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Comparison of the Temporary Dynamics of NGF and BDNF Gene Expression in Rat Hippocampus, Frontal Cortex, and Retina Under Semax Action

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Abstract Neurotrophins are a family of structurally related proteins that regulate the survival, differentiation, and maintenance of function of different neuron populations. Some peptides are able to affect the production and activity of neurotrophins. One of these synthetic peptides is heptapeptide Semax, an analog of the N-terminal adrenocorticotropic hormone fragment 4-10. It is known that Semax has effects on learning and memory formation and exerts some neuroprotective effects in rodents and humans. Male Wistar rats were treated for 20 min, 40 min, 90 min, 3 h, 8 h, and 24 h with Semax. Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) gene expression in rat brain and retina was analyzed by real-time polymerase chain reaction. It was revealed that after Semax administration the multidirectional activation of the expression of the genes under investigation in the hippocampus, frontal cortex, and retina was observed. The expression of both neurotrophin genes was decreased in rat hippocampus and retina 20 min after Semax administration and was increased in the frontal cortex. The expression levels of NGF remained practically constant in the retina at the initial stage, whereas the expression levels of BDNF were significantly increased 90 min after Semax administration.

Keywords Brain-derived neurotrophic factor (BDNF) · Nerve growth factor (NGF) · ACTH · Semax · Gene expression

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Introduction

Neurotrophins are a family of structurally related proteins that regulate the survival, differentiation, and maintenance of function of different neuron populations. The most prominent members of the mammalian neurotrophin family are the nerve growth factor (NGF), the brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). The neurotrophins bind two classes of receptor: the common p75NTR, which is a member of the tumor necrosis factor receptor family (Bothwell 1995; Dechant and Barde 2002), and the tropomyosin-related kinase (trk) receptors, which are members of the large tyrosine kinase receptor family (Lewin and Barde 1996). These neurotrophins function to support the growth and survival of many neuronal populations (Patapoutian and Reichardt 2001; Kirstein and

Farinas 2002). NGF-deficient mice die shortly after birth and show a decrease in sensory and sympathetic neurons (though basal forebrain cholinergic neurons develop relatively normally); however, this perinatal lethality can be rescued by transgenic expression of NGF under the K14 keratin promoter, which restores the sensory and sympathetic neuronal populations (Harrison et al. 2004). These rescued mice exhibit reduced cholinergic innervation in the cortex and hippocampus, which can be restored by intracerebroventricular delivery of NGF (Phillips et al. 2004). Disruption of a single NGF allele causes deficits in memory acquisition and hippocampal cholinergic innervation, which suggests that NGF is required for the formation and maintenance of correct innervations (Chen et al. 1997). BDNF-deficient mice also die shortly after birth because of defects in the brain and sensory, but not motor, neuron development (Jones et al. 1994). Long-term potentiation (LTP) and mechanosensation are impaired in heterozygous BDNF knockout animals (Carroll et al. 1998). Because of their ability to protect multiple neuronal cell types from apoptosis, neurotrophins can stimulate neuronal regeneration in vitro and in model systems (Thoenen 2000; Huang and Reichardt 2001). The fact that neurotrophins can prevent or reverse neuronal cell loss renders them good therapeutic targets in neurodegenerative diseases and in brain or spinal cord injuries (Horner and Gage 2000).

