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ACTION OF THE HYPOTHALAMIC PROLINE-RICH PEPTIDE AND COBRA VENOM DURING VESTIBULAR COMPENSATION FOLLOWING UNILATERAL LABYRINTHECTOMY

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Abstract

The problem and uncovered mechanisms of the vestibular compensation (VC) following the unilateral labyrinthectomy (UL) still remain quite actual. The study on hypothalamic proline-rich peptide (PRP-1) and cobra venom Naja Naja Oxiana (NOX) action on Deiters' lateral vestibular nucleus (LVN) neurons upon dynamics of recovery after UL was carried out. Early and late tetanic, posttetanic potentiation and depression of neurons to bilateral high frequency stimulation of hypothalamic supraoptic and paraventricualar nuclei was studied. The analysis of spike activity by on-line selection and software package was done. The complex averaged peri-event time and frequency histograms were constructed. The increasing of inhibitory and excitatory reactions of Deiters' neurons at the early stage of VC following PRP-1 and NOX injection reaching the norm at the late stage was revealed. Moreover, the shifts of post-stimulus activation were relatively more expressive under high frequency stimulation by 100 Hz. The histochemical study for revealing the activity of Ca^{2+}-dependent acidic phosphatase (AP) was carried out. Data of UL animals with application of PRP-1 indicated to progressing delay of central chromatolysis of Deiters' neurons leading to deep neurodegenerative pattern of cellular shade up to its total disappearance. On the whole, the ferment activity preserved during 19 days after UL and PRP-1 exerted more favorable influence on LVN neurons of the injured side. On intact side PRP-1 led to the gain of phosphorylation, which was more typical for neurons surviving deep stress.

Morphological data concerning the NOX application allows us to assume that delay of deep metabolic disorders resulting in total degeneration in some large neurons takes place. The morphological picture indicates that under the NOX action a hyper activation is observed but it may be assumed that abrupt intensification of ferment activity probably also promotes the purification of affected nerve tissue and tendency to adaptation. Thus, the protective effect of NOX venom after UL is obvious. However, effects are quite expressive and often exceed the norm level, which indicates their abnormal expression. Without diminishing the significance of NOX venom of exogenous nature, one should be convinced of the natural and successful effect of PRP-1: a true endogenous biological modulator.

Keywords: *hypothalamic proline-rich peptide (PRP-1); cobra venom (NOX); Deiters' nucleus; paraventricular nucleus of hypothalamus (PVN); supraoptic nucleus of hypothalamus (SON), single neuronal activity; high frequency stimulation; tetanic- and post-tetanic excitation and depression; acidic phosphatase activity.*

INTRODUCTION

It is known that unilateral labyrinthectomy (UL) causes a syndrome of oculomotor, postural [*Curthoys I., Halmagyi G., 1995; Dieringer N., 1995*] and autonomic system [*Yates B., Bronstein A., 2005*] disorders, which diminish over time in a process of behavioral recovery, known as *vestibular compensation* (VC). The problem of VC remains one of the actual tasks of modern neuroscience directly associated with recovery of vestibular nucleus activity on the injured side. However, its central mechanisms are not completely revealed yet [*Smith F., Curthoys S., 1989*]. Elec-

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trophysiological studies show that the VIII nerve does not undergo a functional recovery; therefore VC has been attributed to CNS plasticity. It was shown that vestibulospinal neurons of the Deiters' lateral vestibular nucleus (LVN) have large interrelations with numerous brain structures and represent as a basic upper segmental center of regulation of the posture, equilibrium and orientation in space [*Sarkisian V., 2000*]. In turn, the hypothalamus being higher integrative link in regulation of autonomic functions realizes its activity by means of numerous reciprocal direct and polysynaptic connections with nuclei of *medulla oblongata* and thoracic segments of spinal cord (SC) [*Swanson L., Kuypers H., 1980; Swanson L. et al., 1981; Sawchenko P., Swanson L., 1982*]. Among the mentioned brain structures hypothalamic neuroendocrine centers, such as paraventricular (PVN) and supraoptic (SON) nuclei of hypothalamus and their direct connections with LVN neurons are of special interest.

Earlier we studied the influence of PVN and SON of hypothalamus on SC lumbar segments neurons in norm and following its hemisection, i.e. during non-specific degeneration [*Abrahamyan S. et al., 2007*]. Data on bilateral hypothalamic-vestibular connections obtained by method of HRP retrograde transport were represented [*Hambardzumyan L. et al., 2009*]. Recently there was found the family of new proline-rich peptides (PRP) produced by neurosecretory cells of hypothalamus PVN and SON. PRP-1, which has large spectrum of biological action on immune and nervous system [*Galoyan A., 2004; Galoyan A., 2008*] is mostly studied among their representatives. Earlier we have shown the protective effect of PRP-1 during peripheral and central neurodegeneration [*Galoyan A. et al., 2000; 2001; 2004; 2005; 2007a; 2007b; 2007c; 2008; Abrahamyan S. et al., 2003; Sarkissian J. et al., 2005*].

During the last decade biomedical research, in which venom components are being investigated for their potential as novel therapeutic agents, has emerged as an interesting option [*Figueroa E. et al., 2005; Chavushyan et al., 2006; Castro F. et al., 2007*]. Snake venom is a natural biological resource that contains several components of potential therapeutic value. Venom has been used for treatment of a variety of patho-physiological conditions in Ayurveda medicine (traditional Indian), homeopathy and folk medicine. With the advent of biotechnology, the efficacy of such treatments has been substantiated by purifying components of venom and delineating their therapeutic properties [*Chavushyan et al., 2006; Koh D. et al., 2006*]. Earlier we have studied the effects of snake venoms in case of neurodegeneration of specific and non-specific origin [*Sarkissian J. et al., 2006a; 2006b; Sarkissian J. et al., 2007*].

