TAC-3'). The housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (sense 5'-GACCACCCAGCCCAGCAAGG-3' and

antisense 5'-TCCCCAGGCCCTCCTGTTGG-3'), was used for the relative quantification of gene expression. For each animal, the results were expressed as relative mRNA levels, with the control defined as 100%. For each group (at 24 h, 7 days, and 8 weeks after SE), the results were expressed as mean percentage $\pm$ standard error of the mean. The data were evaluated by Student's *t*-test or a nonparametric test. *p* values lower than 0.05 were considered statistically significant.

2.4 Tyramide signal amplification (TSA) immunohistochemistry

For the immunohistochemistry analyses, a separate group of rats was used ($n = 4$ in each group) (24 h, 7 days, and 8 weeks after SE). The animals were deeply anesthetized with 2%–3% isoflurane using an animal anesthesia ventilator system (RWD Life Science Co. Ltd, Shenzhen, China) and perfused through the ascending aorta with 200 mL of 0.9% NaCl, followed by 0.1 mol/L sodium phosphate-buffered saline (PBS) containing 4% paraformaldehyde (pH 7.4). The brains were post-fixed in the same solution for 4–6 h, cryoprotected in 0.1 mol/L sodium PBS and 30% sucrose (pH 7.4 (4°C)) for 72 h, and serially sectioned on a cryotome. Subsequently, 30- μ m-thick coronal sections were collected in 0.1 mol/L phosphate buffer and processed for indirect immunofluorescence staining using a TSA Plus TMR/Fluorescence commercial kit (PerkinElmer, Waltham, MA, USA). Thus, the sections were immersed in 0.1% H₂O₂ in PBS, for 10 min, washed with a Tris-NaCl-Tween (TNT) buffer, blocked in TNB blocking buffer for 1 h, and incubated with rabbit anti-galanin antiserum (BachemAG, Bubendorf, Swiss) at a concentration of 1:10,000 overnight at 4 °C. After incubation in horseradish peroxidase-labeled goat anti-rabbit secondary antibody for 2h at room temperature, the sections were washed with TNT buffer for

15 min and were incubated in fluoresce in amplification reagent for 10 min. Subsequently, the sections were washed again with TNT buffer for 15 min, mounted with glycerin, and assessed using an Olympus microscope (Olympus, Tokyo, Japan). The specificity of the antigalantin antiserum was confirmed by absorption with rat galantin.

3 Results

3.1 Pilocarpine-induced SE and subsequent epilepsy

The administration of pilocarpine led to the development of SE in 80% of the animals ($n = 55$). The first Stage 4 seizure occurred between 30 and 120 min after the first pilocarpine administration. SE was characterized by a continuous seizure activity of the limbs, rearing and falling, forelimb clonus, or head nodding. Occasional running and jumping seizures could be observed during SE in some rats, but these rats were either sacrificed or excluded from the study. After recovering from SE, the rats were irritable and hypersensitive to sensory stimulation. Mortality (30%) occurred mainly within 48 h after the induction of SE. The manifestation of spontaneous seizures (e.g., head nodding, forelimb clonus) could be observed in rats reaching the chronic phase (8 weeks after SE). The control rats ($n = 31$) injected intraperitoneally with saline did not develop any of these seizure manifestations.

3.2 mRNA expression of the galantin and galantin receptors in the hippocampus during temporal lobe epileptogenesis

The analysis of the seizure-related changes in the transcript levels in the total RNA extracted at different time points after the induction of SE was done for the galantin, GalR1, GalR2, and GalR3 genes in the dorsal hippocampus. Relative quantification was performed using GAPDH as the endogenous control gene.

As shown in Fig. 1A, a significant up-regulation of galantin (7.5-times higher than the control; $p < 0.01$), GalR1 (37-times higher than the control; $p < 0.01$), and GalR3 (2.6-times higher than the control; $p < 0.05$) at 24 h following pilocarpine-induced SE was observed. When compared with the control group, only the GalR1mRNA level increased by 2.4-fold ($p < 0.05$) (Fig. 1B) and by 1.9-fold ($p < 0.05$) (Fig. 1C) at 7 days and 8 weeks after SE, respectively. No significant differences were observed in the expression levels of the other three transcripts (Fig. 1B, 1C). All of these findings suggest that the up-regulation of GalR1 played a significant role throughout the entire course of the epileptogenesis.

3.3 Galantin immunoreactivities in the hippocampus during temporal lobe epileptogenesis

In control rats, the galantin-positive fibers were found in all hippocampal areas (Fig. 2A, 3A, and

