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COMPARATIVE PHYSIOLOGICAL STUDY OF PROTECTIVE EFFICIENCY OF THE PARATHYROID HORMONE, HYPOTHALAMIC PROLINE-RICH PEPTIDE AND THE CENTRAL ASIAN COBRA VENOM FOLLOWING SCIATIC NERVE CRUSH

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Abstract

On-line registration and program mathematical analysis of impulse activity in the spinal cord motoneurons, at high frequency stimulation of hindlimb flexor (n. Gastrocnemius) and extensor (n. Peroneus communis) nerves, with a view to comparative study of dynamics and degree of development in their neurode- and regenerative processes revealed the formation of excitatory and depressor manifestations of activity on the affected side with respect to prestimulus level. Responses appeared in the form of tetanic potentiation and depression in intact rats in 5-70 days after the sciatic nerve crush without (control) and with the use of parathyroid hormone, proline-rich peptide-1 and Central Asian cobra venom Naja naja oxiana. In the control group a tendency to only extensor nerve regeneration was observed and the lack of the same for flexors.

In the Naja naja oxiana-treated case we also observed slightly better indices for the extensor nerve. In other words, the protective effect of Naja naja oxiana, counteracting in these conditions to the deep reduction of inhibition, apparently, came down to speed up recovery of excitatory effects of spinal cord motoneurons and hence the transmission in damaged peripheral nerves; this latter was consistent with the selective specificity and irreversibility of snake venom effects. In conclusion, the true protective effect of proline-rich peptide-1, as opposed to regulatory action of parathyroid hormone, was shown. In general, the intensity of poststimulus depressor reactions in motoneurons, accompanying the successful regeneration of sciatic nerve before its completion should be also noted.

Keywords: *sciatic nerve crush, motoneurons of spinal cord, high-frequency stimulation, extensor and flexor branch of the sciatic nerve, parathyroid hormone, proline-rich peptide-1 (PRP-1), the Central Asian cobra venom Naja naja oxiana*

Introduction

Despite numerous studies on the mechanisms of sciatic nerve crush development and prevention, the existing facilities and future targets of therapy continue to remain insufficient for progress in this area. Data were presented in favor of astrocytes secretion strong influence on the myelin repair [*Moore C. et al., 2011*]. The key role of resident endoneural macrophages in acrylamide-induced neuropathy was shown, and this latter was merely complemented by hematogenous macrophages in the distal margins of a more pronounced damage [*Müller M. et al., 2008*]. The substantial proliferation of Schwann cells (SCs) was also immunohistologically shown to support axonal regeneration after the nerve injury [*Zhang P. et al., 2008*]. As described, crush damage prevented the ability to conduct nerve impulse, while any minimal damage to myelin significantly affected the activity of ion channels and the subsequent generation of the pulse [*Mert T. et al., 2005*]. At this pathology, several mechanisms were reported to be activated; their knowledge might con-

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tribute to an objective assessment of changes occurring not only at the site of compression, but also beyond it [*Gupta R., Steward O., 2003*]. It was revealed that chronic nerve compression caused competitive apoptosis and proliferation of SCs, with minimal axonal injury, demyelination and moderation of conduction velocity, which reinforced the idea of direct mitogenic effect of mechanical stimuli on SCs [*Gupta R., Steward O., 2003*]. Sprouting of regenerating nerve fibers from the proximal stump promoted "down-regulation" of myelin genes, dedifferentiation and proliferation of SCs expressing surface molecules allowing intergrowth of nerve fibers in the range of 3-4 *mm* per day. Favorable conditions were created in the distal stump through the "up-regulation" of neurotrophins, cytokines, adhesion of nerve cells molecules, etc. [*Stoll G., Müller H., 1999*]. Finally, even in the early studies it was shown that after the nerve crush processes inhibiting nerve growth occurred in the form of retrograde atrophy and degeneration, disproportionate regeneration of small diameter fibers, lack of large regenerated fibers growing in a changed direction, without the restoration of functional re-innervation with cell alterations at the site of injury [*Giannini C. et al., 1989*].

Disability prevention and the search of optimal therapeutic strategy for peripheral nerve injury [*Reyesarmijo E., 1964*], in particular crushing (or compression) of the peripheral nerve (PN), is intensively and comprehensively studied at the interdisciplinary level with the variety of means, including physical effects, growth factors, neurotrophins, hormones, exogenous peptides and other physiologically active compounds [*Bervar M., 2005; Kato N. et al., 2005; Aydin M. et al., 2006; Mohri T. et al., 2006; Fleming C. et al., 2007; Fargo K. et al., 2008; and other references cited*]. A special role is given to the growth factor and neurotrophins.

Thus, the neuroprotective effect of testosterone was obtained in spinal cord motoneurons (MNs) [*Tehranipour M., Moghimi A., 2010*], and the role of androgen was shown in the regulation of the mRNA neuritin levels, wherewith axon regeneration and neurit outgrowth in MNs were activated [*Fargo K. et al., 2008*]. Moreover, neuritin was shown to be involved in the responses of central and peripheral lesions and to serve as a common effector molecule for specific neurotrophic and neurotherapeutic agents [*Fargo K. et al., 2008*]. The application of insulin-like growth factor 1 (IGF-1) and platelet-rich plasma to the damaged nerve accelerated regeneration of the axon [*Emel E. et al., 2011*]. It was established that during the crush of PN dihydroprogesteron significantly reduces up-regulation of the myelinated fibers density and its interaction with progesterone reduceed pain and provided protection [*Roglio I. et al., 2008*]. Further, dihydroprogesteron encouraged SCs myelination, potentiating the regrowth and maturation of myelin, the expression of brain-derived neurotrophic factor (BDNF), being provoked by low-frequency electrical stimulation [*Wan L. et al., 2010 a; b; Zhang S. et al., 2010*]. In turn, the essential significance of SCs proliferation for axonal regeneration support was shown immunohistologically [*Zhang P. et al., 2008; Zhang S. et al., 2010*]. Furthermore, an additional support was shown by further expression of glial cell line-derived neurotrophic factor (GDNF) in the GDNF-modified human amniotic fluid-derived mesenchymal stem cells (AFMSCs) by the 4th week [*Cheng F. et al., 2010*]. In addition, the therapeutic prospects of early recovery were established by peripheral (but not central) delivery of GDNF [*Magill C. et al., 2010*]. The ability of vasoactive agent alprostadil to improve the rehabilitation of neural function by up-regulation of vascular endothelial growth factor (VEGF) expression accompanying the crush damage of PN was demonstrated [*Tang J. et al., 2009*]. Earlier, after the crush damage of PN in mature and rapidly senescent mice, the researchers found that VEGF was not locally up-regulated; that latter provided a proof of the interdependent relationship between age, VEGF, angiogenesis and nerve regeneration [*Pola R. et al., 2004*]. It was established that antagonist of tumor necrosis factor-alpha (TNF-alpha) supported the PN axon regeneration [*Kato K. et al., 2010*]. At the early stages, associated with the regeneration of PN myelination, neurotrophin-3 (NT-3) was essential for the survival of the sciatic nerve (SN) [*Sahenk Z. et al., 2008*]. Development of short-term functional recovery of SN by granulocyte colony-stimulating factor (G-CSF involved a paracrine modulator effect and the effect of mobilizing CD34+ cells of the bone marrow origin [*Pan H. et al., 2009*].

Among other therapeutic means to promote re-

generation of damaged PN we should note the up-regulation role of major histocompatibility complex (MHC) class I in the spinal cord in response to SN damage; it was demonstrated that treatment with interferon beta (IFN beta) enhanced the axonal growth and recovery of motor function [*Zanon R. et al., 2010*]. The important role of endogenous glucocorticoids in myelination through their respective receptors in the SCs after injury of PN was proved [*Morisaki S. et al., 2010*]. The neuroprotection of PN acute crush injuries by erythropoietin was shown to have a probable clinical relevance, because it was effective even if administered during 1 week after the injury [*Elfar J. et al., 2008*]. The key role of meltrin-beta (disintegrin and metalloprotease) in remyelination was revealed and its function as a modulator of a signal from the axon activating transcription factor of myelination (Krox-20) prior to differentiation of SCs was shown [*Wakatsuki S. et al., 2009*]. The mechanism, by which regeneration was not impaired in a long crush-damaged nerve trunk and a large number of mature myelinated axons were formed (i.e., sprouting was facilitated), was unknown [*Xu Q. et al., 2008*]. Moreover, damage to the trunk of the nerve was resistant to endoneural ischemia and despite long-term alterations in the epineural circulation the regenerative sprouting was maintained [*Xu Q. et al., 2010*]. It was also shown that certain stem cells could be differentiated not only in somatic, but also in the vascular cells [*Li M. et al., 2009*].