At present, comprehensive investigations of members of this protein family are being carried out to create new therapeutic drugs for different neurodegenerative diseases. The mechanisms of action of different peptides that are able to affect the production and activity of neurotrophins are also under study. Semax is one of these synthetic peptides and consists of the adrenocorticotropic hormone fragment 4–10 (ACTH 4–10) Met-Glu-His-Phe and a C-terminal Pro-Gly-Pro peptide (Asmarin et al. 1997). The ACTH 4–10 N-terminal fragment is known to stimulate the processes of attention, learning, and memory formation (De Wied and Gispen 1977). The Pro-Gly-Pro fragment of Semax is responsible for its metabolic stability and the relatively long duration of effects. A few ACTH analogs, including Semax, display properties of the melanocortin receptor antagonist. ACTH and its analogs are thus able to affect the dopaminergic and serotonergic brain systems (Eremin et al. 2005). A number of studies have shown that Semax has effects on learning and memory formation and exerts some neuroprotective effects in rodents and humans. This peptide promotes neuronal survival under hypoxia and improves brain circulation in experimental animals and humans (Asmarin et al. 1997; Gusev et al. 1997). There are objective data on the activating influence of Semax on anti-inflammatory postischemic reactions in the brain (Skvortsova et al. 1999). Semax is used in the treatment of human vascular, toxic allergic, and inflammatory diseases of the optic nerve and of partial atrophy of the optic nerve, in parallel with basic neurotrophic and anti-inflammatory therapy (Polunin et al. 2000; Sheremet et al. 2004). Electrophysiological and computer methods of examination demonstrated the advantages of Semax therapy over traditional neuroprotective treatment for glaucoma (Kurysheva et al. 2001). Semax has been successfully used in the treatment of patients with different diseases of the central nervous system, as Semax treatment resulted in significant clinical improvement, stabilization of disease progression, and reduction of the risk of stroke and of transitory ischemic attacks during the disease course (Gusev et al. 2005). Semax also significantly improved the total estimate of life quality, as it ameliorated the emotional state and motivation in motor neuron disease patients (Serdiuk et al. 2007).

Several works have been dedicated to the investigation of Semax mechanisms of action. As this peptide produces no trophic effect on PC12 cells, the protective effects of Semax are obviously mediated by other mechanisms (Safarova et al. 2002). There is a suggestion that the protective action of Semax is caused by stimulation of neurotrophin synthesis. Some experimental data have shown that Semax increases the expression levels of NGF and BDNF in cultures of glial cells obtained from the basal forebrain of newborn rats. The greatest increase in expression was found 30 min after Semax administration (Shadrina et al. 2001). It was also found that Semax application results in the upregulation of BDNF protein levels, the increase of trkB tyrosine phosphorylation levels, and threefold and twofold increases in the levels of rat hippocampal exon III BDNF and trkB mRNA, respectively. In addition, Semax-treated animals exhibit a distinct increase in the number of conditioned avoidance reactions (Dolotov et al. 2006). The intranasal application of Semax at 50 and 250 μg/kg of body weight results in a rapid increase in BDNF levels after 3 h in the basal forebrain but not in the cerebellum. Specific binding sites for Semax were identified in the rat basal forebrain. The binding of Semax is dependent on time, specific, and reversible and meets the main criteria of the ligand-receptor character of binding. Note that the action of the ACTH 4–10 analogs through the known melanocortin receptors is still not confirmed, and the existence of at least one unknown type of the receptors is proposed for these analogs (Dolotov et al. 2004, 2006). Our recent findings showed that a single intranasal application of Semax increased the rat hippocampal expression of BDNF and NGF within 1 h and upregulated the expression of the BDNF gene in the brainstem and cerebellum. In contrast, NGF gene expression decreased in the rat frontal cortex (Agapova et al. 2007). In connection with all these findings, the aim of the present work was to analyze the temporary dynamics of

NGF and BDNF expression under Semax action in rat hippocampus, frontal cortex, and retina.

Materials and Methods

Animal Models

The male Wistar rats (200 g) used in our experiment were kept under a 12-h light/dark cycle with free access to water and food. The animals were divided into six "control" and six "experimental" groups (n=5 per group). All the groups were handled and treated with water (intranasal injection) three times a day every day for 10 days. All animals were treated at the middle of the light phase of the diurnal cycle and had free access to water and food. After this period of preparation, all "control" groups were treated with water as usual, and all "experimental" groups were treated with Semax (water solution, 50 μg/kg, single intranasal application). Animals were then decapitated at 20 min, 40 min, 90 min, 3 h, 8 h, and 24 h after the treatment (all rats of one "control" and one "experimental" group per one time point).

The animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996.

Sample Preparation

Rat brains and eyes were removed immediately, dissected (tissues for further investigation of gene expression changes were obtained from rat hippocampus, frontal cortex, and retina), and kept at −70°C in tubes pretreated with 0.1% diethylpyrocarbonate water solution until needed for the isolation of mRNA.