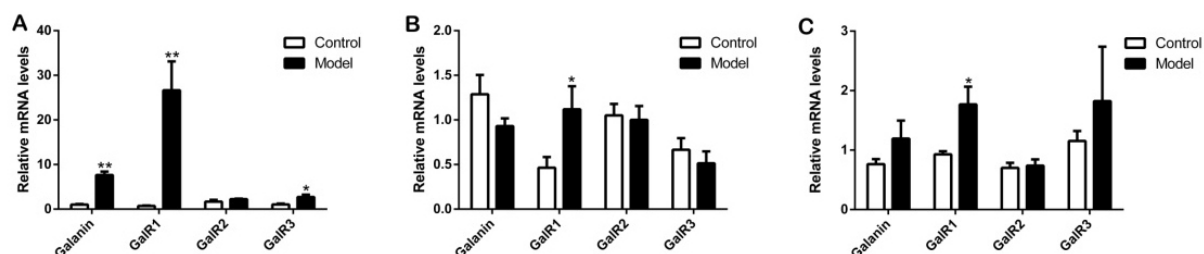


Figure 1 mRNA expression of galantin and galantin receptors in the rat hippocampus during temporal lobe epileptogenesis (A, 24 h after SE; B, 7 days after SE; C, 8 weeks after SE). The relative mRNA levels of the respective genes were determined by RT-PCR and normalized to the housekeeping gene (GAPDH). The asterisks indicate that the groups were significantly different when compared with the control group (** $p < 0.01$; * $p < 0.05$) ($n = 5-6$). Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PCR, polymerase chain reaction; SE, status epilepticus.

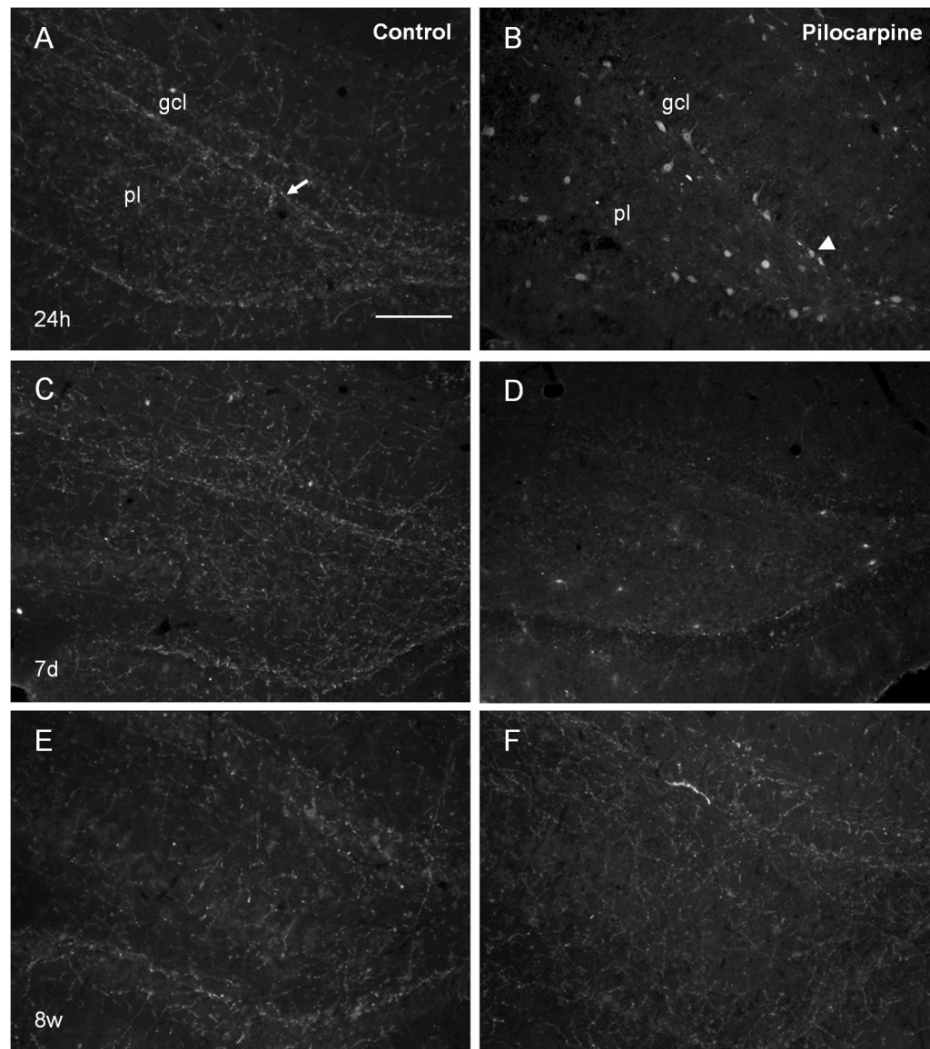


Figure 2 SE-induced changes in galanin-like immunoreactivity in the dentate gyrus of the dorsal rat hippocampus (dentate gyrus) (A and B, 24 h after SE; C and D, 7 days after SE; E and F, 8 weeks after SE; A,C, and E, control group; B,D, and F, model group). More specifically, 24 h after SE, the Gal-IR cells were visible in the hilus of the dentate gyrus, whereas 7 days and 8 weeks after SE, the model and control rats showed no statistical differences. Scale bar, 200 μ m. Arrow, cell fiber. Arrow head, cell body. Abbreviations: Gal-IR, galanin-immunoreactive; SE, status epilepticus; GCL, granule cell layer; PL, polymorph layer of the dentate gyrus.

Fig. 4A), with a high density observed in the hilus of the dentate gyrus (Fig. 2A) and in the stratum lucidum of CA3 (Fig. 4A). No galanin-positive cells were observed throughout all subfields of the hippocampus (Figs. 2A, 3A, and Fig. 4A). These data are consistent with those of a previous study [19].

