Epilepsy & Behavior 96 (2019) 87–91

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Time course of transient expression of pre-α-pro-GDNF and pre-β-pro-GDNF transcripts, and mGDNF mRNA region in Krushinsky–Molodkina rat brain after audiogenic seizures

Georgy Solius [a], Dmitri Panteleev [a], Nikolay Pustogarov [a], Alexander Revishchin [a], Inga Poletaeva [d], Galina Pavlova [a,b,c,*]

[a] Institute of Gene Biology, Russian Academy of Sciences, 34/5, Vavilova Street, Moscow 117312, Russia
[b] Burdenko Neurosurgical Institute, 4-th Tverskaya-Yamskaya st., 16, Moscow 125047, Russia
[c] Sechenov First Moscow State Medical University, Institute of Molecular Medicine, 8 Trubetskaya Street, Bldg. 2, Moscow 119991, Russia
[d] Lomonosov Moscow State University, GSP-1, Leninskie Gory, Moscow 119991, Russia

ARTICLE INFO

Article history:
Received 2 November 2018
Revised 12 February 2019
Accepted 7 March 2019
Available online 18 May 2019

Keywords:
Audiogenic seizures
GDNF transcripts
Rat brain
Brainstem

ABSTRACT

The expression of glial cell line-derived neurothrophic factor (GDNF) transcript forms *pre-(α)pro-gdnf*, *pre-(β)pro-gdnf*, and their common region *m-gdnf* in the pons as well as the inferior (IC) and superior colliculi in Krushinsky–Molodkina (KM) rats and in the strain "0" was analyzed by quantitative real-time polymerase chain reaction (PCR) in the control (unstimulated KM and "0" rats) and 1.5, 4.5, and 8 h after auditory stimulation. Such stimulation induced audiogenic seizures (AS) in KM rats. Audiogenic seizure was not observed in "0" rats, which was obtained by selection for the absence of AS in a population of F2 hybrids between KM and Wistar rats not predisposed to AS. A significant drop in the level of all transcripts was observed 1.5 h after auditory stimulation in both KM and "0" rats. In most cases, the average expression of α and β isoforms and m-region 4.5 h after stimulation was greater than those after 1.5 and 8 h. At the same time, the expression of *pre-(β)pro-gdnf* in the IC of KM rats 4.5 h after the stimulation was significantly lower than after 1.5 or 8 h. This work presents the first demonstration of different time courses of expression of the α and β GDNF isoforms during physiological processes in genotype-specific pathology.

1. Introduction

Animals genetically predisposed to seizures, particularly, rats with audiogenic seizures (AS), are a promising model for studies of epileptogenesis [1]. The involvement of the glial cell line-derived neurothrophic factor (GDNF) in the mechanisms of epileptogenesis has been studied since the discovery of this protein [2–5]. It was shown that the level of GDNF messenger ribonucleic acid (RNA) mRNA in granule cells of the dentate gyrus increased as the result of acute repeated seizures induced by kainic acid [6] or pilocarpine [7] administration.

For instance, kainic acid induces death of hippocampal pyramidal cells. It is of interest to reveal the relationship between GDNF and seizure activity using the models of reflex epilepsy unrelated to drug stimulation. Here, we used Krushinsky–Molodkina (KM) rats as a genetically-based model of reflex epilepsy induced by auditory stimulation. Nearly 100% KM rats demonstrate tonic seizures in response to loud sound, which distinguishes this model of epileptogenesis from "limbic" myoclonic seizures induced by administration of convulsants [1].

The studies mentioned above analyzed the transcripts coding mature GDNF common for the α and β fragments without any distinction between the isoforms. At the same time, the GDNF gene is known to encode two mRNA species: the full-length transcript *pre-(α)pro-gdnf* and the shorter *pre-(β)pro-gdnf* lacking 78 nt in the pro-domain [8,9]. These isoforms are generated by alternative splicing of the GDNF transcript. At the same time, *pre-(α)pro-gdnf* and *pre-(β)pro-gdnf* have different localization in the cell. Moreover, pre-(α)pro-gdnf is thought to be secreted largely via the constitutive pathway, whereas pre-(β)pro-gdnf is largely secreted via the alternative possibly regulated pathway [10].