RNA Isolation and Reverse Transcription

Total RNA was extracted from rat hippocampus, frontal cortex, and retina using RNAgents Total RNA Isolation System (Promega, USA) and treatment with DNase I (RNase-free; Fermentas, Lithuania). Single-stranded cDNAs were synthesized individually for each rat using the RevertAidTM H Minus First-Strand cDNA Synthesis Kit (Fermentas, Lithuania). Then, individual samples cDNAs were combine into one for each group.

Real-Time Quantitative Reverse Transcription-PCR

The relative levels of NGF, BDNF, and Rpl3 cDNAs were analyzed by SYBR Green real-time polymerase chain reaction (PCR) using primers described previously (Agapova et al. 2007). The reactions were carried out using a SYBR Green I Real-Time PCR Kit (Syntol, Russia).

Real-time PCR was performed on an Mx3000PTM Real-Time quantitative PCR system (Stratagene Equipment, USA). Thermal cycling was carried out as follows: (1) 95°C for 180 s; (2) 45 cycles of 20 s at 95°C, 20 s at a specific primer annealing temperature described previously (Agapova et al. 2007), and 15 s at 72°C; and (3) 15 s at 25°C.

All reactions were repeated five times for cDNAs of each tissue type using specific gene primers. Reaction efficiencies were determined for all tissue cDNA samples from each "control" and "experimental" group.

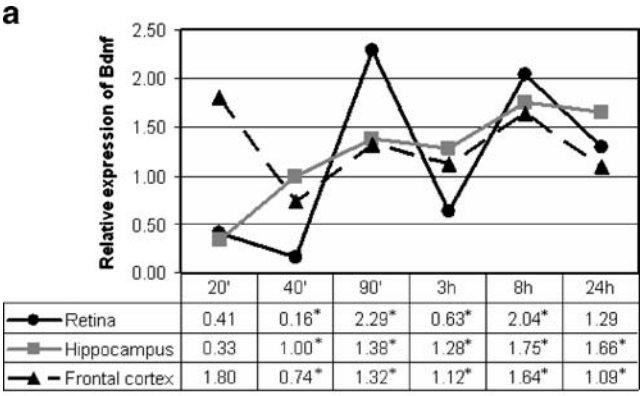
Statistical Analyses

Ct (threshold cycle) meanings obtained after analysis of the real-time PCR reaction curves were then normalized and analyzed using the Relative Expression Software Tool-384 (Pfaffl 2001, 2002, 2004). Rpl3 expression results were used as the reference for the normalization of NGF and BDNF mRNA expression data. The Ct values for NGF and BDNF were normalized to the Ct value of the reference Rpl3 mRNA among the tissues of each group and for each time point.

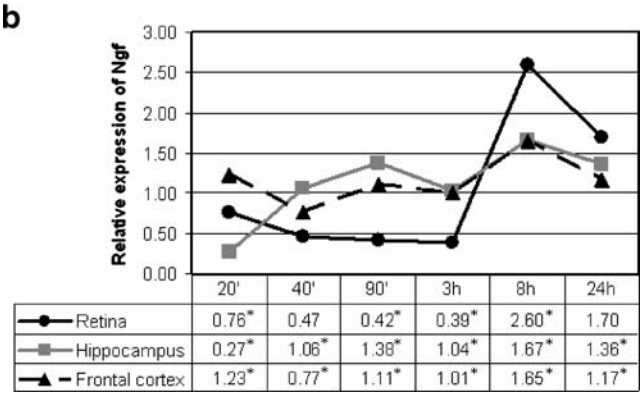
Results

We investigated the temporary dynamics of BDNF and NGF mRNA expression levels in rat hippocampus, frontal cortex, and retina 24 h after Semax intranasal application. We chose these tissues for analysis because the hippocampus and frontal cortex take part in cognitive processes and memory formation, and retina cells are directly connected to the hippocampus and frontal cortex through the optic nerve. At the same time, several studies have shown that Semax has an effect on learning and memory formation (Asmarin et al. 1997). In addition, Semax is used in the treatment of human vascular, toxic allergic, and inflammatory diseases of the optic nerve and of partial atrophy of the optic nerve (Polunin et al. 2000; Sheremet et al. 2004). As Semax influences cognitive processes, memory formation, and apoptosis of optic nerve and retina cells, we decided to study rapid and distant gene expression in these brain structures.