In the acute seizure phase (24 h after SE), a significant reduction in the number of galanin-immunoreactive (Gal-IR) fibers in all layers of the hippocampal formation was observed (Fig. 2A with 2B, 3A with 3B, 4A with 4B). Meanwhile,

Gal-IR cell bodies appeared in all subfields of the hippocampus, mainly in the hilus of the dentate gyrus (Fig. 2B) and the stratum lucidum of the CA3 (Fig. 4B). Moreover, double labeling revealed that those cells were neurons as they were found to also be Neun-positive (Fig. 5A, 5B, 5C) but glial fibrillary acidic protein (GFAP)- and cluster determinant 11b (CD11b)-negative (Fig. 5D, 5E, 5F, and Fig. 5G, 5H, 5I).

In the latent phase (7 days after SE), some Gal-IR fibers with a lower density compared with the controls (Fig. 2C, Fig. 3C, and Fig. 4C)

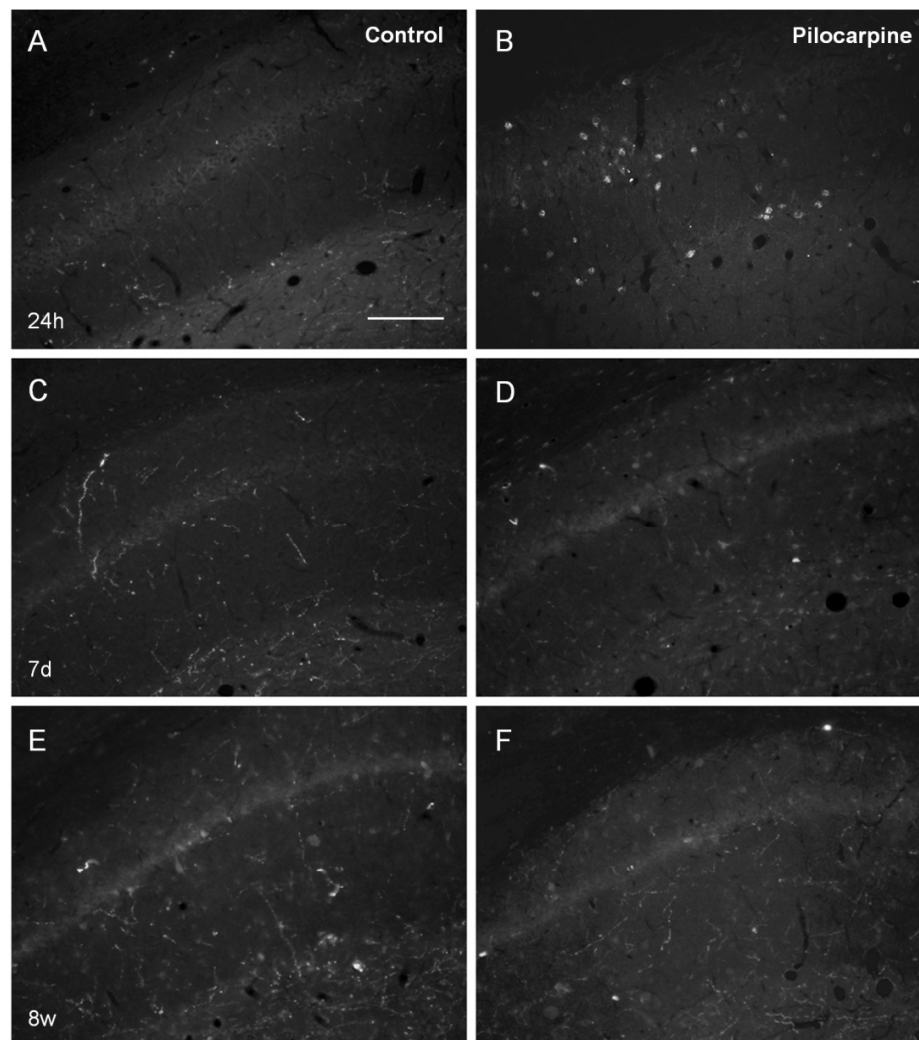


Figure 3 SE-induced changes in the galanin-like immunoreactivity of the CA1 region of the dorsal rat hippocampus (A and B, 24 h after SE; C and D, 7 days after SE; E and F, 8 weeks after SE; A,C, and E, control group; B,D,F, pilocarpine group). More specifically, 24 h after SE, the Gal-IR cells were visible in the stratum lacunosum-moleculare, stratum radiatum, and stratum oriens of CA1, whereas at 7 days and 8 weeks after SE, the pilocarpine-treated and control rats showed no statistical differences. Scale bar, 200 μ m. Abbreviations: Gal-IR, galanin-immunoreactive; SE, status epilepticus.

reappeared in all hippocampal areas, whereas the number and intensity of the Gal-IR cells decreased (Figs. 2D, 3D, and 4D).

In the chronic epileptic phase (8 weeks after SE), the Gal-IR fibers recovered in all parts of the hippocampal formation, whereas the Gal-IR cells were absent (Figs. 2F, 3F, and 4F). In fact, no significant difference was observed between the model and control rats (Fig. 2E, 3E, 4E). Furthermore, such observations strongly indicate a robust release of galanin in the rat hippocampus during the acute phase of the pilocarpine model.