Here, we studied the time course of expression of *pre-(α)pro-gdnf* and *pre-(β)pro-gdnf* as well as their common region m-*gdnf* encoding mature GDNF after auditory stimulation in KM and rats of "0" strain without audiogenic seizures. The latter strain "0" was selected for no AS in F2 hybrids between KM and Wistar rats not prone to AS [1,11]. The brainstem origin of tonic AS has been clearly

* Corresponding author at: Institute of Gene Biology, Russian Academy of Sciences, 34 Vavilov st., 119334, Russia.
E-mail address: lkorochkin@mail.ru (G. Pavlova).

https://doi.org/10.1016/j.yebeh.2019.03.007
1525-5050/

demonstrated [1]. In this context, the expression time course of isoforms was studied in the pons as well as the superior (SC) and inferior (IC) colliculi.

2. Methods

All in vivo experiments were performed in accordance with European regulation 2010/63/EU. Expression of the full-length *pre-(α)pro-gdnf*, short *pre-(β)pro-gdnf* transcripts, and their common *m-gdnf* region was analyzed in 12 KM and 12 "0" rats (males at the age of 4–6 months).

An old auditorium bell sound of 100 dB for 30 s was used as a stimulus. After 1.5, 4.5, and 8 h, the animals were anesthetized by lethal dose of chloral hydrate and the brain samples of pons varolii, IC, and SC were frozen in liquid nitrogen. Each time point was studied in 3 KM and 3 "0" rats; 3 more KM and "0" animals were analyzed without stimulation.

Total RNA was isolated from frozen tissue samples homogenized by active pipetting in 800 μl (IC and SC) or 1 ml (pons) of RNAzol (MRC, #RN 190) using the manufacturer's protocol. The obtained RNA was used to synthesize the first complementary DNA (cDNA) strand with Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (Evrogen, #SK021). The resulting cDNA served as the template in quantitative real-time polymerase chain reaction (PCR) (qRT-PCR). The quantitative PCR (qPCR) was performed with use of PCR-Komplekt kit in the presence of the SYBR Green dye (Syntol). For the GDNF transcripts amplification, the following primer pairs were used: F-alfa and R-1 for *(α)pro-gdnf* transcript; F-beta and R-1 for *(β)pro-gdnf* transcript; and F-1 and R-1 for their common m-region (Fig. 1). Initially, the housekeeping genes encoding hypoxanthine guanine phosphoribosyl transferase gene (HPRT; primers HPRT-f and HPRT-r) and beta-2-microglobulin (B2m; primers b2m-f & b2m-r) were used for reference; however, using HPRT alone was equally informative and B2m was excluded. Primer efficiency was calculated for all pairs. The expression levels for different GDNF regions were corrected for the primer efficiency.

The following primers were used in this study (Fig. 1), presented in 5′ to 3′ direction.

F-alfa GCC GGT AAG AGG CTT CTC GAA
F-beta CCC GCT GCC CGC CGC CAA TAT
F-1 AGC CCA GAG AAT TCC AGA GG
R-1 GCT TCA CAG GAA CCG CTA CA
HPRT-f CCC AGC GTC GTG ATT AGT GAT
HPRT-r CCA GCA GGT CAG CAA AGA AC
b2m-f TGC TTG CCA TTC AGA AAA CTC C
b2m-r ATA CAT CGG TCT CGG TGG GT

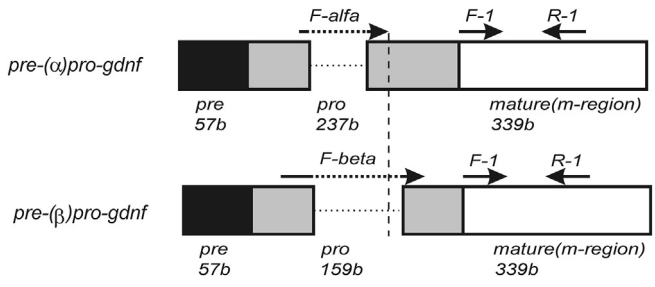

Fig. 1. Arrangement of RT-qPCR primers on the *pre-(α)pro-gdnf* (upper) and *pre-(β)pro-gdnf* (lower) transcripts. Dotted line in the pro-region of the β-form marks the 78-nt gap distinguishing the α- and β-isoforms. The F-alfa covers positions 70–90 relative to the start codon (the AUG codon) of the α-isoform. The region complementary to F-beta covers the larger region upstream of the gap (positions 60–73) and the smaller downstream region (152–158), which rules out the possibility of annealing to the α-isoform. The F1 primer covers the beginning of the m-region common for the α- and β-isoforms (295–314). The reverse primer positions are 433–452 of m-region.