Figure 1 (a, b) depicts the multidirectional activation of the expression of the genes under investigation in the hippocampus, frontal cortex, and retina, after Semax administration (up to 3 h). Tissue comparison showed a decrease in neurotrophin gene expression in the rat retina (the expression levels of BDNF and NGF were 0.41 and



	20'	40'	90'	3h	8h	24h
Retina	0.41	0.16*	2.29*	0.63*	2.04*	1.29
Hippocampus	0.33	1.00*	1.38*	1.28*	1.75*	1.66*
Frontal cortex	1.80	0.74*	1.32*	1.12*	1.64*	1.09*



	20'	40'	90'	3h	8h	24h
Retina	0.76*	0.47	0.42*	0.39*	2.60*	1.70
Hippocampus	0.27*	1.06*	1.38*	1.04*	1.67*	1.36*
Frontal cortex	1.23*	0.77*	1.11*	1.01*	1.65*	1.17*

Figure 1 Temporary dynamics of BDNF (**a**) and NGF (**b**) gene expression in rat retina, hippocampus, and frontal cortex after single intranasal application of Semax. Gene expression level in the control tissue samples has been accepted for 1. (*$p \leq 0.05$)

0.76 times the level in the control samples, respectively) and hippocampus (the expression levels of BDNF and NGF were 0.33 and 0.27 times the level in the control samples, respectively) 20 min after Semax administration. In contrast, the expression of these genes was increased in the frontal cortex (expression levels of BDNF and NGF were 1.80 and 1.23 times the level in the control samples, respectively). The expression levels of NGF remained practically constant in the retina at the initial stage, whereas the expression levels of BDNF were significantly increased (2.29 times) 90 min after Semax administration. In the hippocampus and frontal cortex, we can observe as neurotrophin gene expression decrease and neurotrophin gene expression increase, especially in 90 min, at the initial stage; however, it should be noted that there was a decline in neurotrophin gene expression in all tissues at the 3-h time point when compared with the previous time point.

At the late stage (>3 h after Semax administration), we observed the unidirectional activation of the neurotrophin genes in the hippocampus, frontal cortex, and retina after Semax administration. An expression peak was observed for both genes at the 8 h time point in all tissues (>1.5 times the levels detected in control samples).

Discussion

Previously, we established that the expression levels of the BDNF and NGF genes were increased 1 h after Semax treatment in glial cell cultures from rat basal forebrain and in different rat brain structures (Shadrina et al. 2001; Agapova et al. 2007). Here, we observed similar changes in the expression levels of these genes. These data seem to confirm the contention that the mechanism of Semax action overlaps with the intracellular signaling pathways induced by neurotrophin.

As mentioned above, neurotrophins are a family of structurally related proteins that regulate the survival, differentiation, and maintenance of the function of different neuron populations. Neurotrophin receptors are differentially expressed in the central and peripheral nervous systems. Different members of the neurotrophin family have distinct and not always overlapping neuroprotective actions. The low-affinity transmembrane receptor p75NTR binds all neurotrophins. In addition, each neurotrophin binds with high affinity to one of the trk family of transmembrane receptors: NGF to trkA, BDNF and NT-4/5 to trkB, and NT-3 to trkC (and to trkA with a lower affinity; Bothwell 1995; Lewin and Barde 1996; Dechant and Barde 2002). NGF and BDNF, the expression of which was investigated in this work, regulate the expression of several functionally important proteins, which include neurotransmitters, receptors, and voltage-regulated ion channels (Priestley et al. 2002; McMahon et al. 2006). NGF treatment induces BDNF expression in virtually all trkA-expressing dorsal root ganglion neurons. Recent data indicate that the nature of the signaling cascades that are activated by neurotrophins and the biological responses that ensue are specified not only by the ligand itself but also by the temporal pattern and spatial location of the stimulation. Studies on neurotrophin signaling have revealed variations in the Ras/MAP kinase, PI3 kinase, and phospholipase C pathways, which transmit spatial and temporal information (Segal 2003). In particular, it is known that BDNF and NGF participate in the formation of long-term synaptic plasticity and long-term memory storage in the hippocampus. A number of experiments involving the inhibition of endogenous BDNF and signaling through its trkB receptor tyrosine kinase suggest that BDNF is required for the generation of late LTP (Chen et al. 1999; Minichiello et al. 1999; Patterson et al. 2001). Some data confirm that BDNF activation of its receptor trkB is necessary for the consolidation of stable extinction memories (Chhatwal et al. 2006); however, the exact mechanism underlying these interactions is unknown. Semax is known to influence both memory formation and neurotrophin gene expression (Shadrina et al. 2001; Dolotov et al. 2006). This peptide