This study represents data on characteristics of deafferented neurons of Deiters' LVN after treatment by PRP-1 and Central Asian cobra venom *Naja Naja Oxiana* (NOX), as well as dynamics of their rehabilitation after UL.

MATERIAL AND METHODS

Experiments were carried out in normal and UL animals (adult male Albino rats; body weight = 230±30 *g*) without (sham control) and with PRP-1 (0.1 *mg/kg i/p* once the next day after UL) and NOX (5% LD50, 1 *mg/кg, i/m* three days after UL) administration. During acute experiments animals were immobilized by 1% dithylinum (25 *mg/kg, i/p*) and under artificial ventilation the section of SC at T_1-T_3 level (with ultrasound scalpel) was done to achieve *encephale isole* preparation. In stereotaxic apparatus the trepanation of the skull was done from *bregma* till *lambda* and *dura mater* was removed. Stereotaxic orientated glass electrodes of 1-2 *μM* tip diameter were filled with 2 *M* NaCl and inserted into LVN for bilateral recording of single neurons spikes flow activity evoked by bilateral high frequency stimulation (HFS) of ipsi- (i) and contralateral (c) $PV_{i,c}$ and $SO_{i,c}$ (rectangle current pulses - 0.05 *ms*, 0.12-0.18 *mV*, 0.32 *mA* and frequency of 50 and 100 *Hz* during 1 *s*). Stimulating electrodes were inserted according to stereotaxic coordinates of the rat atlas [*Paxinos G., Watson C., 2005*]: NSO (AP – 1.3, L ±1.8, DV+ 9.4 *mm*); NPV (AP – 1.8, L±0.6, DV+7.8 *mm*). The recording electrodes were inserted by coordinates: LVN (AP -11.5, L±2.5, DV+7.0 *mm*). Post stimulus activity was revealed as tetanic potentiation (TP) and depression (TD) following with posttetanic potentiation (PTP) and depression (PTD) of different latency,

intensity, and duration. On-line registration was done on the basis of program providing selection of the spikes by mean of amplitude discrimination. After selection the pulse flow was analyzed by means of special mathematical program before and after stimulation for getting "raster" of single neurons pre- and post stimulus spike flows in real time. There are also shown the histograms of the sum and averaged frequency histograms of the spikes presented in raster, as well as PETH, Cumulative and Frequency histogram. For selected comparable groups of neuronal spiking the similar complex averaged PETH (*PETH Average*), cumulative (*Cumulative Average)* and frequency (*Frequency Average*) histograms were constructed. On average, during each record up to 10-15 post stimulus trials were carried out. This special mathematical program allows separating stimuli, superposed on action potential during their close succession in the process of TP and TD and avoiding traditional complex intracellular recording approach of long-term tetanic potentiation and depression. This allows taking into consideration strictly permanent tetanic effects in comparison with less stable posttetanic ones. At the end of each experiment the sites of stimulation and registration were verified histologically.

For histochemical investigation, parts of the brain stem were fixed for 2-3 days in 5% neutral formalin prepared on phosphate buffer. The frontal frozen sections (40-50 μM) were processed using the new approach of revealing the activity of Ca^{2+}-dependent acidic phosphatase (AP) [*Meliksetyan I., 2007*]. After washing, the sections were developed in 3% solution of Na_2S and covered with Canadian balsam.

RESULTS

The study of impulse activity flow of the single LVN neurons evoked by bilateral stimulation NPV and NSO was carried out in spinal rats in norm (7 rats, 128 neurons), after administration of PRP-1 (7 rats with UL, 133 neurons) and NOX (9 rats with UL, 219 neurons).

Electrophysiological study: TP and TD followed by PTP and PTD in response to hypothalamus PVN and SON stimulation with administration of PRP-1 were analyzed (Figure 1). The following data were registered (Figure 1 A, B) to signify stimulation of PVc-i with HFS 50 *Hz* at 4-18th days after UL in comparison with pre stimulus level. Stimulation of the PVi (recordings from the injured side) led to activity elevation (from 1.5 to 4 times) with the maximum at the 7th day reaching 1.5 level at the end of the tests with consequent early and late PTPs and activity up to initial level (Figure 1 A, Groups A-D). As to stimulation of PVc (recordings from the intact side) activity of TP, it increased from 4.8 to 8 times with maximum at the 4th day (8 times) and minimum at the 9th day (4.8 times) followed by early and late PTPs, especially expressed at the 4th day of the tests (Groups E-F). In norm TP reaches only the 2.2-fold overshoot (Group G). This allows to conclude on successful protective action of PRP-1 and expressive compensatory gain of activity on the intact side. TD in response to PVi stimulation in comparison with pre stimulus level had 1.6-2.5 times less activity with their maximum at the 18th day (2.5 times). Likewise the norm, stimulation of PVc was accompanied by 1.6 times depression with consequent stationarity (Figure 1 B, Groups A-G). This indicates that effects tend to the norm on intact and even exceed on injured sides, respectively. Under stimulation of the same nuclei by 100 *Hz* there were recorded the following displacements of TP and TD (Figure 1 C, D). Activity of TP to PVi stimulation increased 2.8-4.8 times at the 7-18th day with the maximum at the 18th day and consequence stationarity. To stimulation of PVc TP had 9 to 2.4 times increase at the 4th and the 9th day, respectively (Groups A-C) and were accompanied by PTPs. In norm TP experienced only twofold increase but less than during UL which testifies to obvious protection of PRP-1 (Groups D-F). TD in the mentioned conditions of PVi stimulation decreased in the range of 1.5-7.3 with the maximum at the 9th day and minimum at the end of tests (Figure 1 D, Groups A-D). Stimulation of PVc gave rise to activity decrease 3.2 times (with PTP) at the 9th day and to stimulation of PVi in intact side (Groups E, F) actually approaching the norm. In other words, in response to both PVc-i stimulation in case of PRP-1 application on injured side the tetanic excitatory and depressor effects approach the norm and quite often

exceed it, whereas on intact side their compensatory increase was registered.