The fold change of the relative mRNA expression of each *gdnf* form was calculated with the 2-ΔΔCT method. Statistical analysis was performed using the SPSS software. The values were compared by the Mann–Whitney *U* test. Statistical significance was accepted at $p < 0.05$.

3. Results

Expression analysis of *pre-(α)pro-gdnf*, *pre-(β)pro-gdnf*, and their common m-region by RT-qPCR has demonstrated decreased mean levels of these mRNAs relative to the control (unstimulated KM and "0" rats) 1.5 and 8 h after stimulation in the pons, IC, and SC of KM and "0" rats (Fig. 2). In many cases, the poststimulation levels significantly differed from the control according to the one-sided Mann–Whitney test ($p < 0.05$). In particular, the expression levels after 1.5 h were significantly different from the control in KM and "0" rats except for m-region in the pons in "0" rats (Fig. 2A) and *pre-(α)pro-gdnf* in the IC of both strains (Fig. 2B). The expression levels 8 h after auditory stimulation were significantly lower than the control except for *pre-(α)pro-gdnf* in the IC of both strains (Fig. 2B) and *m-region* in the IC and SC of "0" rats (Fig. 2B and C). "0" rats demonstrated increased mean expression of all studied transcripts in all studied structures 4.5 h after auditory stimulation relative to the corresponding values after 1.5 and 8 h. The mean levels of these transcripts in KM 4.5 h after the stimulation were also higher than those after 1.5 and 8 h except for *pre-(β)pro-gdnf* in the IC. The levels of *pre-(α)pro-gdnf* and *m-region* in the SC of KM rats 4.5 h after the stimulation were significantly higher than those after 1.5 and 8 h, while the mean *pre-(β)pro-GDNF* levels in KM and "0" as well as of *m-region* in "0" rats were even (insignificantly) higher than levels in control. The expression of *pre-(β)pro-gdnf* in this structure significantly decreased ($p < 0.05$) after 4.5 h relative to 1.5 and 8 h after stimulation and seizure.

The total expression of *pre-(α)pro-gdnf* and *pre-(β)pro-gdnf* was in most cases lower than the expression of their common *m-gdnf*, which can be attributed to the existence of other than *pre-(α)pro-gdnf* and *pre-(β)pro-gdnf* isoforms. Additional alternative splice variants of the GDNF gene transcripts were found in a mouse [1] and in humans [12–15]. The mean level of *m-region* mRNA in the control in KM rats was (insignificantly) higher than that in "0" rats in the IC and SC (Fig. 2 A and B), and the fold change of the relative mRNA expression of each gdnf form was calculated with the 2-ΔΔCT method (Fig. 3).

4. Discussion

Here, a significant decrease in the mean level was demonstrated for all studied *GDNF* transcripts 1.5 and 8 h and less frequently 4.5 h after audiogenic seizure-inducing auditory stimulation both in audiogenic seizure-prone KM rats and in "0" strain. Considering that the effect is observed in both strains, this response is likely unrelated to AS.

Studies on other models of epilepsy demonstrated increased protein and mRNA levels of GDNF in the dentate gyrus and hilus after chemically-induced seizures [6,7]. One can propose that convulsant-induced increase in GDNF level is due to its damaging effect rather than to seizure activity. Our study demonstrated no increase in the level of GDNF transcription after audiogenic seizure-inducing auditory stimulation in KM rats relative to the background, which can be interpreted in favor of the above proposal. However, we have revealed a complex pattern of changes in GDNF transcription after auditory stimulation. A significant decrease was followed by an increase in the mean expression levels after 4.5 h (in all but one case). Another drop was observed 8 h after the stimulation. The revealed transcription dynamics can result from the interplay between