stimulates operative memory and attention, increases resistance to hypoxia, and improves brain circulation in experimental animals and human beings over a prolonged period (20–24 h after intranasal administration). Semax significantly improves memory and attention in healthy men under extreme conditions of activity.

Moreover, NGF and BDNF have been shown to act on cells of the visual system. NGF receptors are expressed in the retina of chick embryos, as well as in Müller cells, photoreceptors, and retinal ganglion cells (RGCs) of developing and adult rodents (Carmignoto et al. 1991; Siliprandi et al. 1993). Rat RGCs have been shown not only to express their receptors but also to transport NGF in a retrograde and anterograde fashion along their axons, which together comprise the optic nerve 8. In animal models of ocular disease, intraocular administration of NGF improves RGC degeneration after optic nerve transection, ocular ischemia, or induced ocular hypertension (Siliprandi et al. 1993; Lambiase et al. 1997). Exogenous BDNF induces dendritic growth in cultured dissociated rat RGCs (Bosco and Linden 1999); however, intraocular injection of BDNF antisense oligonucleotides into the mouse retina blocks the accelerated refinement induced by enriched environments (Landi et al. 2007). Semax is used in the treatment of human vascular, toxic allergic, and inflammatory diseases of the optic nerve and of partial atrophy of the optic nerve, in parallel with basic neurotrophic and anti-inflammatory therapy (Polunin et al. 2000; Sheremet et al. 2004). It has been established that intranasal introduction of Semax is the most effective. Pharmacokinetic investigation of Semax demonstrated that ten to 15 times higher content of Semax can be found in the brain 2 min after its intranasal administration in comparison with its injection in blood. A higher content of Semax in the rat brain and blood was observed for the first minute after its intranasal administration (Shevchenko et al. 2006).

Here, we identified the temporary dynamics of NGF and BDNF expression in rat hippocampus, frontal cortex, and retina after intranasal Semax application. We found a rapid and prolonged up- or downregulation of these genes after injection of the peptide. We observed a significant expression change 20 min after Semax administration, and we observed an expression peak of both genes in all tissues at 8 h. These findings suggest the rapid and specific activation of intracellular signaling pathways induced by Semax. The prolonged and identical changes in the expression of neurotrophins in rat hippocampus, frontal cortex, and retina are probably related to autoregulation of the investigated genes. Finally, it would be interesting to determine which neurotrophin signaling pathways are induced by Semax.

References

Agapova, T. Y., Agniullin, Y. V., Shadrina, M. I., et al. (2007). Neurotrophin gene expression in rat brain under the action of Semax, an analogue of ACTH 4–10. *Neuroscience Letters, 417*, 201–205.

Asmarin, I. P., Nezavibat'ko, V. N., Myasoedov, N. F., et al. (1997). A nootropic adrenocorticotropin analog 4–10-semax (15 years experience in its design and study). *Zhurnal Vysshei Nervnoi Deiatelnosti Imeni I. P. Pavlova, 47*, 420–430.

Bosco, A., & Linden, R. (1999). BDNF and NT-4 differentially modulate neurite outgrowth in developing retinal ganglion cells. *Journal of Neuroscience Research, 57*, 759–769.

Bothwell, M. (1995). Functional interactions of neurotrophins and neurotrophin receptors. *Annual Review of Neuroscience, 18*, 223–253.

Carmignoto, G., Comelli, M. C., Candeo, P., et al. (1991). Expression of NGF receptor mRNA in the developing and adult rat retina. *Experimental Neurology, 111*, 302–311.

Carroll, P., Lewin, G. R., Koltzenburg, M., Toyka, K. V., & Thoenen, H. (1998). A role for BDNF in mechanosensation. *Nature Neuroscience, 1*, 42–46.