An evidence for PRP-1 protective effect may serve the similar post stimulus displays of activity to stimulation of the same nuclei in the control experiments (without application of PRP-1). In response to stimulation of PVi-c by 50 *Hz* at the 3rd-17th days after UL there were seen the following manifestations of activity (Figure 1 E-H). TP to stimulation of PVi exceeded the pre stimulus level of activity 1.4-2.3-fold with the maximum at the 9th day (Figure 1 E, Groups A-C).

Stimulation of PVc evoked TP with 1.76-2.66 times higher activity reaching the maximum at the 9th day but in norm 2.4 times higher (Groups D-F), which indicate to absence of compensatory increase of activity on the intact side and its decrease at the end of tests on injured side. TD to stimulation of PVi showed two-fold decrease of activity at the 9th and 14-17th days and absence of reactions at the 3rd day. Stimulation of PVc accompanied by TD activity decrease in the range of 1-2.5 times in the same time constraint, with maximum at the end of tests and was 1.66 times lower than in norm (Figure 1 F, Groups A-G) which indicate compensatory deepening of depression on the intact side and some increase of TP on injured side at the end of tests (defined increase of depressor reactions). TP to stimulation of PVi by 100 Hz at the 3rd-17th days after UL increased 1.75-3 times reaching the maximum at the 9th day. At stimulation of PVc increase was 2-2.8 times with maximum also at the 9th day and in norm to stimulation of PVi – 2.5 times (Figure 1 G, Groups A-F). TD to stimulation of PVi suffered 2.25-3 times depression with maximum again at the 9th day. TD to stimulation of PVc depressed 2-5 times, but with minimum at the 9th day and 3 times in norm (Figure 1 H, Groups A-G). This indicates to levels of TD lower than the norm both on injured and intact sides.

Under stimulation of SOc-i by 50 *Hz* at the 4-18th day after UL with PRP-1 administration the following effects were revealed (Figure 2 A-D): to stimulation of SOi at the 18th day the maximum increase of TP was 5 times; stimulation of SOi at the 4th and the 9th days (Groups D, E) led to 6.4 times increase followed by PTP and in norm stimulation of the SOc – only up to 2.7 times increase (Group F), which is less than two times of those in pathology. TD in the same conditions had 1.4-2.3 times decrease of activity with the maximum at the 9th day after UL (Figure 2 B, Groups A-D). Stimulation of SOi at the 4th and 9th day gave rise to TD in the range of 3.0-3.5 times of activity decrease but in norm up to 7 times. In norm stimulation of SOc decreased the activity 7 times (Groups E-G), which is much higher of those in pathology even on intact side. In all cases at the end of the tests activity became stationary. Stimulation of the same nuclei by 100 *Hz* (Figure 2 C, D) evoked TP in response to stimulation of SOc maximum 8 times higher at the 18th day of the test (Figure 2 C, Groups A-D). To stimulation of SOi at the 4th and 9th days –5.8 and 4.2 times higher, respectively. In norm 2.4 times higher to SOc stimulation (Groups E-G), which is twice less of the level in pathology and point to obvious protective effect of PRP-1. Finally, TD to stimulation of SOc had maximum effect at the 9th day (8 times), and at the end of tests – 3 times (Figure 2 D, Groups A-D). To SOi stimulation TD in average showed 7.5 times decrease and in norm – 7 times (Groups E-G). In other words, in response to both SOc-i stimulation in case of PRP-1 application on injured side the tetanic excitatory and depressor effects approach the norm and quite often exceed it whereas on intact side their compensatory increase was registered.

To stimulation of SOc-i by 50 *Hz* in control experiments the following post stimulus shifts were recorded (Figure 2 E, F): TP exceeded the pre stimulus level of activity 1.1-1.4 times with the maximum at the 9th day (Figure 2 E, Groups A-C); to stimulation of SOi minimum values, as well as absence of reactions were registered at the 9th day (Groups D-F) and in norm to stimulation of SOc triple enhancement was recorded (Groups D-G), which was twice higher the levels on injured and intact sides. TD to stimulation of SOc at all days of tests reaches the triple decrease of activity with pronounced PTP (2.2 times) (Figure 2 F, Groups A-C). Stimulation of SOi at the same days led to twofold depression and 8 times in norm (Figure 2 F, Groups D-G), which on the whole abruptly differs from the untreated pathol-