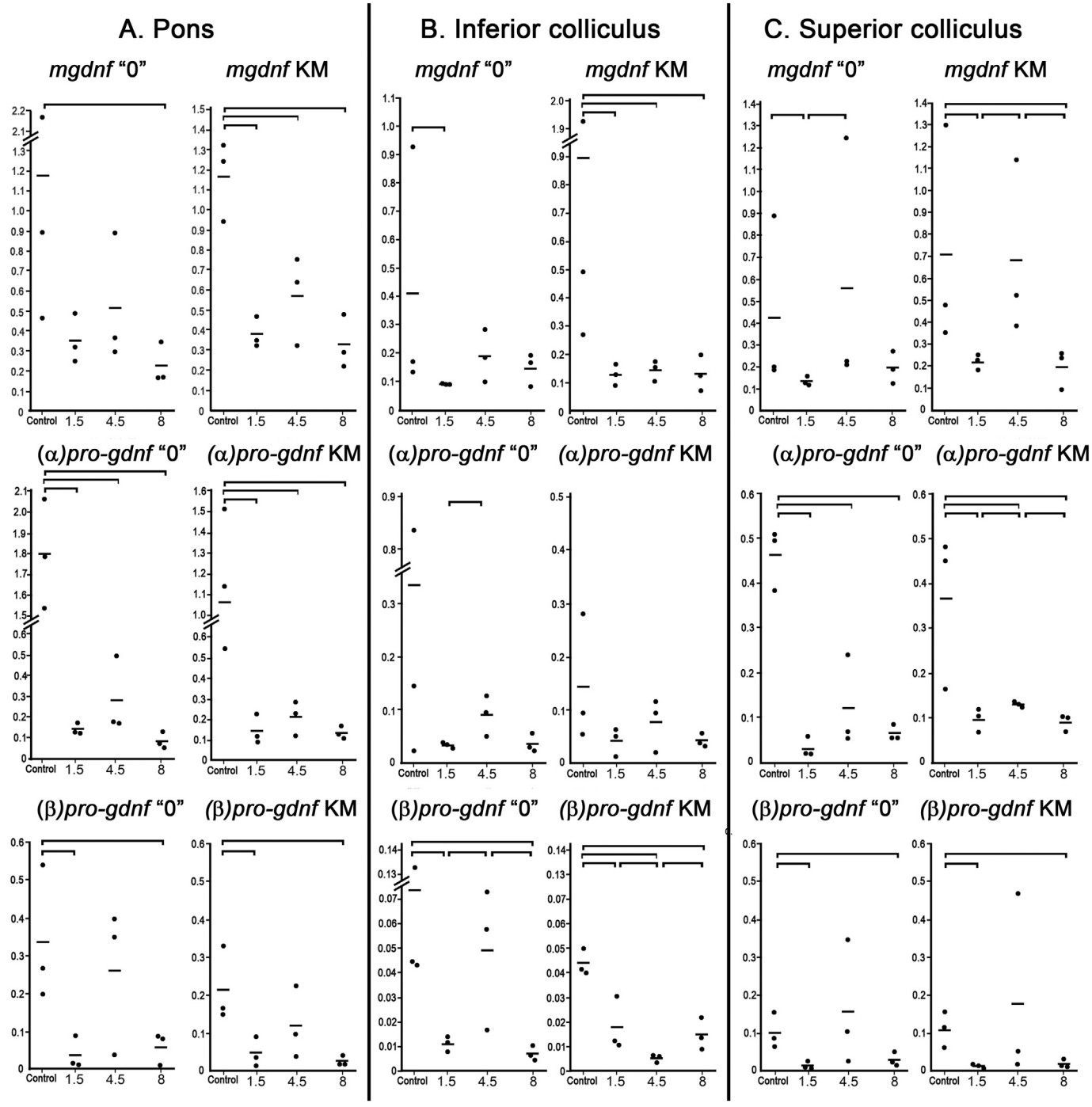

Fig. 2. Time course of *pre-(α)pro-gdnf*, *pre-(β)pro-gdnf*, and *m-region* expression in the pons (A), IC (B), and SC (C) (ordinate). Amounts are relative to HPRT. The abscissa shows time after auditory stimulation. Bars indicate the main values. Significantly differing values according to the one-sided Mann–Whitney test ($p < 0.05$) are indicated by lines above.

positive and negative factors. The balance between these factors can differ in KM and "0" rats.