However, due attention was not paid to flexor nerve regeneration under conditions of crush, as evolutionarily newer structure, naturally lagging in regeneration of the extensor nerve, because of the greater commitment to supra segmental control and delay in the development of regenerative processes. In addition, no data is available on the varying degrees of severity and rate of regeneration in flexors and extensors innervation of the related muscles during nerve crush and development of neurode- and regenerative processes under the segmental and supra segmental control. Considering this phenomenon is of great practical significance in terms of directional use of endo- and exogenous modulators of a broad spectrum of action.

Along with known therapeutic agents in the PN crush, the parathyroid hormone (PTH), proline-rich peptide (PRP-1) and the Central Asian cobra venom *Naja naja oxiana* (NOX) are of interest as promising therapeutic agents.

This paper presents a comparative electrophysiological study on the dynamics and degree in development of the neurode- and regenerative processes of flexor (*n. Gastrocnemius* – G) and extensor (*n. Peroneus communis* – P) nerves of the hind limb after the SN crush with and without PTH, PRP-1 and NOX venom application.

Material and Methods

Experiments were conducted in 49 male Albino rats (250 ± 30 *g*): intact (n = 7), subjected to unilateral SN crush (control, n = 11) and those intramuscularly (*i/m*) administered PTH (n = 7), PRP-1 (n = 6) and NOX (n = 4) from the day after SN crush. PTH was injected daily for 7 days at 0.35 *ml* (10^{-9} M solution), PRP-1 was administered daily for 3 days at 0.1 *mg/kg* and NOX – daily for 3 days (5% of the LD_{50} = 1 *mg/kg*). The comparative analysis of sensory (reflex test of abduction) and motor (static sciatic index) indicators of the functional recovery after crush was conducted. In the electrophysiological experiments after 5, 13, 16, 21, 25, 32, 35, 70 days after crush in control, in 1, 3, 5, 7, and 9 days after PTH injection, in 3, 7 and 9 days after PRP-1 injection, as well as in 5, 10 and 17 days after injection of NOX and fixation in the stereotaxic apparatus, the kraneotomy was done under nembutal anesthesia, as well as dorsal laminectomy of the lumbosacral spinal cord and separating of distal flexor and extensor branches of the crushed SN. Then the animals were immobilized by 25 *mg/kg* intraperitoneally (*i/p*) administerd 1% ditillinum and transferred to artificial respiration. All procedures were performed in accordance with the "rules for the care of laboratory animals" (NIH publication No. 85-23, revised in 1985), as well as specific guidance provided by the Committee of National Health Service for animals care. For extracellular recording of spike activity the glass microelectrodes with tip diameter of 1 μM were filled with 2 M solution of NaCl and inserted in the anterior horn of the lumbar segments gray matter (L4-L5) in the area of spinal cord motoneurons (MNs) (VIII-IX plates by Rexed). High-frequency stimulation (HFS) (0.05 *ms*, 0.10-0.16 *mA*, 50 *Hz* for 1 *sec*) of G and P nerves (distal stump on the side of

damage) of the hind limbs was performed by bipolar silver electrodes.

To identify the MNs, suprasegmental structures of control, the giant-cell red nucleus (GRN) and the lateral vestibular nucleus (LVN), were stimulated using cylindric bipolar electrodes stereotaxically oriented in line with the brain atlas [*Paxinos G., Watson C., 2005*] at current parameters: 0.05 *ms*, 0.08 *mA*, 50 *Hz* for 1 *sec*. Moreover, we used the pair reciprocity of central structures stimulation effects managing the relief of flexion and inhibition of the extension and *vice versa*, or the peripheral structures of the certain well-known direction.

To determine the statistical significance of differences in the duration of interspike intervals before and after the action of stimulus, the nonparametric test for homogeneity of two independent samples – two-sample Wilcoxon-Mann-Whitney test – was used. Since the number of recorded spikes was large enough (several hundred spikes per 20-second interval after the stimulus action), a variation of this test was utilized, taking into account its asymptotic normality – z-test. The comparison of critical values with the tabulated values of the normal distribution at significance level 0.05, 0.01 and 0.001 (for various tests) showed that as a result of HFS for most samples of neuronal activity spiking had a statistically significant change at least with a significance level of 0.05.

Results

The analysis of spike activity of individual MNs in norm (128 cells), in control at the 5-70th day without (116 cells, 440 tests) and with the use of PTH on days 1-9 (130 cells, 350 tests), PRP-1 on days 5-9 (97 cells, 123 tests), NOX on days 5, 10 and 17 (28 cells, 94 trials) after SN crush revealed formation of responses to HFS of flexor and extensor nerves in the corresponding spinal cord MNs on the injured side, in the form of tetanic potentiation (TP) and depression (TD), with subsequent manifestations of posttetanic potentiation (PTP) and depression (PTD).

On-line registration and program mathematical analysis of impulse activity in the control found the following reactions in corresponding MNs of spinal cord during dynamics of recovery at the 5-70th days after the SN crush (represented in Figures 1 and 2). In response to HFS of G nerve TP from the 5th to 25th day exceeded the initial level only 2-fold, while growing progressively 3.25 and 4.2 times by days 32-35 and 70, respectively; TP in norm reached 8-fold excess of the baseline. Under HFS of the P nerve on day 5 TP showed only about 1.33-fold excess of prestimulus level, at the 13th day – within a 4-fold excess, but twice subsided at the 21st and 25th days, slightly increasing at days 32-35 (2.66 times) and reaching the maximum on the 70th day: in the normal range (5.25-fold excess). Regarding the TD, as a response to HFS of G and P nerves the lowest level of decreased prestimulus level (about 1.4-fold) was recorded; then at days 13 and 21 it was increasing up to 2 and 4 times for the G nerve and 2.33 and 3.5 times for P nerve. At the 25th day to HFS of G there was recorded TD sharp decrease up to 1.5 times, but for P it achieved 5-fold decrease and reached the same 2-fold level for both nerves at days 32-35, then increased sharply at the 70th day: within 6 and 9 times for nerves G and P, thus exceeding the normal level for the same nerves 2 and 2.5 times, respectively. In other words, there was only regeneration of the extensor nerve, in the absence of flexor regeneration, as considered only by TP maximum indices in norm, but not those of TD, because these indices, as anticipated, were much higher than at norm, according to its protective purpose that will be discussed in detail in the Discussion part of the paper.

In terms of systemic administration of PTH values of TP and TD to HFS of the same nerves were distributed as follows in the dynamics of recovery from days 1 to 9 after the SN crush and in comparison with the control and norm (Figures 3 and 4). To HFS of the G nerve TP first increased to the maximum from day 1 to days 3 and 5 (within 1.5, 2.6 and 4-fold excess of the prestimulus level, respectively), then at the 7th and 9th days it fell (3.7 and 3 times, respectively), reaching the value of TP in control at days 32-35 (3.25 times) and 2.66 times lower than that of the norm. To HFS of the P nerve TP was growing saltatory, being at the 1st and 3rd day within the limits of 1.8 and 3.5-fold excess, respectively; on days 5 and 7 the excess made 2.23 and 1.6-fold, reaching at day 9 the value equal to that of the control at days 32-35 (2.66 times), which was also below the norm (reaching 5.25-fold excess), but to a lesser extent (1.97 times). TD to