4 Discussion

In the present study, we observed a significant increase in the mRNA expression level of the following: (i) galanin, GalR1, and GalR3 in the dorsal hippocampal formation at 24h after the SE induction and (ii) GalR1 in the dorsal hippocampal formation at 7 days and 8 weeks after the induction of SE. A strong reduction in the galanin immunoreactivity in fibers and a significant increase in the number of galanin-

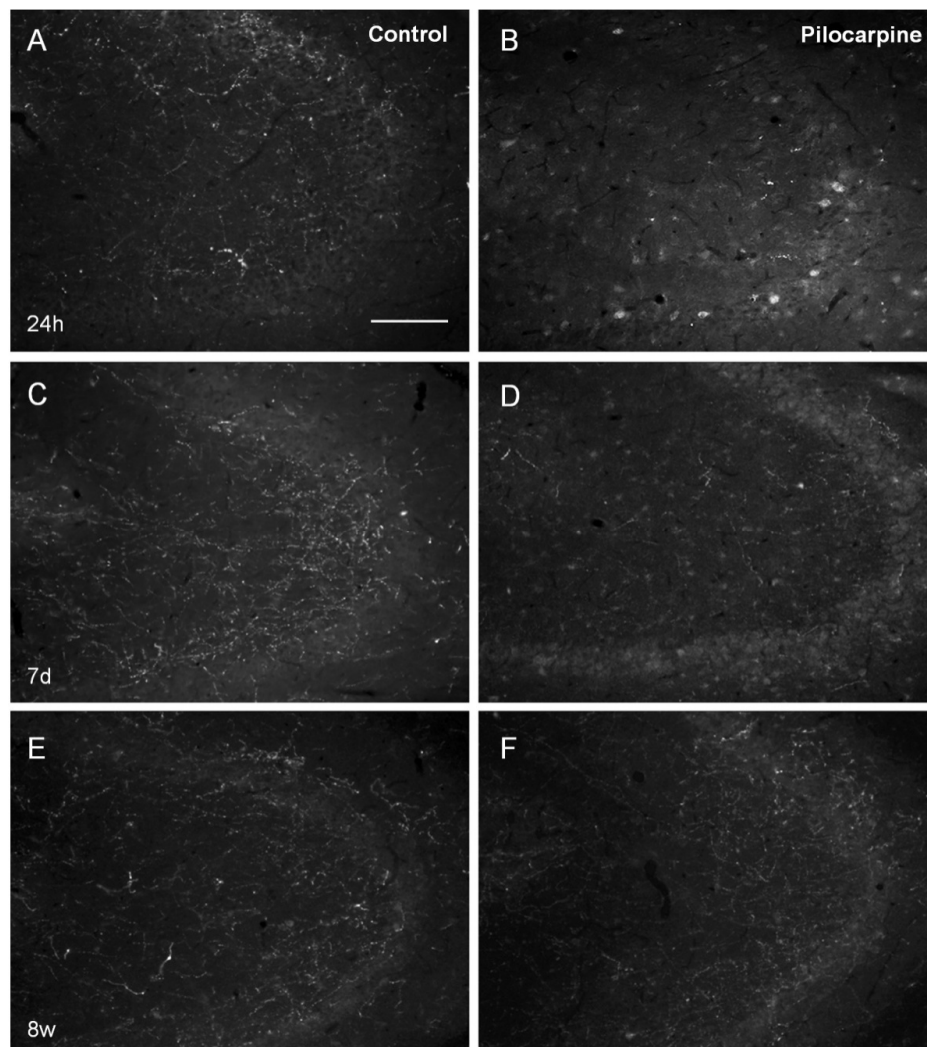


Figure 4 SE-induced changes in the galanin-like immunoreactivity of the CA3 region of the dorsal rat hippocampus (A and B, 24 h after SE; C and D, 7 days after SE; E and F, 8 weeks after SE; A,C, and E, control group; B,D, and F, pilocarpine group).More specifically, 24 h after SE, the Gal-IR cells were visible in the stratum lucidum of CA3, whereas at 7 days and 8 weeks after SE, the pilocarpine-treated and control rats showed no significant differences. Scale bar, 200 μ m. Abbreviations: Gal-IR, galanin-immunoreactive; SE, status epilepticus.

positive neurons in the dorsal hippocampal formation at 24h after the induction of SE were also observed, thereby suggesting that adaptive changes occur in the galanin system during epileptogenesis and that the galanin signaling may play a role as an endogenous anticonvulsant.

TLE accompanied by hippocampal sclerosis stands out as the prevailing form of partial complex seizure in adulthood [20]. Most of its characteristics can be reproduced in chronic animal models of TLE, particularly in the SE animal model [21]. The pilocarpine model is one of the SE models in which pilocarpine can be simply

injected intraperitoneally in order to induce a convulsive SE, chronic recurrent seizures appear after a latent period of about 2 weeks, and a hippocampal sclerosis develops [17]. In order to assess the seizure-related changes occurring in the gene expression of galanin and that of its receptors after pilocarpine-induced SE, RT-PCR was used for analysis and relative quantification was performed using GAPDH as an endogenous control gene. In an attempt to identify changes related to critical epileptic stages in the course of epileptogenesis, the rats were decapitated and analyzed at three different time points after SE