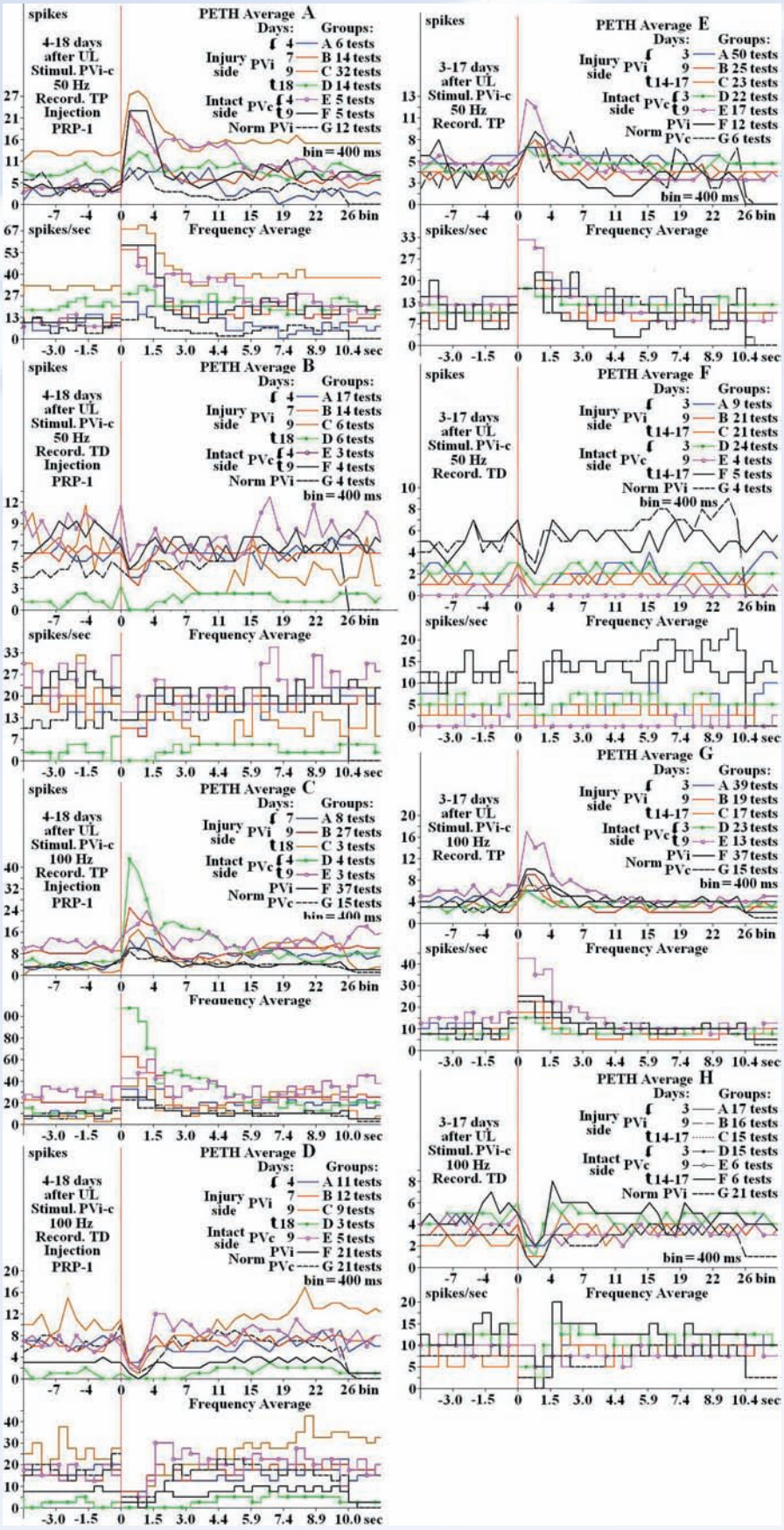


Figure 1. *A-H – Complex average (PETH Average) and frequency (Frequency Average) histograms of neurons activity spiking with (A-D) and without (E-G) PRP-1 use in rats after UL and those of intact ones (Norm) by bilateral recording of excitatory (A, C, E, G) and inhibitory (B, D, F, H) tetanic potentiation (TP) and depression (TD), respectively) at HFS of 50 Hz (A, B, E, F) and 100 Hz (C, D, G, H) PVc and PVi on intact and injury sides. Next to each group the number of tests is given.*