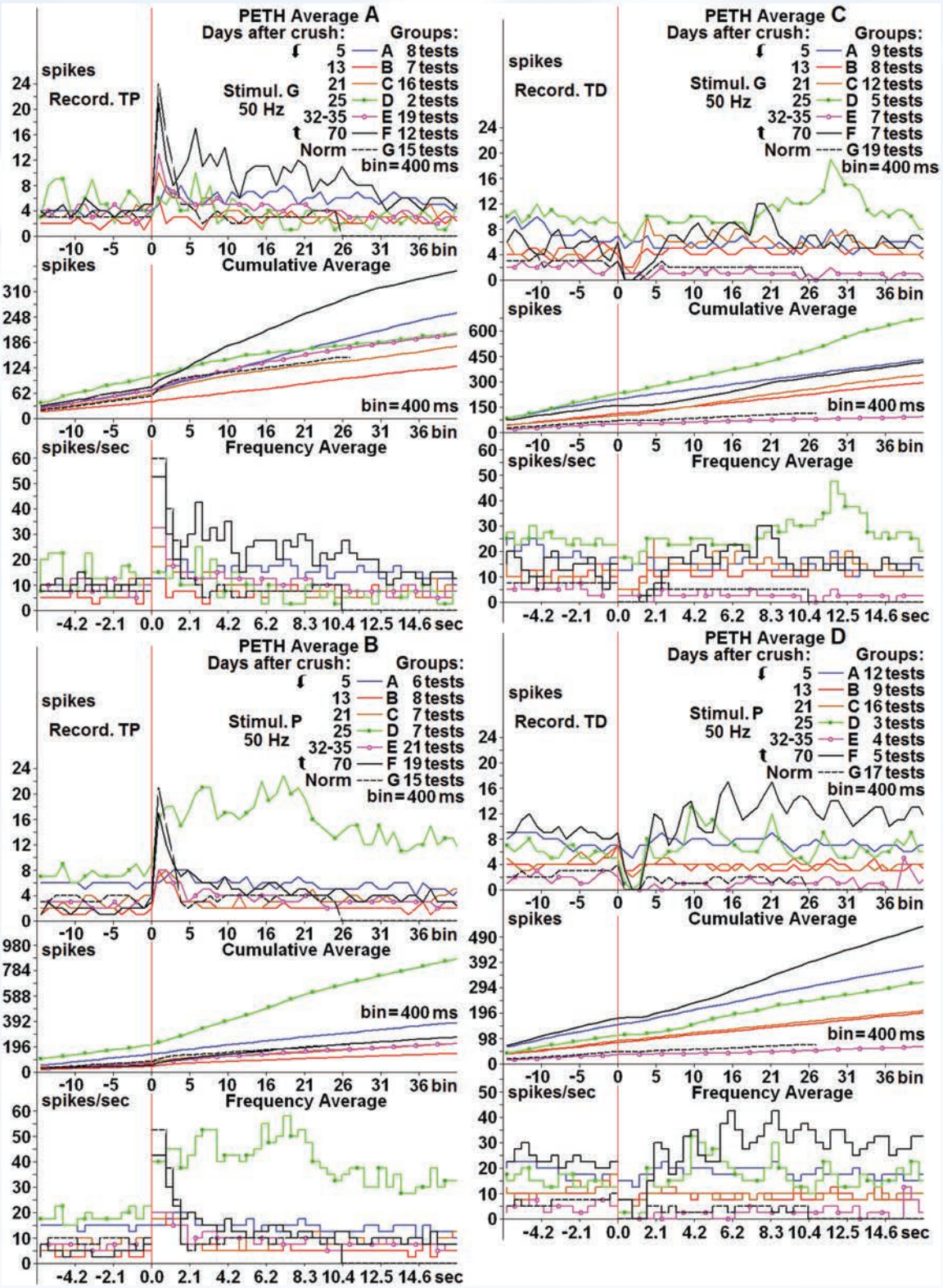

Figure 1. *Averaged peri-event time (PETH Average), cumulative (Cumulative Average) histograms and histograms of frequency (Frequency Average) of excitatory (A, B) and depressor (B, D) poststimulus manifestations of activity of the spinal cord motoneurons to HFS of G (A, C) and P (B, D) nerves on days 5-70 after the SN crush in the control (Groups A to F) and in norm (Group G). Hereinafter: stimulation (stimul.), recording (record.), tetanic and posttetanic potentiation (TP, PTP) and depression (TD, PTD), G and P (n. Gastrocnemius and n. Peroneus communis, respectively).*

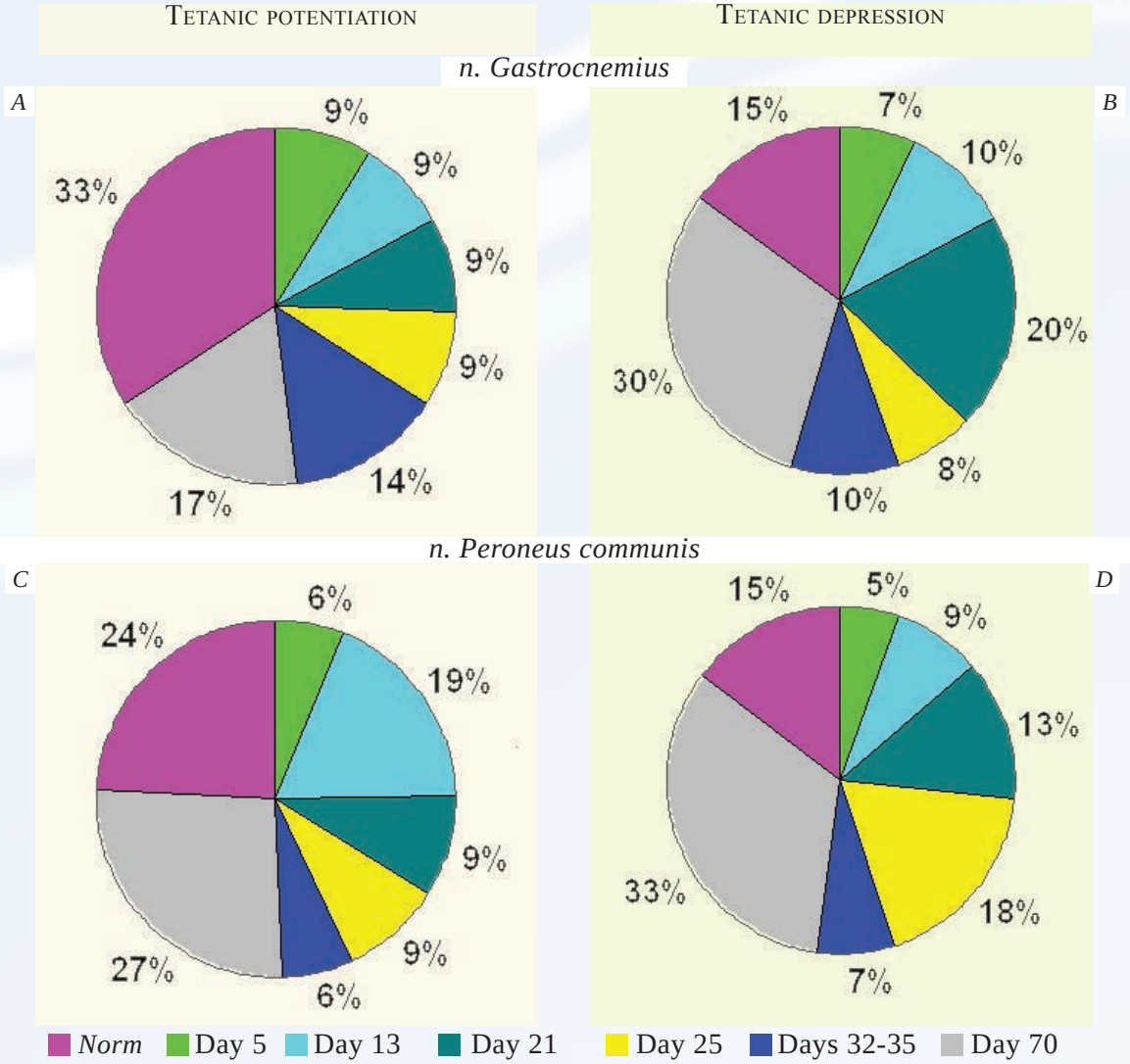

FIGURE 2. *Comparative disc charts of the ratio of excitatory (TP – A, B) and depressor (TD – C, D) reactions in the spinal cord motoneurons to HFS of G (A, C) and P (B, D) nerves by a multiple percentage in difference of averaged prestimulus frequency level of activity and the subsequent poststimulus tetanic effect in the norm and 5-70 days after SN crush (A-D). The remaining notations are in the figure.*

HFS of G nerve at the 1st and 3rd days were estimated about 2.5 and 2-fold underestimation, and on days 7 and 9 reached 3.5 and 3-fold underestimation, respectively, which was 1.5 times greater than TD on days 32-35 (2 times) and was equal to the value of those in norm (3 times). TD to HFS of P nerve first increases to 1 and 3 days (2.66 and 4 times), then fell decreased at the 5th day (2 times) and again grew up to the maximum at the 7th (3.5-fold) and 9th days (6 times), which was 3 times higher than the value for days 32-35 (2-fold) and 1.5 times higher than the value of the norm (4 times).