Chen, K. S., Nishimura, M. C., Armanini, M. P., Crowley, C., Spencer, S. D., & Phillips, H. S. (1997). Disruption of a single allele of the nerve growth factor gene results in atrophy of basal forebrain cholinergic neurons and memory deficits. *Journal of Neuroscience, 17*, 7288–7296.

Chen, G., Kolbeck, R., Barde, Y. A., Bonhoeffer, T., & Kossel, A. (1999). Relative contribution of endogenous neurotrophins in hippocampal long-term potentiation. *Journal of Neuroscience, 19*, 7983–7990.

Chhatwal, J. P., Stanek-Rattiner, L., Davis, M., & Ressler, K. J. (2006). Amygdala BDNF signaling is required for consolidation but not encoding of extinction. *Nature Neuroscience, 9*, 870–872.

Dechant, G., & Barde, Y. A. (2002). The neurotrophin receptor p75 (NTR): novel functions and implications for diseases of the nervous system. *Nature Neuroscience, 5*, 1131–1136.

De Wied, D., & Gispen, W. H. (1977). Behavioral effects of peptides. In H. Gainer & J. L. Barker (Eds.), *Peptides in Neurobiology* (pp. 397–448). New York: Plenum Press.

Dolotov, O. V., Karpenko, E. A., Inozemtseva, L. S., et al. (2006). Semax, an analog of ACTH(4–10) with cognitive effects, regulates BDNF and trkB expression in the rat hippocampus. *Brain Research, 1117*, 54–60.

Dolotov, O. V., Zolotarev, Y. A., Dorokhova, E. M., et al. (2004). The binding of Semax, ACTH 4–10 heptapeptide, to plasma membranes of the rat forebrain basal nuclei and its biodegradation. *Bioorganicheskaia Khimiia, 30*, 241–246.

Eremin, K. O., Kudrin, V. S., Saransaari, P., et al. (2005). Semax, an ACTH(4–10) analogue with nootropic properties, activates dopaminergic and serotoninergic brain systems in rodents. *Neurochemical Research, 30*, 1493–1500.

Gusev, E. I., Skvortsova, V. I., Miasoedov, N. F., Nezavibat'ko, V. N., Zhuravleva, E. Y., & Vanichkin, A. V. (1997). Effectiveness of Semax in acute period of hemispheric ischemic stroke (a clinical and electrophysiological study). *Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova, 97*, 26–34.

Gusev, E. I., Skvortsova, V. I., & Chukanova, E. I. (2005). Semax in prevention of disease progress and development of exacerbations in patients with cerebrovascular insufficiency. *Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova, 105*, 35–40.

Harrison, S. M., Davis, B. M., Nishimura, M., Albers, K. M., Jones, M. E., & Phillips, H. S. (2004). Rescue of NGF-deficient mice I: transgenic expression of NGF in skin rescues mice lacking

endogenous NGF. *Brain Research. Molecular Brain Research, 122*, 116–125.

Horner, P. J., & Gage, F. H. (2000). Regenerating the damaged central nervous system. *Nature, 407*, 963–970.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. *Annual Review of Neuroscience, 24*, 677–736.

Jones, K. R., Farinas, I., Backus, C., & Reichardt, L. F. (1994). Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell, 76*, 989–999.

Kirstein, M., & Farinas, I. (2002). Sensing life: regulation of sensory neuron survival by neurotrophins. *Cellular and Molecular Life Sciences, 59*, 1787–1802.

Kurysheva, N. I., Shpak, A. A., Ioileva, E. E., et al. (2001). Semax in the treatment of glaucomatous optic neuropathy in patients with normalized ophthalmic tone. *Vestnik Oftalmologii, 117*, 5–8.

Lambiase, A., Centofanti, M., Micera, A., et al. (1997). Nerve growth factor (NGF) reduces and NGF antibody exacerbates retinal damage induced in rabbit by experimental ocular hypertension. *Graefes Archive for Clinical and Experimental Ophthalmology, 235*, 780–785.

Landi, S., Cenni, M. C., Maffei, L., & Berardi, N. (2007). Environmental enrichment effects on development of retinal ganglion cell dendritic stratification require retinal BDNF. *PLoS ONE, 2*, e346.