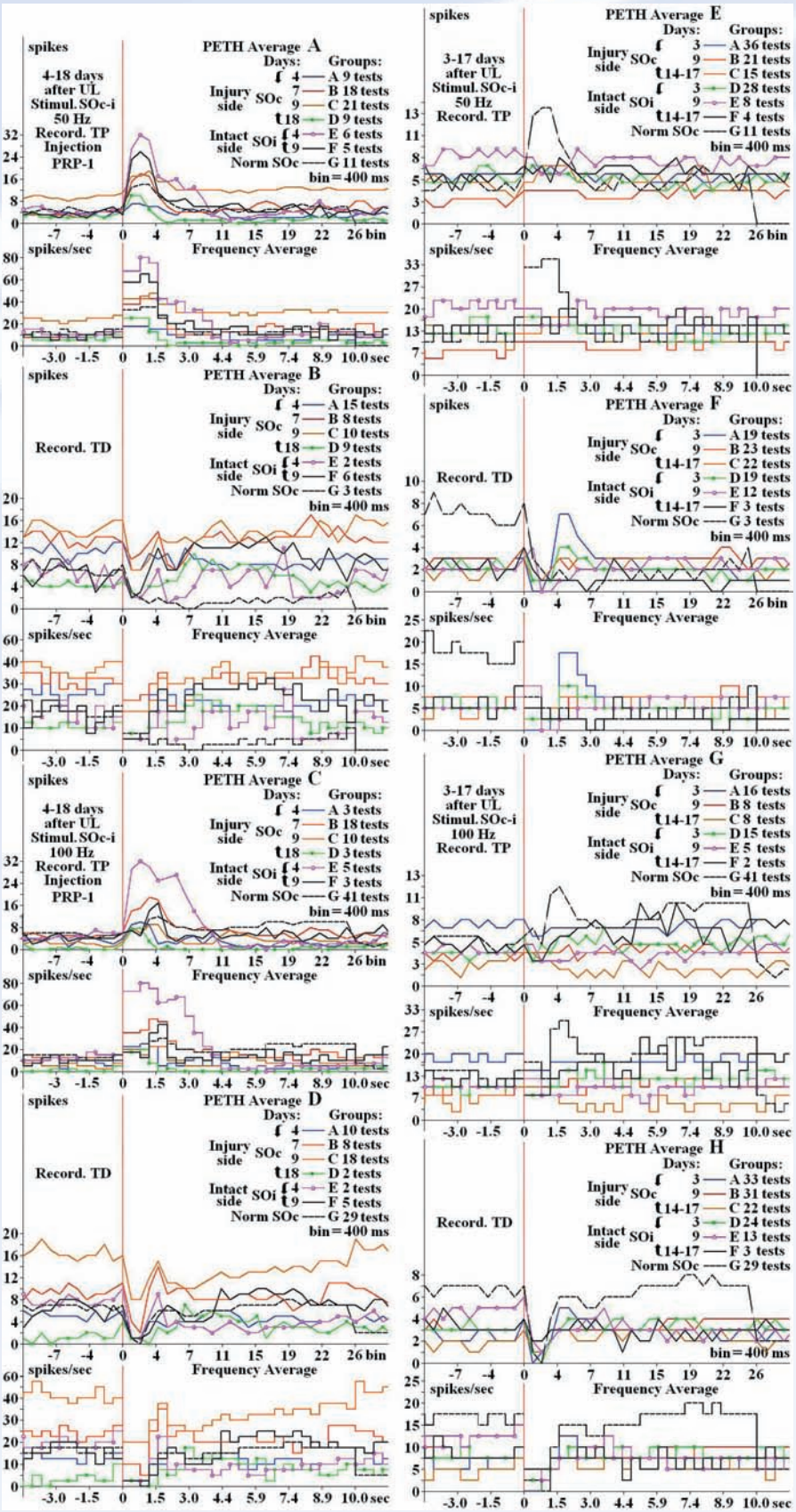


Figure 2. *Complex average (PETH Average) and frequency (Frequency Average) histograms of neurons activity spiking with (A-D) and without (E-G) PRP-1 use in rats after UL and those of intact ones (Norm) by bilateral recording of excitatory (A, C, E, G) and inhibitory (B, D, F, H) tetanic potentiation (TP) and depression (TD), respectively) at HFS of 50 Hz (A, B, E, F) and 100 Hz (C, D, G, H) SOc and SOi on intact and injury sides. Next to each groups the number of tests is given.*