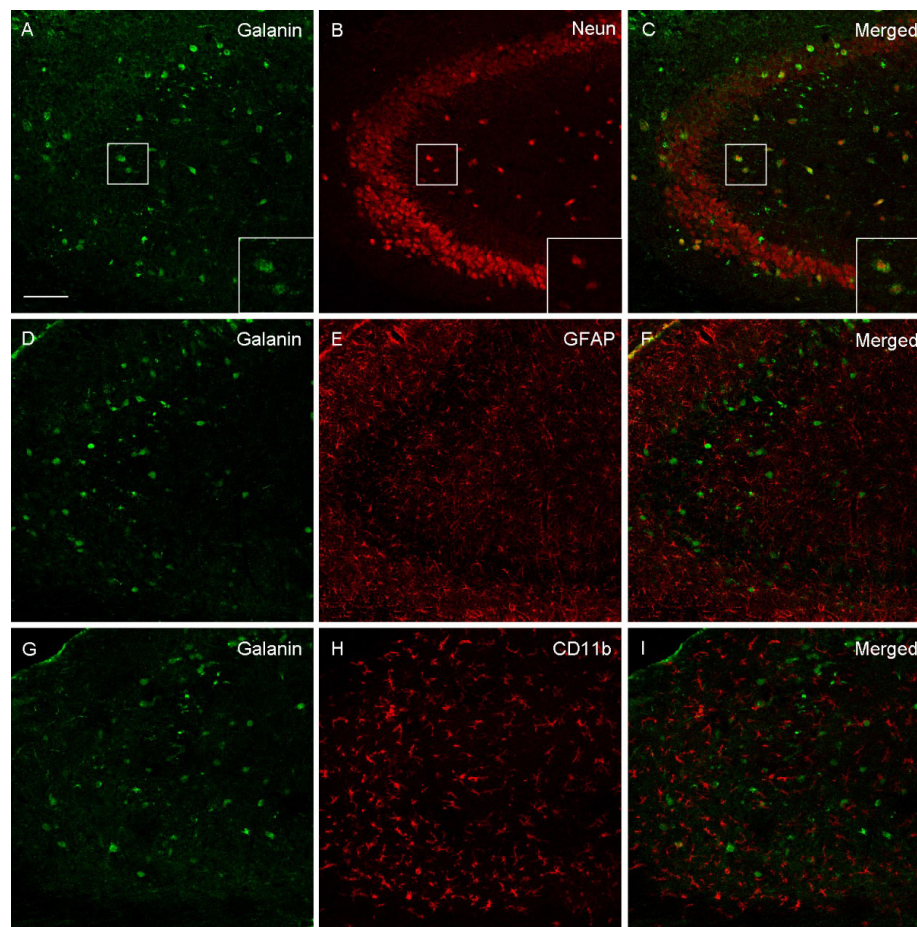


Figure 5 SE-induced galanin-like immunoreactivity was observed in neurons in the CA3 of the dorsal hippocampus. The galanin-IR cells (A, D, and G) were NeuN-positive (B) but also GFAP- and CD11b-negative (E and H, respectively). Scale bar, 100 μ m. Abbreviations: CD11b, cluster determinant 11b; GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclei; SE, status epilepticus. (C, F, and I) Merged with the green channel and the red channel.

induction: at the acute phase (24 h after SE), latent phase (7 days after SE), and chronic epileptic phase (8 weeks after SE).

Exogenous galanin has been shown to exhibit antiepileptic effects in numerous studies. For instance, the injection of galanin or adeno-associated vectors containing the galanin gene into the hippocampus is known to cause a seizure-suppressant effect in rodents [8, 22–25]. Resistance to seizure induction is increased in GALOE mice [9–11], while the propensity to develop seizures is increased in galanin knock out mice [9]. In the present study, an up-regulation of galanin both at the mRNA and peptide levels in the acute phase after SE induction was observed in the dorsal hippocampus of the pilocarpine-induced

SE model rats, indicating an increase in galanin synthesis during the acute phase, which is consistent with the findings of previous studies [26, 27]. Meanwhile, the galanin immunoreactivity in the fibers was significantly decreased in the acute phase and recovered in the chronic epileptic phase, thereby indicating a strong release of galanin during the acute phase. These data indicate that both the synthesis and the release of galanin in the rat hippocampus are up-regulated during the acute phase in a pilocarpine model. The inhibitory effect of galanin on glutamate release has been confirmed by biochemical [28] and electrophysiological [10] studies on rat hippocampal slices. A direct inhibitory effect of galanin fragments, such as galanin (1–15), on the

dorsal hippocampal CA3 pyramidal cells has also been observed [29]. In addition to its neuromodulating effects, galanin might also play a tropic role, as it does in the injury of other neuronal systems [30–33]. In general, these data suggest that the up-regulation of galanin in the rat hippocampus after the induction of SE may be indicative of this neuropeptide acting as an endogenous anticonvulsant peptide.