Lewin, G. R., & Barde, Y. A. (1996). Physiology of the neurotrophins. *Annual Review of Neuroscience, 19*, 289–317.

McMahon, S. B., Bennett, D. L., & Bevan, S. (2006). Inflammatory mediators and modulators. In S. B. McMahon & M. Koltzenburg (Eds.), *Textbook of Pain* (pp. 49–72). London: Elsevier.

Minichiello, L., Korte, M., Wolfer, D., et al. (1999). Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron, 24*, 401–414.

Patapoutian, A., & Reichardt, L. F. (2001). Trk receptors: mediators of neurotrophin action. *Current Opinion in Neurobiology, 11*, 272–280.

Patterson, S. L., Pittenger, C., Morozov, A., et al. (2001). Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. *Neuron, 32*, 123–140.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research, 29*, e45.

Pfaffl, M. W., Horgan, W. G., & Dempfle, L. (2002). Relative Expression Software Tool (REST©) for group wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research, 30*, e36.

Pfaffl, M. W., Tichopad, A., Prgomet, C., & Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper-Excel-based tool using pair-wise correlations. *Biotechnological Letters, 26*, 509–515.

Phillips, H. S., Nishimura, M., Armanini, M. P., Chen, K., Albers, K. M., & Davis, B. M. (2004). Rescue of NGF-deficient mice II: basal forebrain cholinergic projections require NGF for target innervation but not guidance. *Brain Research. Molecular Brain Research, 124*, 1–11.

Polunin, G. S., Nurieva, S. M., Baiandin, D. L., Sheremet, N. L., & Andreeva, L. A. (2000). Evaluation of therapeutic effect of new Russian drug Semax in optic nerve disease. *Vestnik Oftalmologii, 116*, 15–18.

Priestley, J. V., Michael, G. J., Averill, S., Liu, M., & Willmott, N. (2002). Regulation of nociceptive neurons by nerve growth factor and glial cell line derived neurotrophic factor. *Canadian Journal of Physiology and Pharmacology, 80*, 495–505.

Safarova, E. R., Shram, S. I., Grivennikov, I. A., & Myasoedov, N. F. (2002). Trophic effects of nootropic peptide preparations cerebrolysin and semax on cultured rat pheochromocytoma. *Bulletin of Experimental Biology and Medicine, 133*, 401–403.

Segal, A. (2003). Selectivity in neurotrophin signaling: theme and variations. *Annual Review of Neuroscience, 26*, 299–330.

Serdiuk, A. V., Levitskiy, G. N., Myasoedov, N. F., & Skvortsova, V. I. (2007). The study of chronic partial denervation and quality of life in patients with motor neuron disease treated with Semax. *Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova, 107*, 29–39.

Shadrina, M. I., Dolotov, O. V., Grivennikov, I. A., et al. (2001). Rapid induction of neurotrophin mRNAs in rat glial cell cultures by Semax, an adrenocorticotropic hormone analog. *Neuroscience Letters, 308*, 115–118.

Sheremet, N. L., Polunin, G. S., Ovchinnikov, A. N., et al. (2004). An experimental substantiation for using the "Semax" neuroprotector in the treatment of optic-nerve diseases. *Vestnik Oftalmologii, 120*, 25–27.

Shevchenko, K. V., Nagaev, I. Y., Alfeeva, L. Y., et al. (2006). Kinetics of Semax penetration into the brain and blood of rats after its intranasal administration. *Bioorganicheskaia Khimiia, 32*, 64–70.

Siliprandi, R., Canella, R., & Carmignoto, G. (1993). Nerve growth factor promotes functional recovery of retinal ganglion cells after ischemia. *Investigative Ophthalmology and Visual Science, 34*, 3232–3245.

Skvortsova, V. I., Nasonov, E. L., Zhuravleva, E. Y., et al. (1999). Clinico-immunobiochemical monitoring of factors of focal inflammation in the acute period of hemispheric ischemic stroke. *Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova, 99*, 27–31.

Thoenen, H. (2000). Neurotrophins and activity-dependent plasticity. *Progress in Brain Research, 128*, 183–191.