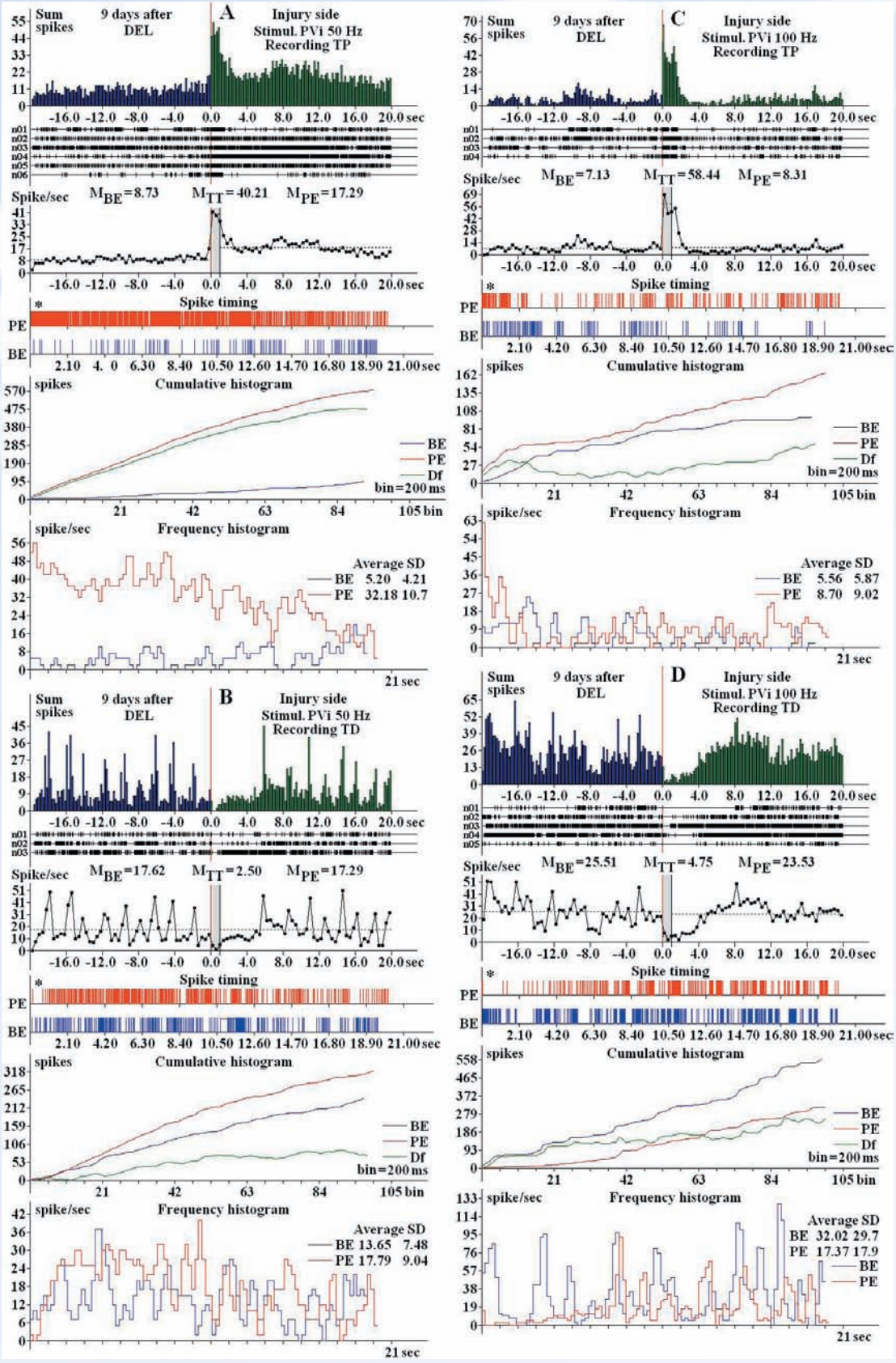


Figure. 3. A-D – *peristimulus histograms of sum spikes (from above) constructed on the basis of raster of pre- and poststimulus excitatory – TP (A, C), depressor – TD (B, D) effects and manifestations of spike activity of LVN single neurons under HFS 50 (A, B)) and 100 (C, D) Hz (during 1 sec) PVi at the 9th day after UL with the use of PRP-1; below: diagrams of frequency of spikes presented in raster with average meanings and detailed analysis (Spike timing, Cumulative and Frequency histograms) of occasionally selected single neurons (shown by* in raster).*

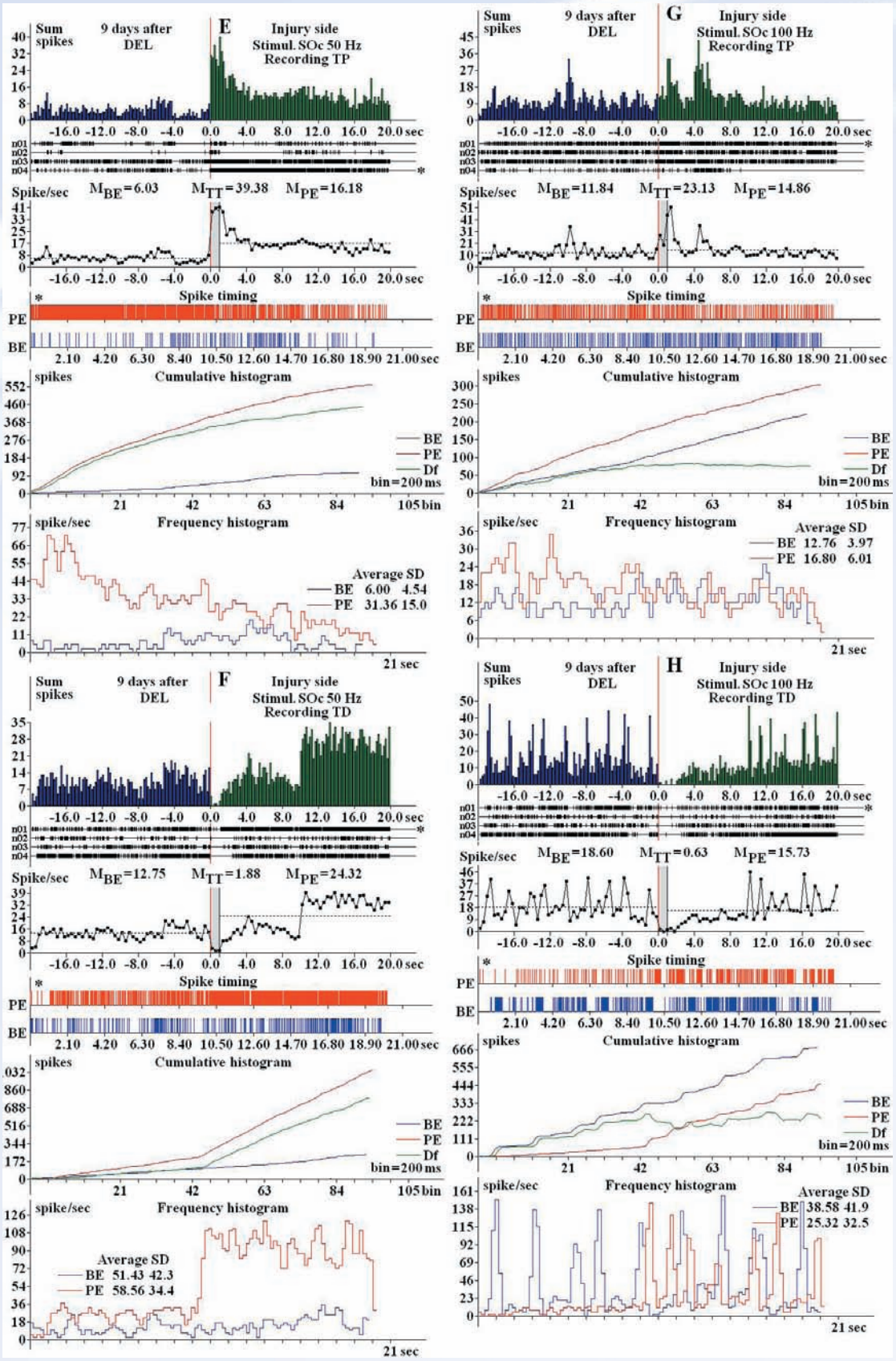


Figure 4. *A-D – peristimulus histograms of sum spikes (from above) constructed on the basis of raster of pre- and post-stimulus excitatory – TP (A, C), depressor – TD (B, D) effects and manifestations of spike activity of LVN single neurons under HFS 50 (A, B)) and 100 (C, D) Hz (during 1 sec) SOc at the 9th day after UL with the use of PRP-1; below: diagrams of frequency of spikes presented in raster with average meanings and detail analysis (Spike timing, Cumulative and Frequency histograms) of occasionally selected single neurons (shown by* in raster).*