Thus, TD, in contrast to TP exceeded the values in control and norm, and this was more expressed for the extensor nerve that seemed indicative of the worst protection in case of the flexor nerve. In all the aforementioned cases PTP was also recorded, mostly early ones, which were followed by the actual stationary activity by the end of the test.

In terms of PRP-1 systemic administration the values of TP and TD to HFS of the same nerves in the dynamics of regeneration development from day 5 to 9 after the SN crush and compared with the control and norm changed as reflected in Figures 5 and 6. The maximum level of TP to HFS of G nerve was detected on the 7th day after the SN crush (within the limits of 14.86-fold excess of prestimulus level) and on the 5th and 9th days (about 3.67 and 4.47 times, respectively), while in

control at days 32-35 it did not exceed 3.25 times that in comparison with the norm (8 times) was about 1.79 times less. To HFS of P nerve the TP gradually grew and somewhat decreased at the 9th day calculated in the ranges of 3.04, 6.57 and 6.13-fold increase of the prestimulus level, respectively at the 5th, 7th and 9th days, while in control at days 32-35 it did not exceed 3.25-fold increase, which in comparison to norm with the use of PRP-1, reached 1.97-fold excess in the last day of testing, thus slightly differing from the changes in TP to HFS of G nerve. TD to HFS of G nerve at the

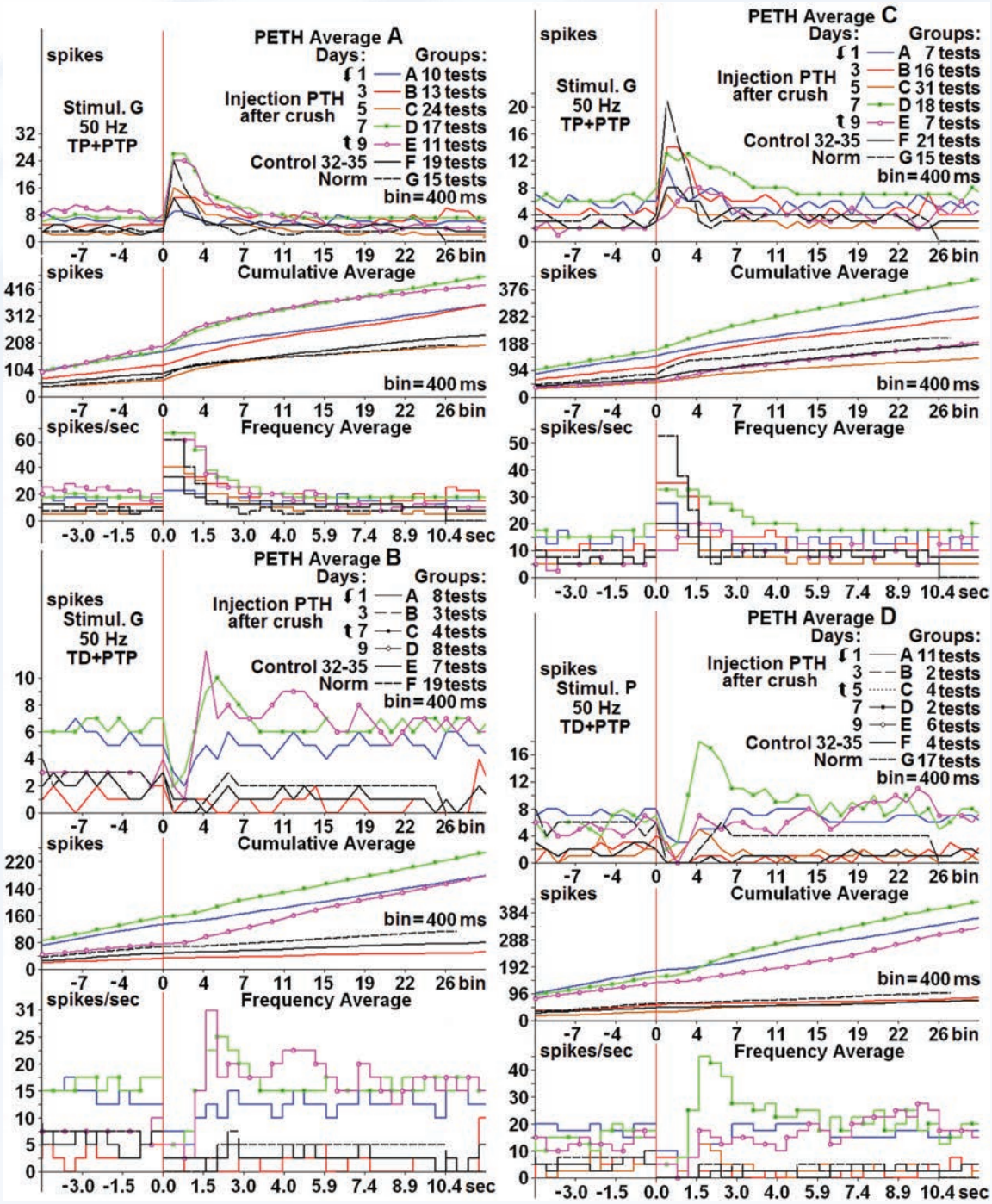

Figure 3. *Averaged peri-event time (PETH Average), cumulative (Cumulative Average) histograms and histogram of frequency (Frequency Average) excitatory (A, C) and depressor (B, D) poststimulus manifestations of activity of the spinal cord motoneurons on the injured side in terms of PTH application at days 1-9 (Groups A-E) in the control group (Group F) and in norm (Group G) to HFS (50 Hz, 1 sec) of G (A, C) and P (B, D) nerves in rats after the SN crush.*