5. Conclusions

Of particular interest is the revealed correlation between epileptic seizures in KM and decreased expression of *pre-(β)pro-gdnf* in the IC 4.5 h after stimulation. This has not been observed in "0". Thus, this study presents the first demonstration of different expression of GDNF α- and β-isoforms in the living body in pathology determined by genotypic factors.

Acknowledgments

This work was supported by the Russian Foundation for Basic Research (project nos. 16-04-01094 and 17-29-01001) and by the Program of the Presidium of the Russian Academy of Sciences "Foundations of Technology of Physiological Adaptations."

Conflicts of interest

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in

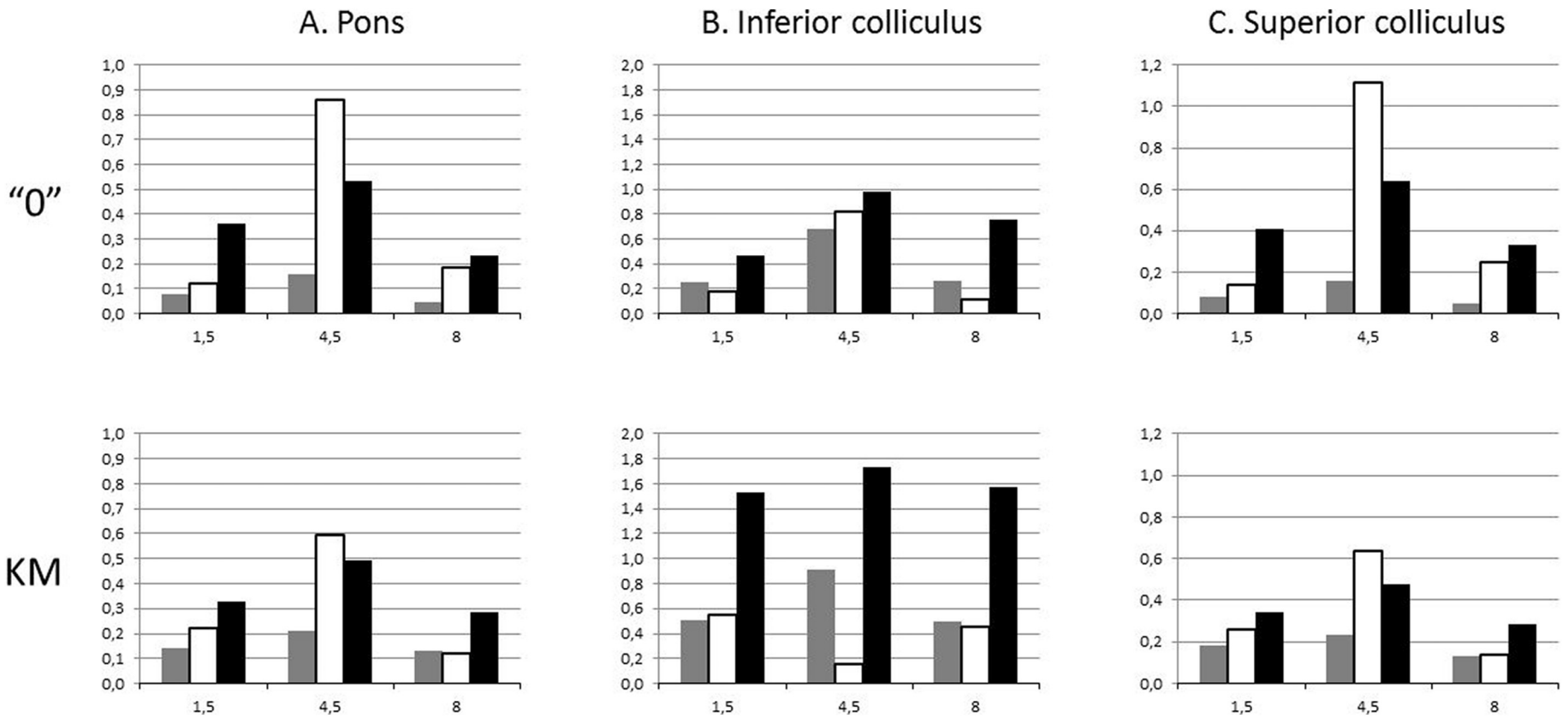

Fig. 3. The fold change of the relative *pre-(α)pro-gdnf*, *pre-(β)pro-gdnf*, and m-region mRNA expression in the pons (A), IC (B), and SC (C) (ordinate) calculated with the 2-ΔΔCT method.

ethical publication and affirm that this report is consistent with those guidelines.