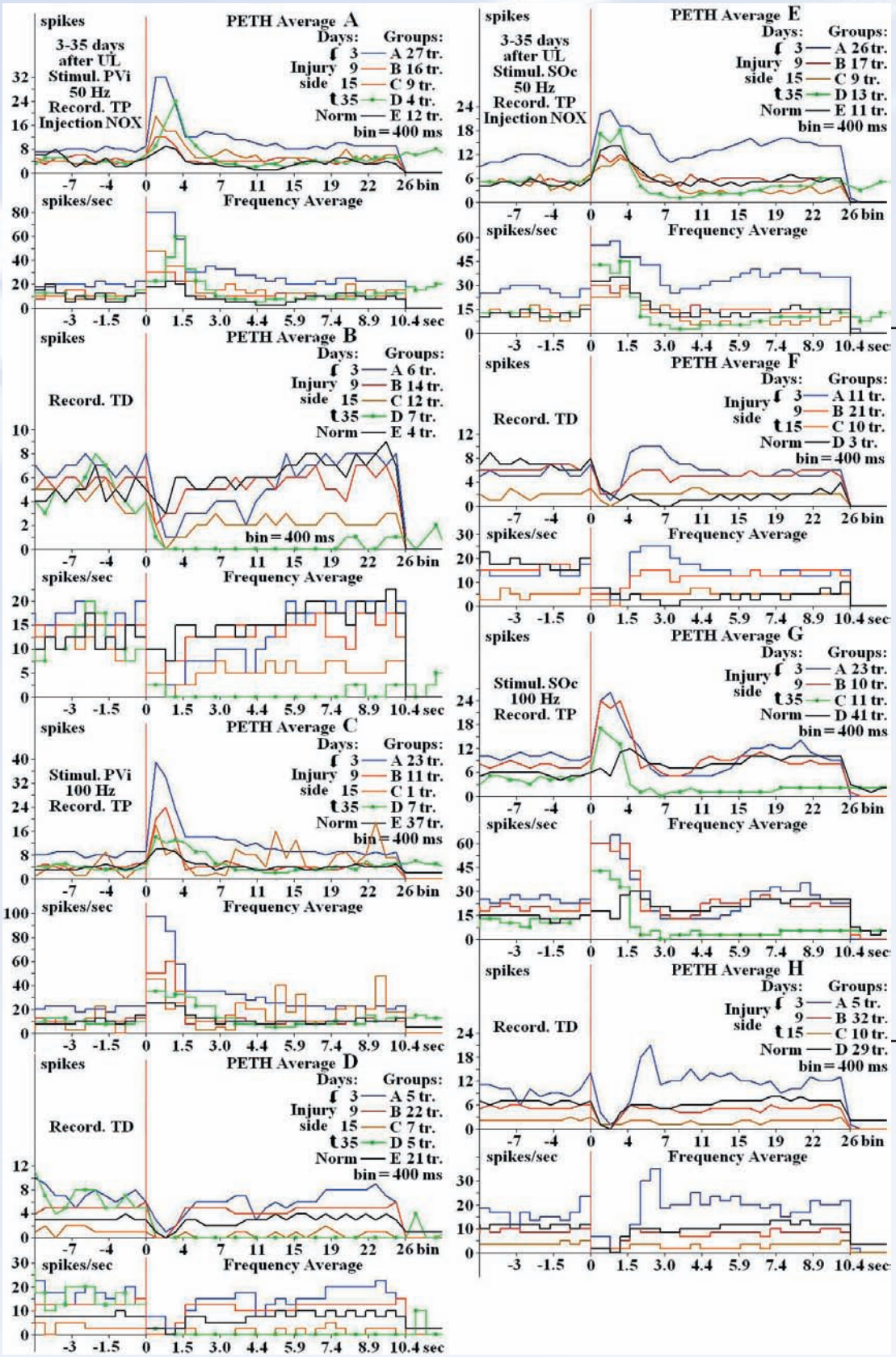


Figure 5. *A-H – Complex average (PETH Average) and frequency (Frequency Average) histograms of neurons activity spiking to PVN (A-D) and SON (E-H) with the use of NOX in rats after UL and of those of intact ones (Norm) by bilateral recording of excitatory (A, E, C, H) and inhibitory (B, D, F, H) tetanic potentiation (TP) and depression (TD), respectively at HFS of 50 Hz (A, B, E, F) and 100 Hz (C, D, G, H) PVi and SOc on injury side. Next to each groups the number of tests is given.*

ogy. TP to stimulation of SOc by 100 *Hz* exceeded the pre stimulus level of activity just at the end of tests 1.3 times (Figure 2 G, Groups A-C). To stimulation of SOi TP were not growing at all, but in norm they increased for 1.4 times with PTP gain 1.4 times (Groups D-G). At least TD to stimulation of SOc decreased in limits of 1.5-3 times with maximum at the end of tests. TD during stimulation of SOi at the same time strain decreased up to 1.5-5 times and in norm – 7 times (Figure 2 H, Groups A-G) which is also incomparably higher than presented values in pathology and on intact side 2.3 and 4.6 times, respectively. Thus, the difference in effects of TP and TD in control as compared with those in condition of PRP-1 administration is obvious.

As typical examples of above-mentioned reactions Figures 3 and 4 represent raster and detailed analysis of single neurons pre and post stimulus patterns of activity to stimulation of PVN and SON at the 9th day after UL and PRP-1 administration.