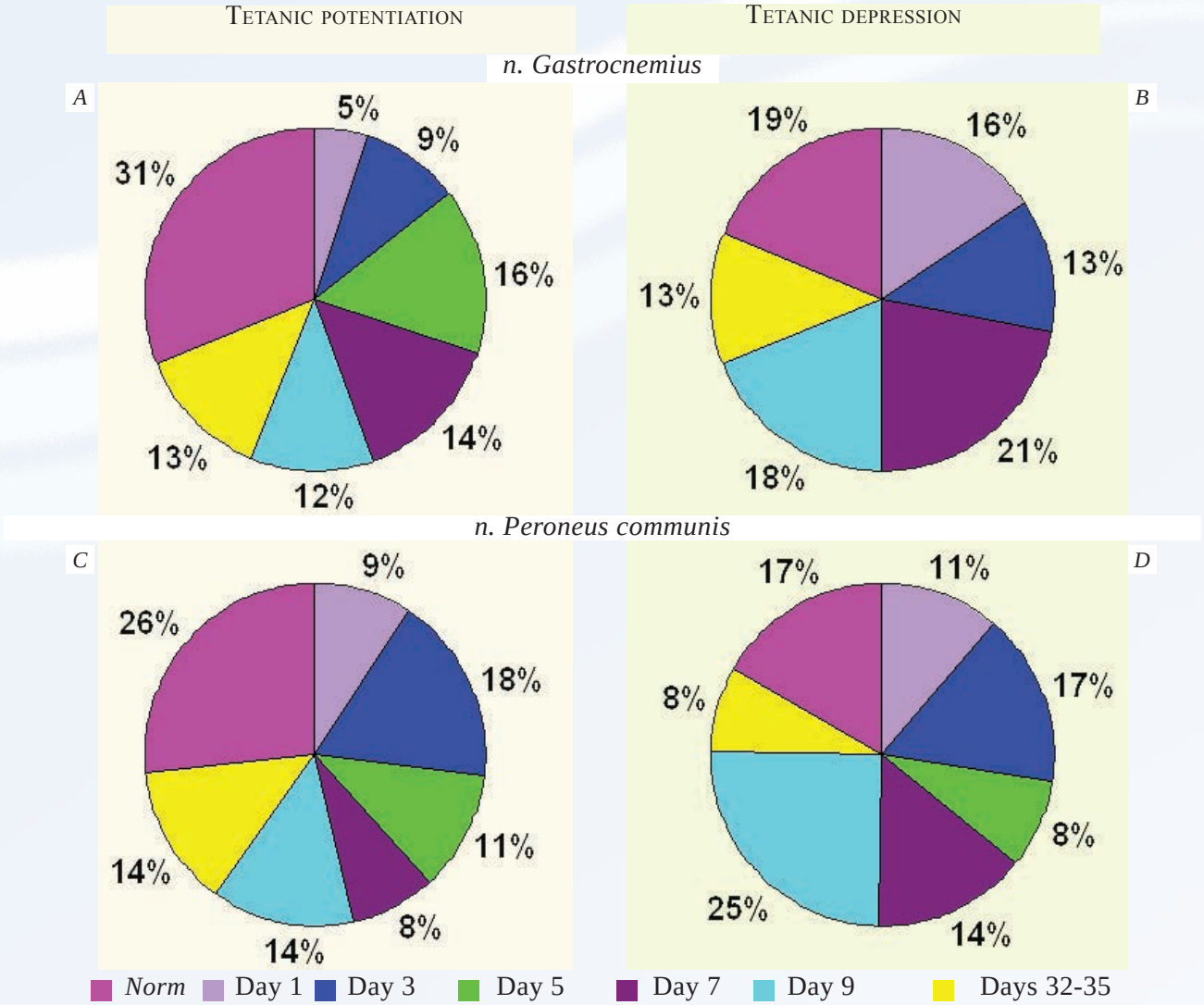

FIGURE 4. *Comparative disc charts of the ratio of excitatory (TP – A, B) and depressor (TD – C, D) reactions in spinal cord motoneurons to HFS of G (A, C) and P (B, D) nerves by a multiple of the average percentage ratio of the prestimulus frequency level of activity followed by tetanic poststimulus effect in 1-9 days after the SN crush in terms of PTH application, on days 32-35 in control and in the norm. The remaining notations are in the figure.*

5th and 9th test days after the SN crush in terms of PRP-1 application reached the value of about 4-fold decrease compared to the prestimulus level, which was twice higher than the value in control (for days 32-35) and 1.33 times higher *vs.* the norm. Finally, to HFS of the P nerve TD reached considerably greater decrease from 3.5 to 10-fold at days 5, 7, respectively, and 10 times on day 9, which was 5 times higher the same values in control and 2.5 times higher than in norm. In other words, in this case TD as compared with PTH group was significantly more pronounced.

Under the impact of NOX venom (Figures 7 and 8) the following fluctuations of TP and TD in the corresponding MNs of spinal cord were recorded. To HFS of G nerve by day 5 after the SN crush the highest level of activity within 7.33-fold excess of its prestimulus level was recorded. On the 10th day TP decreased up to 4-fold value and then again increased on day 17, but up to 5.7 times, which was 1.76 times higher than at days 32-35 and in comparison with the norm. To HFS of P nerve, although at the 5th and 10th days TP was less than in case of G nerve response to HFS, at day 17 the TP sharply increased up to 8.19-fold excess, which was 3 times higher than in control and even 3.64 times greater than in norm. In other words, the relative expression of regeneration compared with G nerve was obvious. As for the TD, to HFS of G nerve the highest level in understating of the prestimulus level was recorded at the 10th day (8 times) and approximately the same

level was recorded at the 5th and 17th days of tests (3 and 3.07 times, respectively), which was also higher than in control (1.5 times), but less than in norm (1.3 times). Finally, at HFS of P nerve conversely, the highest level of understating was recorded at day 17 (7.2 times), compared with days 5 and 10 after the SN crush (4 and 1.1 times, respectively), which 3.6 times exceeded the level in control and was 2.88 times above the norm. Thus, according to values of both TP and TD to HFS recorded for the extensor nerve they not only achieved, but even exceeded the norm.

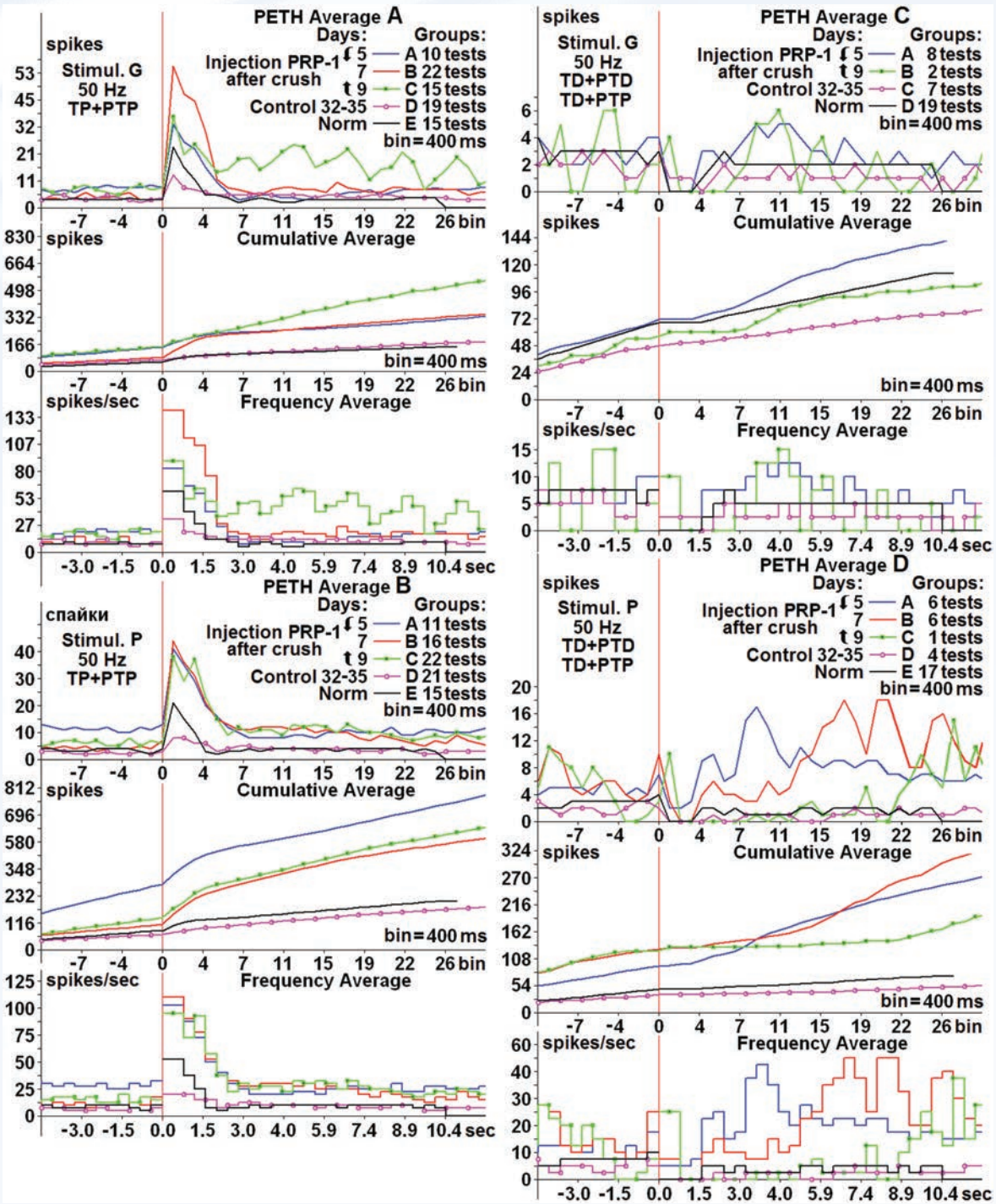

Figure 5. *Averaged peri-event time (PETH Average), cumulative (Cumulative Average) and frequency histogram of frequency (Frequency Average) excitatory (A, B), depressor and mixed (C, D) poststimulus manifestations of activity of spinal cord motoneurons on the injured side at days 5-9 in conditions of PRP-1 application (Groups A-C), in the control group (Group D) and in norm (Group E) to HFS (50 Hz, 1 sec) of the G (A, C) and P (D, D) nerves after the crush.*