Among the galanin receptors cloned so far, the activation of GalR1 or that of GalR3 mainly causes inhibitory effects, whereas GalR2 mediates an excitatory action. In the present study, a significant up-regulation of the GalR1 transcripts was observed in all three assessed time points (24 h, 7 days, and 8 weeks) after the pilocarpine-induced SE. It has been shown that the activation of GalR1 can mainly result in inhibitory effects by coupling Gi/o proteins [34, 35]. Moreover, GalR1 knockout (GalR1 KO) mice can exhibit spontaneous epilepsy [12]. When compared with wild-type littermates, GalR1 KO mice tend to have more severe seizures and a more profound injury in the hippocampus upon Li-pilocarpine administration [13] or systemic kainate administration [36]. Thus, the up-regulation of GalR1 might play an anticonvulsant role in epilepsy. It has been indicated that in addition to its inhibitory effect on neuronal activity, GalR1 in the dorsal hippocampus can protect neurons from excitotoxicity. Thus, the up-regulated GalR1 may also mediate the neurotropic role of galanin during the chronic epilepsy phase. Therefore, the functional implications of the observed GalR1 transcript up-regulation need to be fully investigated in order to determine its role in epilepsy. A recent study has shown that the transcription factor Scratch2 can lower the expression of the GalR1 receptor by binding to an E-box in the GalR1 promoter [37]. Moreover, the extent to which Scratch2 is involved in the regulation of GalR1 in epilepsy needs to be determined.

GalR3 has been shown to be involved in depression [38], obesity [39], fluid homeostasis [40], and alcoholism [41]. The present study is the first to show that the GalR3 mRNA level is up-regulated in the dorsal hippocampus 24 h after the induction of SE, suggesting that GalR3, as GalR1, also plays an important role in epilepsy. In fact, both receptors exert inhibitory effects. It has been shown that GalR3 induces an inhibitory effect on dorsal raphe neurons [42]. However, the functional implications of the seizure-induced GalR3 gene expression up-regulation need to be further studied in a pilocarpine model of TLE.

GalR2 has also been shown to be involved in the inhibition of seizures. The down-regulation of GalR2 in the rat hippocampus can significantly increase both the severity and duration of SE [14]. The application of CYM2503, a GalR2-positive allosteric modulator, is known to delay epileptogenesis [15]. However, we did not find any significant differences in the transcript levels of GalR2 between the SE rats and their controls in any of the assessed time points. However, this finding is contrary to that of an earlier study in which the GalR2 mRNA level was found to be down-regulated in the rat hippocampus 20h after the induction of SE [43]. In the latter study, the SE rats were induced by pilocarpine administration without the use of diazepam. In fact, we also observed a ~30% decrease in the GalR2 mRNA level of the rat dorsal hippocampus when using our protocol without diazepam (data not shown). Thus, the observed down-regulation of GalR2 in the earlier study may be related to the application of diazepam.

In conclusion, the alterations observed in galanin expression and receptor activity within the hippocampus following SE strongly suggest the presence of an innate adaptive mechanism that is focused on mitigating the hyperexcitability associated with epileptic activity. These galanin system modifications may indeed signify an

intrinsic response to hinder the progression of epilepsy. To achieve a comprehensive understanding of the role of the galanin system in epilepsy and to develop targeted therapeutic strategies, further research is needed.

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Author contribution

J.S. and Z.-Q.D.X. designed research; J.S., S.X. and X.L. performed research; J.S., S.X., X.L. T.L., C.L., J.Z., G.L and Z.-Q.D.X. analyzed data; and J.S. and Z.-Q.D.X. wrote and revised the manuscript. All authors commented on previous versions of the manuscript and approved the final version.

Declaration of conflicting interests

The authors declare no conflict of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics approval

The study protocol was approved by the Ethics

Committee of the Capital Medical University (Ethical approval number AEEI-2016-015), and it was compliant with the Helsinki Declaration of 1975, as revised in 2008.

Consent to participate

Not applicable.

References

- [1] Falco-Walter, J. Epilepsy-Definition, Classification, Pathophysiology, and Epidemiology. *Semin Neurol*, **2020**, 40(6): 617–623.
- [2] Tekgul, H., Simsek, E., Erdoğan, M. A., Yiğittürk, G., Erbaş, O., Taşkiran, D. The potential effects of anticonvulsant drugs on neuropeptides and neurotrophins in pentylenetetrazol kindled seizures in the rat. *Int J Neurosci*, **2020**, 130(2): 193–203.
- [3] Sultana, B., Panzini, M. A., Veilleux Carpentier, A., Comtois, J., Rioux, B., Gore, G., Bauer, P. R., Kwon, C. S., Jetté, N., Josephson, C. B., Keezer, M. R. Incidence and Prevalence of Drug-Resistant Epilepsy: A Systematic Review and Meta-analysis. *Neurology*, **2021**, 96(17): 805–817.
- [4] Löscher, W., Potschka, H., Sisodiy, a S. M., Vezzani, A. Drug Resistance in Epilepsy: Clinical Impact, Potential Mechanisms, and New Innovative Treatment Options. *Pharmacol Rev*, **2020**, 72(3): 606–638.
- [5] Melander, T., Hokfelt, T., Rokaeus, A. Distribution of galaninlike immunoreactivity in the rat central nervous system. *J Comp Neurol*, **1986**, 248(4): 475–517.
- [6] Skofitsch, G., Jacobowitz, D. M. Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. *Peptides*, **1985**, 6(3): 509–546.
- [7] Branchek, T., Smith, K. E., Walker, M. W. Molecular biology and pharmacology of galanin receptors. *Ann N Y Acad Sci*, **1998**, 863: 94–107.
- [8] Mazarati, A.M., Halaszi, E., Telegdy, G. Anticonvulsive effects of galanin administered into the central nervous system upon the picrotoxin-kindled seizure syndrome in rats. *Brain Res*, **1992**, 589(1): 164–166.
- [9] Mazarati, A. M., Hohmann, J. G., Bacon, A., Liu, H., Sankar, R., Steiner, R. A., Wynick, D., Wasterlain, C. G. Modulation of hippocampal excitability and