References

[1] Poletaeva II, Surina NM, Kostina ZA, Perepelkina OV, Fedotova IB. The Krushinsky–Molodkina rat strain: the study of audiogenic epilepsy for 65years. Epilepsy Behav 2017;71:130–41.

[2] Chen AC, Eisch AJ, Sakai N, Takahashi M, Nestler EJ, Duman RS. Regulation of GFRalpha-1 and GFRalpha-2 mRNAs in rat brain by electroconvulsive seizure. Synapse 2001;39:42–50.

[3] Duveau V, Fritschy JM. PSA-NCAM-dependent GDNF signaling limits neurodegeneration and epileptogenesis in temporal lobe epilepsy. Eur J Neurosci 2010;32:89–98.

[4] Martin D, Miller G, Rosendahl M, Russell DA. Potent inhibitory effects of glial derived neurotrophic factor against kainic acid mediated seizures in the rat. Brain Res 1995; 683:172–8.

[5] Yoo YM, Lee CJ, Lee U, Kim YJ. Neuroprotection of adenoviral-vector-mediated GDNF expression against kainic-acid-induced excitotoxicity in the rat hippocampus. Exp Neurol 2006;200:407–17.

[6] Humpel C, Hoffer B, Stromberg I, Bektesh S, Collins F, Olson L. Neurons of the hippocampal formation express glial cell line-derived neurotrophic factor messenger RNA in response to kainate-induced excitation. Neuroscience 1994;59:791–5.

[7] Schmidt-Kastner R, Tomac A, Hoffer B, Bektesh S, Rosenzweig B, Olson L. Glial cell-line derived neurotrophic factor (GDNF) mRNA upregulation in striatum and cortical areas after pilocarpine-induced status epilepticus in rats. Brain Res Mol Brain Res 1994;26:325–30.

[8] Suter-Crazzolara C, Unsicker K. GDNF is expressed in two forms in many tissues outside the CNS. Neuroreport 1994;5:2486–8.

[9] Trupp M, Ryden M, Jornvall H, Funakoshi H, Timmusk T, Arenas E, et al. Peripheral expression and biological activities of GDNF, a new neurotrophic factor for avian and mammalian peripheral neurons. J Cell Biol 1995;130:137–48.

[10] Lonka-Nevalaita L, Lume M, Leppanen S, Jokitalo E, Peranen J, Saarma M. Characterization of the intracellular localization, processing, and secretion of two glial cell line-derived neurotrophic factor splice isoforms. J Neurosci 2010;30: 11403–13.

[11] Fedotova IB, Kostyna ZA, Surina NM, Poletaeva II. Laboratory rat selection for the trait "the absence of audiogenic seizure proneness". Genetika 2012;48:685–91.

[12] Tanaka M, Ito S, Kiuchi K. Novel alternative promoters of mouse glial cell line-derived neurotrophic factor gene. Biochim Biophys Acta 2000;1494:63–74.

[13] Grimm L, Holinski-Feder E, Teodoridis J, Scheffer B, Schindelhauer D, Meitinger T, et al. Analysis of the human GDNF gene reveals an inducible promoter, three exons, a triplet repeat within the 3'-UTR and alternative splice products. Hum Mol Genet 1998;7:1873–86.

[14] Airavaara M, Pletnikova O, Doyle ME, Zhang YE, Troncoso JC, Liu QR. Identification of novel GDNF isoforms and cis-antisense GDNFOS gene and their regulation in human middle temporal gyrus of Alzheimer disease. J Biol Chem 2011;286:45093–102.

[15] Baecker PA, Lee WH, Verity AN, Eglen RM, Johnson RM. Characterization of a promoter for the human glial cell line-derived neurotrophic factor gene. Brain Res Mol Brain Res 1999;69:209–22.