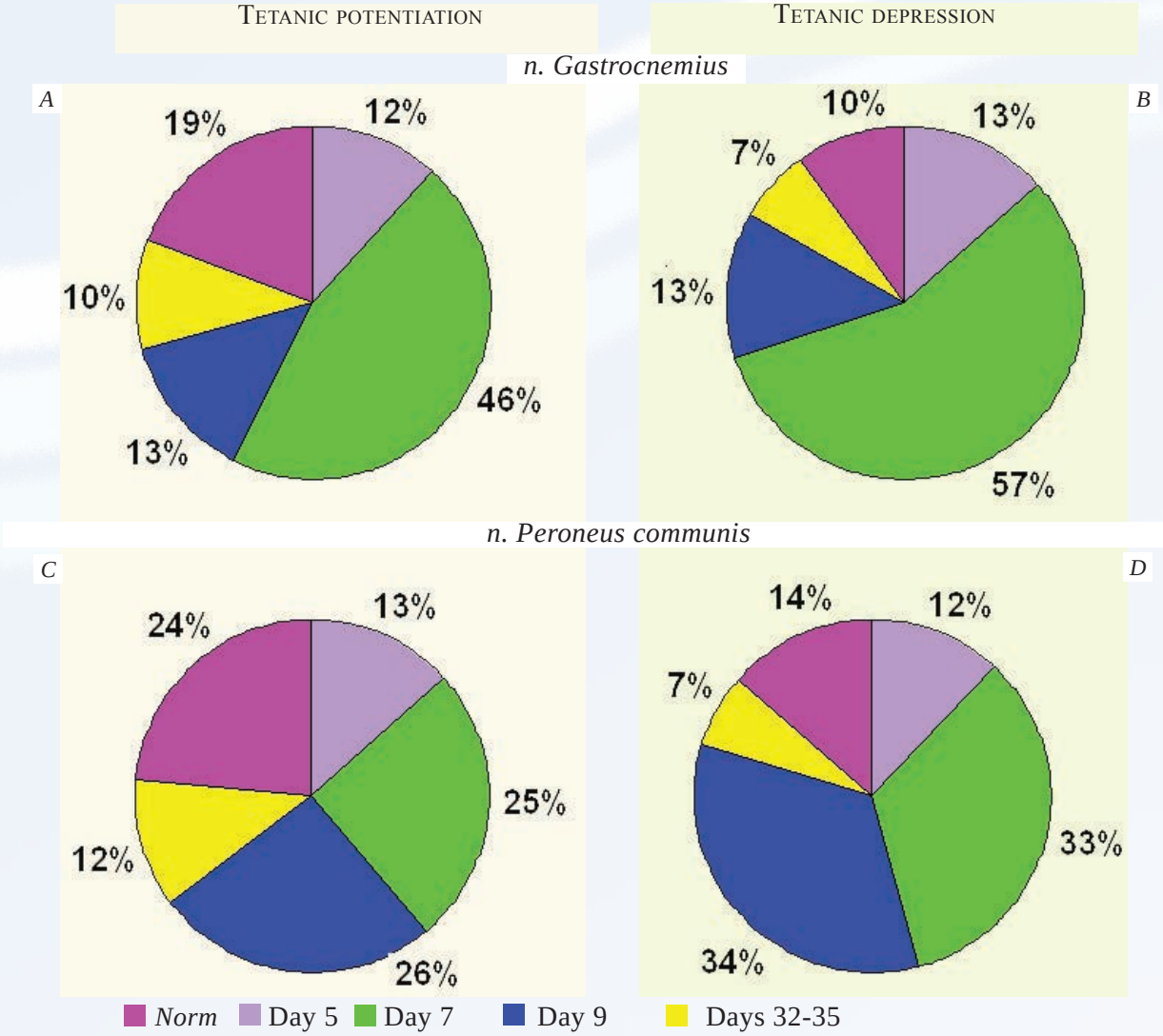

***FIGURE* 6.** *Comparative disc charts of the ratio of excitatory (TP – A, B) and depressor (TD – C, D) reactions in the spinal cord motoneurons to HFS of G (A, C) and P (B, D) nerves by a multiple of the average percentage ratio of the prestimulus frequency level of activity followed by tetanic poststimulus effect in the norm, 32-35 days after the SN crush in control and 5-9 days later – under PRP-1 injection (A-D). The remaining notations are in the figure.*

DISCUSSION

On-line registration and program mathematical analysis of motoneurons (MNs) impulse activity to high frequency stimulation (HFS) of spinal cord flexor (G) and extensor (P) nerves showed excitatory and inhibitory activity manifestations in intact rats (norm) at days 5-70 without (control) and with PTH, PRP-1 and NOX venom application.

Further search for effective therapeutic agents of peripheral nerves (PNs) neurodegeneration is still a topical need, in spite of intensive interdisciplinary research in this area. Therefore, we should agree with G. Stoll and H. Muller that a full recovery of PNs requires more prolonged time frames and pharmacological intervention [*Stoll G., Muller H., 1999*]. In turn, at long-term reinnervation by the 9th month post PN section in mature cats a significantly increased proportion of slow muscle fibers was marked in relation to fast ones [*Foehring R. et al., 1986 a; b; Fugleholm K. et al., 2000*].

In the present study we used a number of biomodulators, the use of which needs to be validated. PTH is of interest, because the peptide attributable to him, parathyroid hormone-related peptide (PTHrP), dramatically increases the number of undifferentiated Schwann cells (SCs), their migration along the axonal membrane and restoration of axons' growth in the explant [*Macica C. et al., 2006*]. In other words, PTHrP acts through the activation of immature SCs critically needed for successful nerve regeneration