- seizures by galanin. *J Neurosci*, **2000**, 20(16): 6276–6281.
- [10] Kokaia, M., Holmberg, K., Nanobashvili, A., Xu, Z. Q., Kokaia, Z., Lendahl, U., Hilke, S., Theodorsson, E., Kahl, U., Bartfai, T., Lindvall, O., Hökfelt, T. Suppressed kindling epileptogenesis in mice with ectopic overexpression of galanin. *Proc Natl Acad Sci U S A*, **2001**, 98(24): 14006–14011.
- [11] Schlifke, I., Kuteeva, E., Hökfelt, T., Kokaia, M. Galanin expressed in the excitatory fibers attenuates synaptic strength and generalized seizures in the piriform cortex of mice. *Exp Neurol*, **2006**, 200(2): 398–406.
- [12] McColl, C. D., Jacoby, A. S., Shine, J., Iismaa, T. P., Bekkers, J. M. Galanin receptor-1 knockout mice exhibit spontaneous epilepsy, abnormal EEGs and altered inhibition in the hippocampus. *Neuropharmacology*, **2006**, 50(2): 209–218.
- [13] Mazarati, A., Lu, X., Shinmei, S., Badie-Mahdavi, H., Bartfai, T. Patterns of seizures, hippocampal injury and neurogenesis in three models of status epilepticus in galanin receptor type 1 (GalR1) knockout mice. *Neuroscience*, **2004**, 128(2): 431–441.
- [14] Mazarati, A., Lu, X., Kilk, K., Langel, U., Wasterlain, C., Bartfai, T. Galanin type 2 receptors regulate neuronal survival, susceptibility to seizures and seizure-induced neurogenesis in the dentate gyrus. *Eur J Neurosci*, **2004**, 19(12): 3235–3244.
- [15] Lu, X., Roberts, E., Xia, F., Sanchez-Alavez, M., Liu, T., Baldwin, R., Wu, S., Chang, J., Wasterlain, C. G., Bartfai, T. GalR2-positive allosteric modulator exhibits anticonvulsant effects in animal models. *Proc Natl Acad Sci U S A*, **2010**, 107(34): 15229–15234.
- [16] Fetissov, S. O., Jacoby, A. S., Brumovsky, P. R., Shine, J., Iismaa, T. P., Hökfelt, T. Altered hippocampal expression of neuropeptides in seizure-prone GALR1 knockout mice. *Epilepsia*, **2003**, 44(8): 1022–1033.
- [17] Glien, M., Brandt, C., Potschka, H., Voigt, H., Ebert, U., Loscher, W. Repeated low-dose treatment of rats with pilocarpine: low mortality but high proportion of rats developing epilepsy. *Epilepsy Res*, **2001**, 46(2): 111–119.
- [18] Racine, R. J. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol*, **1972**, 32(3): 281–294.
- [19] Xu, Z. Q., Shi, T. J., Hökfelt, T. Galanin/GMAP- and NPY-like immunoreactivities in locus coeruleus and noradrenergic nerve terminals in the hippocampal formation and cortex with notes on the galanin-R1 and -R2 receptors. *J Comp Neurol*, **1998**, 392(2): 227–251.
- [20] Bruxel, E. M., Bruno, D. C. F., do Canto, A. M., Geraldini, J. C., Godoi, A. B., Martin, M., Lopes-Cendes, I. Multi-omics in mesial temporal lobe epilepsy with hippocampal sclerosis: Clues into the underlying mechanisms leading to disease. *Seizure*, **2021**, 90: 34–50.
- [21] Morimoto, K., Fahnestock, M., Racine, R. J. Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol*, **2004**, 73(1): 1–60.
- [22] Lin, E. J., Richichi, C., Young, D., Baer, K., Vezzani, A., During, M. J. Recombinant AAV-mediated expression of galanin in rat hippocampus suppresses seizure development. *Eur J Neurosci*, **2003**, 18(7): 2087–2092.
- [23] Haberman, R. P., Samulski, R. J., McCown, T. J. Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. *Nat Med*, **2003**, 9(8): 1076–1080.
- [24] McCown, T. J. Adeno-associated virus-mediated expression and constitutive secretion of galanin suppresses limbic seizure activity in vivo. *Mol Ther*, **2006**, 14(1): 63–68.
- [25] Kanter-Schlifke, I., Toft, S. A., Ledri, M., Kuteeva, E., Hökfelt, T., Kokaia, M. Galanin gene transfer curtails generalized seizures in kindled rats without altering hippocampal synaptic plasticity. *Neuroscience*, **2007**, 150(4): 984–992.
- [26] Mazarati, A. M., Liu, H., Soomets, U., Sankar, R., Shin, D., Katsumori, H., Langel, U., Wasterlain, C. G. Galanin modulation of seizures and seizure modulation of hippocampal galanin in animal models of status epilepticus. *J Neurosci*, **1998**, 18(23): 10070–10077.
- [27] Wilson, D. N., Chung, H., Elliott, R. C., Bremer, E., George, D., Koh, S. Microarray analysis of postictal transcriptional regulation of neuropeptides. *J Mol Neurosci*, **2005**, 25(3): 285–298.
- [28] Zini, S., Roisin, M. P., Langel, U., Bartfai, T., Ben-Ari, Y. Galanin reduces release of endogenous excitatory amino acids in the rat hippocampus. *Eur J Pharmacol*, **1993**, 245(1): 1–7.
- [29] Xu, Z. Q., Ma, X., Soomets, U., Langel, U., Hökfelt, T. Electrophysiological evidence for a hyperpolarizing,

- galanin (1-15)-selective receptor on hippocampal CA3 pyramidal neurons. *Proc Natl Acad Sci U S A*, **1999**, 96(25): 14583–14587.
- [30] Hökfelt, T., Wiesenfeld-Hallin, Z., Villar, M., Melander, T. Increase of galanin-like immunoreactivity in rat dorsal root ganglion cells after peripheral axotomy. *Neurosci Lett*, **1987**, 83(3): 217–220.
- [31] Villar, M. J., Meister, B., Cortes, R., Schalling, M., Morris, M., Hökfelt, T. Neuropeptide gene expression in hypothalamic magnocellular neurons of normal and hypophysectomized rats: a combined immunohistochemical and in situ hybridization study. *Neuroscience*, **1990**, 36(1): 181–199.
- [32] Cortes, R., Villar, M. J., Verhofstad, A., Hökfelt, T. Effects of central nervous system lesions on the expression of galanin: a comparative in situ hybridization and immunohistochemical study. *Proc Natl Acad Sci U S A*, **1990**, 87(19): 7742–7746.
- [33] Li, Y., Mei, Z., Liu, S., Wang, T., Li, H., Li, X.-X., Han, S., Yang, Y., Li, J., Xu, Z. Q. Galanin Protects from Caspase-8/12-initiated Neuronal Apoptosis in the Ischemic Mouse Brain via GalR1. *Aging Dis*, **2017**, 8(1): 85–100.
- [34] Xu, Z. Q., Zheng, K., Hökfelt, T. Electrophysiological studies on galanin effects in brain-progress during the last six years. *Neuropeptides*, **2005**, 39(3): 269–275.
- [35] Bai, Y. F., Ma, H.T., Liu, L. N., Li, H., Li, X. X., Yang, Y. T., Xue, B., Wang, D., Xu, Z. Q. D. Activation of galanin receptor 1 inhibits locus coeruleus neurons via GIRK channels. *BiochemBiophys Res Commun*, **2018**, 503(1): 79–85.
- [36] Schauwecker, P.E. Galanin receptor 1 deletion exacerbates hippocampal neuronal loss after systemic kainate administration in mice. *PLoS One*, **2010**, 5(12): e15657
- [37] Yang, Y., Li, Y., Liu, B., Li, C., Liu, Z., Deng, J., Luo, H., Li, X., Wu, J., Li, H., Wang, C.Y., Zhao, M., Wu, H., Lallemand, F., Svenningsson, P., Hökfelt, T. G. M., Xu, Z.Q.D. Involvement of Scratch2 in GalR1-mediated depression-like behaviors in the rat ventral periaqueductal gray. *Proc Natl Acad Sci U S A*, **2021**, 118(24): e1922586118.
- [38] Kuteeva E., Hökfelt T., Wardi.T, Ogren S. O. Galanin, galanin receptor subtypes and depression-like behaviour. *Exp Suppl*, **2010**, 102: 163–181.
- [39] Kim, A., Park, T. Diet-induced obesity regulates the galanin-mediated signaling cascade in the adipose tissue of mice. *Mol Nutr Food Res*, **2010**, 54(9): 1361–1370.
- [40] Schmidhuber, S. M., Rauch, I., Kofler, B., Brain, S. D. Evidence that the modulatory effect of galanin on inflammatory edema formation is mediated by the galanin receptor 3 in the murine microvasculature. *J Mol Neurosci*, **2009**, 37(2): 177–181.
- [41] Belfer, I., Hipp, H., Bollettino, A., McKnight, C., Evans, C., Virkkunen, M., Albaugh, B., Max, M. B., Goldman, D., Enoch, M. A. Alcoholism is associated with GALR3 but not two other galanin receptor genes. *Genes Brain Behav*, **2007**, 6(5): 473–481.
- [42] Swanson, C. J., Blackburn, T. P., Zhang, X., Zheng, K., Xu, Z. Q., Hökfelt, T., Wolinsky, T. D., Konkel, M. J., Chen, H., Zhong, H., Walker, M. M., Durkin, M., Artymyshyn, R. P., Smith, K. E., Jones, K. J., Craig, D. A., Gerald, C. P., Branchek, T. A. Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal3) antagonists SNAP 37889 and SNAP 398299. *Proc Natl Acad Sci U S A*, **2005**, 102(48): 17489–17494.
- [43] Lu, X., Mazarati, A., Sanna, P., Shinmei, S., Bartfai, T. Distribution and differential regulation of galanin receptor subtypes in rat brain: effects of seizure activity. *Neuropeptides*, **2005**, 39(3): 147–152.