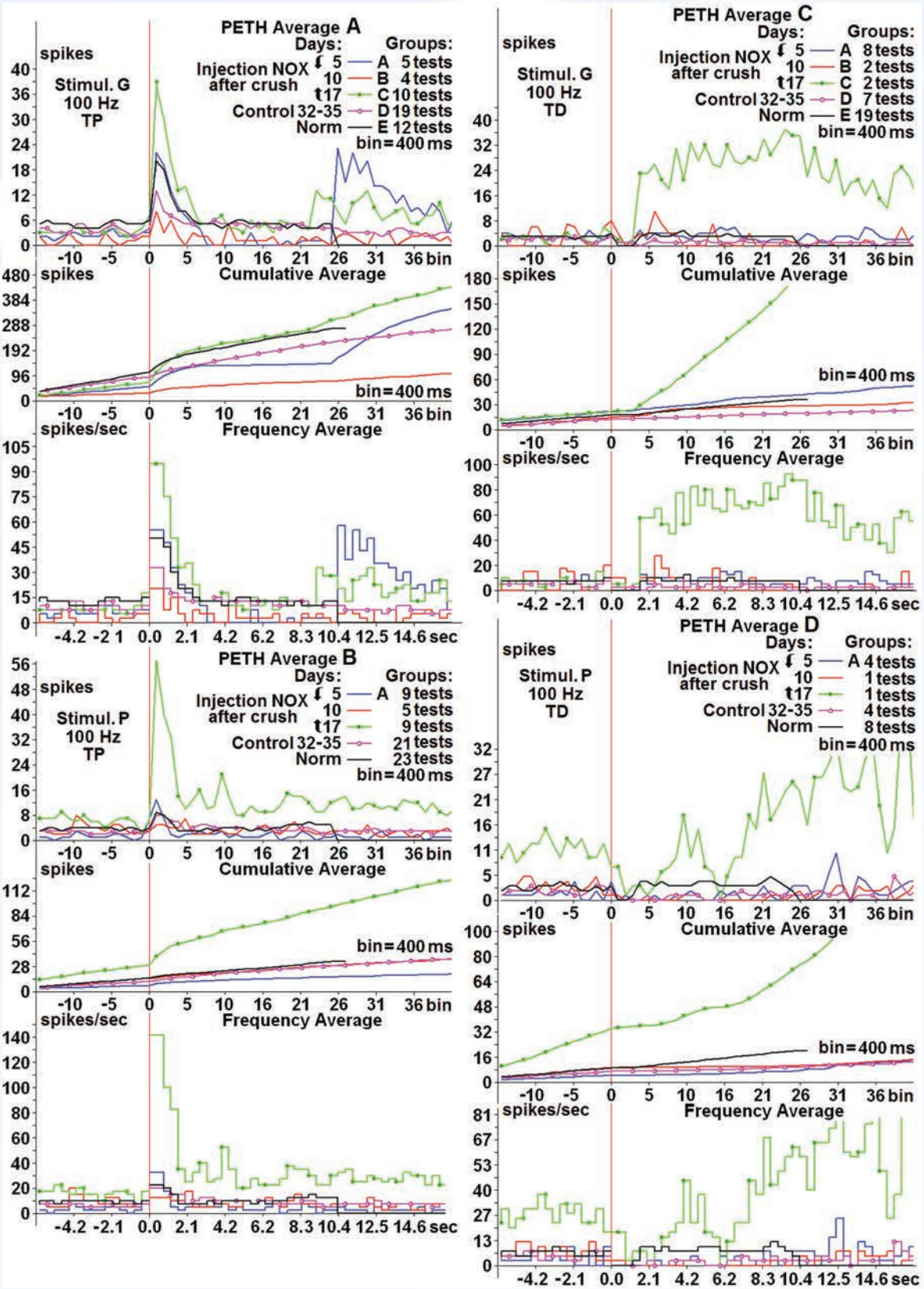

Figure 7. *Averaged peri-event time (PETH Average), cumulative (Cumulative Average) histograms and histogram of frequency (Frequency Average) of excitatory (A, B), depressor and mixed (C, D) poststimulus manifestations of activity of spinal cord motoneurons on injured side to HFS of G and P nerves at days 5-17 after the SN crush in conditions of protection by NOX venom (Groups A-C), in control at days 32-35 (Group D) after the SN crush and in norm (Group E).*

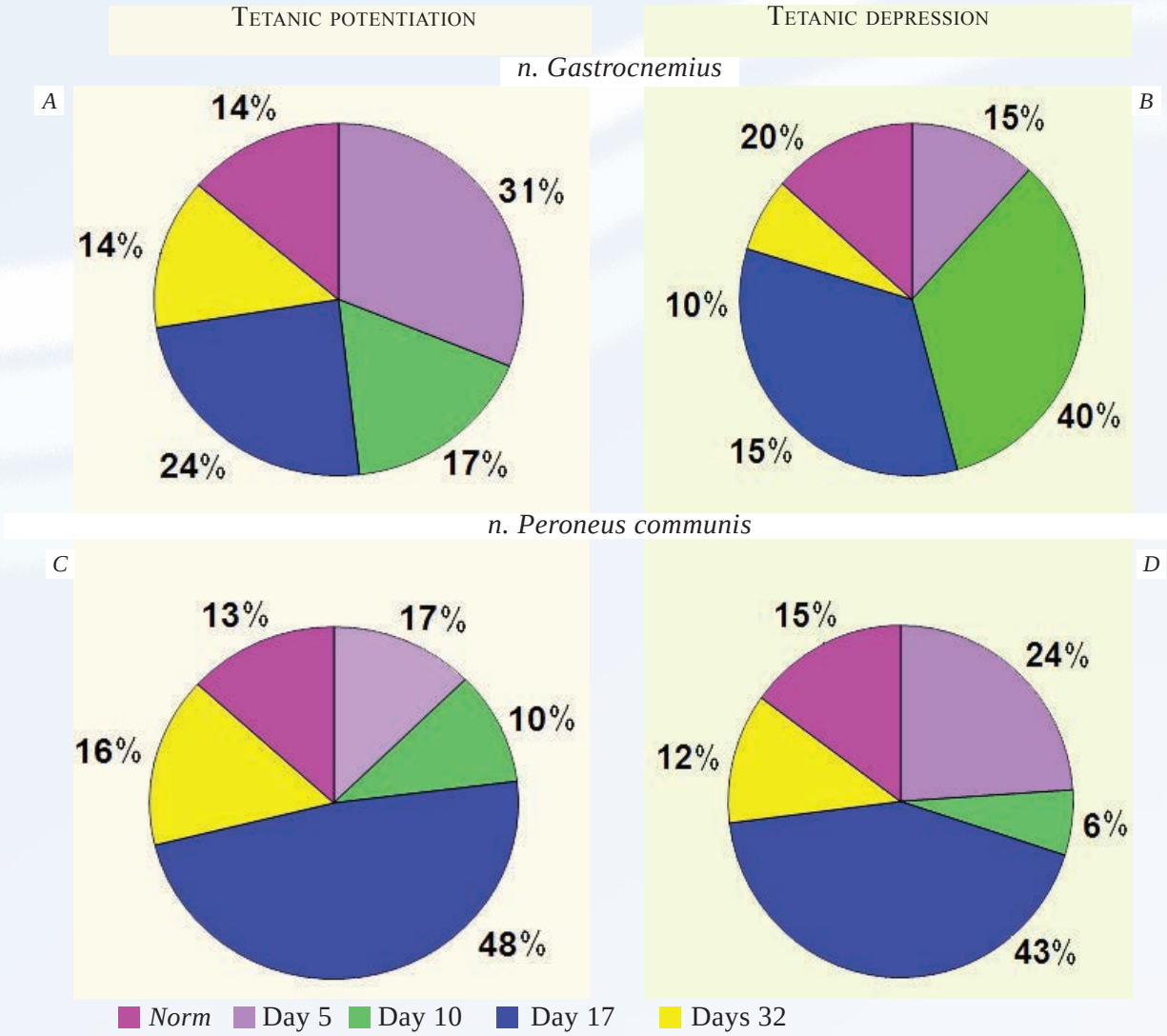

FIGURE 8. *Comparative disc charts of the ratio of excitatory (TP – A, B) and depressor (TD – C, D) reactions in spinal cord motoneurons to HFS of G (A, C) and P (B, D) by a multiple of the average percentage ratio of the prestimulus frequency level of activity followed by tetanic poststimulus effect in norm, after 32-35 days in control and 5-17 days after the SN crush in conditions of NOX venom action (A-D). The remaining notations are in the figure.*

[*Macica C. et al., 2006*]. Its successful protective effect was shown in our model of Alzheimer's disease [*Khudaverdyan D. et al., 2008*].

A wide spectrum of biological activity of neurotrophine-like PRP-1 peptide (an immunomodulator) produced by neurosecretory cells of hypothalamic NPV and NSO nuclei was revealed [*Galoyan A., 2001; 2008*]. The neuroprotective effects of PRP-1, in contrast to the complexities of multi-component therapy in acute and chronic nonspecific neurodegeneration of toxic (poisoning with animal toxins) and traumatic (spinal cord hemisection, transection of PNs) origin stimulates regrowth of transected spinal tracts, promotes survival of neuronal elements of the gray matter in both damaged area and beneath it counteracting the formation of scar, proliferation, migration and accumulation of glial elements at the site of injury, with subsequent recovery of lower extremity motor function on the side of injury [*Galoyan A. et al., 2000; 2001; 2005 a; b; 2007* a*; b; c*; *Abrahamyan S. et al., 2001; 2003*; *Sarkissian J. et al., 2005*]. PRP-1 might be a potential therapeutic agent for specific neurodegenerative diseases (Alzheimer's disease) as well [*Galoyan A. et al., 2004; 2008; Yenkoyan K. et al., 2011*]. The above-mentioned explains its use in the present study based on the therapeutic benefit, in general, related to the prevention of neurodegenerative processes, modulation of the apoptotic cascade, regulation of anti-inflammatory and neuroprotective events. Finally, poisons of animal origin, especially the snake venoms (SVs) obtain the increasing im-

portance for neuroprotection. The appropriate interest is due to the prospect of unlimited use of toxins and enzymes of SVs due to their high selective specificity and irreversible effects that determine the duration of their action, which in turn, determines the necessity of their combination with medications [*Bowman W., Sutherland G., 1986; Cook N., 1990*]. In addition, based on *Dendroaspis polylepis* (*Elapidae* family) dendrotoxines (DTXs), to which the NOX also belongs, compounds were synthesized that selectively block some subtypes of potential-dependent fast-activating K^+ channels in neurons, by which their excitability was controlled; moreover, both their activity (selective blockade of subtypes of potential-dependent K^+ channels in Alzheimer's disease), and inhibition (K^+ channel activators as anticonvulsants for epilepsy) [*Rudy B., 1988; Cook N., 1990*] were provided. Thus, the excitability of injured neurons in neurodegenerative diseases was controlled adaptively [*Rudy B., 1988; Cook N., 1990*]. In addition, DTXs were shown to trigger restart in neurons, promoting the release of neurotransmitters [*Harvey A., 1997; 2001*]. On the other hand, the presence of multiple K^+ conductances in neurons sensitive to DTXs was characteristic [*Pelchen-Matthews A., Dolly J., 1989*].

Finally, the neurotrophic growth factor (NGF) of SVs is of interest. Its bioactivity from Chinese cobra *Naja naja atra* SV promoting the growth of fibers without the enzymatic, toxicologic and teratogenic activities was revealed. In the present study the successful protective effect of NOX venom, apparently associated with NGF, was shown. We have previously proven the protective effect of SV in the non-specific neurodegeneration of peripheral and central origin [*Chavushyan V. et al., 2006; Sarkissian J. et al., 2006 a; b; Abrahamyan S. et al., 2007; Galoyan A. et al., 2010*] and specific neurodegeneration [*Sarkissian J. et al., 2007*].

At PTH application the excitatory tetanic effects of flexor nerve grew up to day 5 of tests, but were twice as low compared to norm, then dropped on days 7 and 9 of tests. Excitatory tetanic manifestations of the extensor nerve abruptly increased and dropped, reaching the level almost twice lower than that in control. By the end of testing in both cases there was an actual achievement of control level at days 32-35 of tests, but exceeded it on days 5 and 3 for nerves G and P, respectively. Depressor responses for the same flexor nerve on day 7 and for the extensor nerve on day 9 reached the values higher than those not only in control, but also in normal conditions, indicating the activation of depressor processes accompanying the protection. However, indicators of TP and TD were relatively pronounced for the extensor nerve. In general, upon stimulation of the nerve P to the end of the test we observed the TP recovery up to the norm being accompanied by approaching the norm of TD. In all the aforementioned cases predominantly early PTPs were also recorded to be followed by the actual stabilizing of activity by the end of tests.

The highest level of excitatory activity of spinal cord MNs to stimulation of G (TP + PTP) nerve in animals treated with PRP-1 was detected on day 7 after SN crush (nearly 4 times higher than values recorded by day 5 and 1.85 times higher than the norm), which at day 9 decreased up to 3.3 times, yet not reaching the norm (1.8 times). Moreover, the lowest excitatory effect occurred in the control group. Upon stimulation of P nerve the excitatory displays of spinal cord MNs activity showed high levels above the norm as early as day 7, which by day 9 continued to exceed the norm and were almost 2.3-fold above the control level. Nevertheless, with such a successful restoration of activity in SN branches treated by PRP-1 the best results for the extensor nerve should be noted compared with those of flexor.

A conclusion might be drawn about the true protective effect of PRP-1, unlike rather regulatory action of PTH , which allows the successful regeneration of the nerve, in response to distal stimulation of which on the damaged site the excitatory responses in MNs of spinal cord were much more greater than those of norm already at the first week of tests, while in control (until the end of the test) the regeneration of SN's flexor branch was not observed, and there was only a weak tendency of regeneration in SN's extensor collaterals.

Under the application of NOX venom, in general, with increasing time-frames after SN crush, in activation of the extensor nerve there was a tendency of TP overestimation in spinal cord MNs, more pronounced by day 17 of trials (3-fold higher than control and 3.64 times above normal), but greatly reduced on day 10. The TP of G nerve 2.27 times exceeded the level of norm on day 5, then

slightly decreased, but continued to exceed the norm on days 10 and 17 (1.23 and 1.76 times, respectively). The TD to HFS of the flexor nerve gradually increased from the 5th to 10th days and abruptly decreased on day 17 unlike extensor nerve, the activation of which was accompanied with by far less expressed changes in dynamics, but with similar effect at the end of tests. In other words, at regeneration of both branches of the SN and restoring of spinal cord MNs in NOX-treated animals slightly better indices were observed again for the extensor nerve, as compared to flexor one. In conclusion, the protective effect of NOX, counteracting in these conditions with deep reduction of inhibition, apparently, came down to speed up recovery of excitatory poststimulus manifestations of spinal cord MNs activity and, therefore, the conduction in damaged PNs. This is consistent with high selective specificity and irreversible effects of SVs.

In terms of the protective effects success, it is of interest to track both dynamics of expression degree and the dynamics of aforementioned depressor and excitatory reactions growth in time. The significance of inhibitory tetanic manifestations, as contributing to protection and better expressed at the initial stage of recovery and accompanying the damaged nerve restoration should be noted. It is known that poststimulus manifestations of activity in the form of TD and PTD mediate the inhibitory monoamines, which at the spinal cord level act as GABA or glycine. As inhibition is the basis of depression, the possibility of its assistance in protection is of interest. In turn, the true inhibition, in contrast to depression of disfasilitatory origin, might be of very different origin. The available data suggested that GABA and glycine might play an important and perhaps a different role in developing and mature central vestibular system [*Owens D., Kriegstein A., 2002*]. A review on complex function of inhibitory synapses in the CNS was recently published [*Birke G., Draguhn A., 2010*]. The review presented numerous modern studies on cellular and network levels showing that synaptic inhibition cannot be assessed only as opposed to synaptic excitation, but might additionally serve highly specific functions in the nervous system of mammals [*Birke G., Draguhn A., 2010*]. In addition, the effect of PRP-1 on the activity of neurotransmitter amino acids glutamine – glutamate – GABA system is of interest [*Hambartsumyan D. et al., 2003*]. To confirm the assumption on universal protective function of GABA-ergic inhibition there are also published data showing that in some systems during the nervous system development GABA acts as a factor affecting various features including proliferation, migration, as well as differentiation and maturation of synapses, cell death and expression of the $GABA_A$ receptor [*Cuppini R. et al., 2002*]. Research data suggested that GABA and glycine might play an important and perhaps a different role in developing and mature central vestibular system [*Tighilet B., Lacour M., 2001*]. On the other hand, the crucial role of events mediated by GABA receptor in neurons of the vestibular nuclei in recovery of functions after unilateral labyrinthectomy known as vestibular compensation was established [*Yamanaka T. et al., 2000; Johnston A. et al., 2001; Tighilet B., Lacour M., 2001; Giardino L. et al., 2002*]. Finally, the metabolic effect plays an important role in the mechanism of PRP-1 impact on the crush of the nerve. In particular, it was found that it increases glucose utilization in various organs, and in the brain it is almost 10 times higher the norm [*Kevorkian G. et al., 2001*].

In this paper, as a protector category, the involvement of GABA in deepening of inhibition during neurodegeneration was suggested to be intensified by application of biomodulators we used. In other words, in the present study, in terms of their therapeutic effects in the process of de- and regeneration of the crushed PNs, it is possible to suppose the involvement of true GABA-ergic inhibition in TD and PTD. According to our preliminary data, depressor responses are more intensively involved at both non-specific (peripheral, central) and specific neurodegeneration in different brain regions. Thus, the protective purpose of GABA was shown, in particular, in the nucleus of Deiters (after unilateral labyrinthectomy) and in the *substantia nigra* (on the model of Parkinson's disease), as well as in the hippocampus (on the model of Alzheimer's disease), mainly in neurons of the GABA-ergic nature, in which earlier rapidly involved depressor reactions accompany the recovery process until its completion [*Galoyan A. et al., 2008; 2010; Sarkissian J. et al., 2007; Sarkisyan S. et al., 2008*].

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