Application of NOX venom under similar experimental conditions led to exposure of effects represented in the following figures 5-7. Under stimulation of PVi by 50 *Hz* 3-35 days after UL TP arose 2.6-4.8 times in excess and reached the maximum at the end of test, whereas in norm exceeding of about 2.25 times (Figure 5 A, Groups A-F) was recorded being almost twice higher the indices of norm. To stimulation of PVi TD decreased 3-7 times in the same terms with maximum at the 3rd day and 5 times at the 35th day of the test and in norm it was 2.5 times (Figure 5 B, Groups A-E) which was also twice higher the indices of norm. To stimulation of PVi by 100 *Hz* TP exceeded the initial level 3.5-6 times in the same terms after UL with maximum at the 9th day and minimum at the end of tests and 3.3 times in norm (Figure 5 C, Groups A-E), which approaches the values of pathology at the 35th day and argue in favour of gaining excitatory processes in previous terms of tests. TD to stimulation of PVi showed about 1-7 times decrease of activity in the same terms of tests with maximum at the 3rd day and about 6 times at the 35th day, but 3 times in norm (Figure 5 D, Groups A-E), which was at least twice higher the values in norm and indicated to the gain of inhibitory effects. TP to stimulation of SOc by 50 *Hz* after UL arose in the limits of 2.1-3.6 times enhancement with maximum at the end of tests and about 2.8 times in norm (Figure 5 E, Groups A-E), which indicated to approaching the norm of TP readings already at the 15th day of test, but at the 35th day exceeded them (1.3 times). TD to stimulation of SOc underwent 2-10 times decrease with maximum at the 3rd day and minimum at the 15th day of tests and 7 times in norm (Figure 5 F, Groups A-D). This indicates to increase of depressor effects already at the 3rd day of test (above the norm), but with abrupt decrease at the 15th day (3.5 times lower the norm). At the 9th day and at the end of tests TP to stimulation of SOc by 100 *Hz* was recorded by 2.9-3.4 times activity increase and by 1.8 times in norm (Figure 5 G, Groups A-D), which indicated to almost twice gain of effects in comparison with norm already at the 9th day of test. TD to stimulation of SOc undergo 2-11 times activity decrease with maximum already at the 3rd day of test and minimum at the end of tests and 6 times in norm (Figure 5 H, Groups A-D), testifying to approaching the normal level already at the 9th day, but with twice decrease of depressor effects at the 25th day of test.

As typical examples of above mentioned reactions on the following two figures (Figures 6 and 7) there are represented rasters of characteristic samples and detailed analysis of single neurons pre and post stimulus activity displays in response to stimulation of PVN and SON at the 9th day after UL and with NOX administration.

Thus, the protective effect of NOX venom after UL is obvious. However, effects are quite expressive and often exceed the norm level, which indicates their abnormal expression and should be elucidated in the future concerning their possible application without undesirable consequences. Without diminishing the significance of NOX venom of exogenous nature, one should be convinced of natural and successful effect of PRP-1: a true endogenous biological modulator.

Morphological study: Most of intact rats Deiters' neurons are of large polygonal pattern (Picture 1 Aa) and abundantly provided with processes and collide with them in the nucleus limits. The thickness of processes at long distance is almost the

same. The gross granular suspend of lead phosphate is uniformly distributed over the soma and processes, but the zone of cytoplasm around nucleus dyed most dark and along with elimination from the cell body suspend in processes localized somewhat reduced (Picture 1 B, C). In most of neurons processes can be pursued at long distance from the cell body, giving rare collaterals in the given plane. In dendrites the ferment activity is higher than in axons. At the 7th day after UL on injured side the phenomenon of central chromatolysis can be observed in rats. The shape of cells is disturbed and the processes are significantly shortened (Picture 1 G). Some of the cells are swelled and round. A significant part of the cell cytoplasm with thin and stressed cellular membrane become clarified (due to abrupt decrease of ferment activity), whereas the other half of the neuron seems intensively labeled.

Perhaps, in the certified part of a neuron there lies an intensively swelled ectopic nucleus (Picture 1 D). There are also neurons with phenomenon of central chromatolysis and centrally located nucleus. In a number of cases high ferment activity of the cell nucleus is observed which may indicate to a phosphorylating process, proper to the stress condition. Shapeless Hamori-positive granulations with high ferment activity are also observed (Picture 1 E). The morphological pattern is expression of etiological diversity, shortening of processes (Picture 1 F).

Following disorder of cellular metabolism some decrease of proper to non-specific neuronal lesions was observed. The effect of stereotype response to stimulation was a reaction of the satellite glia. On intact side UL-evoked condition of stress accompanied by ferment activity could be seen. In response to cell pathological-morphological display a reaction of the satellite glia was observed. Thus, in response to UL at the 7th day after injury besides prevailing reversible processes there might be found more heavy lesions with effect of chromatolysis up to cellular shade. There were no special differences in character of LVN neurons responses of intact and injured sides at the 3rd day following UL in combination with PRP-1. In intercellular space of both sides the nuclei of glial and small triangle cells were responded. Here and there, one could see large, slightly dyed small corpuscles with centrally disposed nucleus. On intact side ectopia of the nucleus, some intensification of ferment activity in the cells bodies and processes was registered but with preservation of suspended granules. There was a pericellular edema (Picture 2 B), slight Hamori-positive dust-like granules and high ferment activity (Picture 2 D). The close convergence of neurons was typical. At UL side in LVN neurons suspend of the plumbum phosphate preserved its granules, processes became longer and very often the body of a giant cell gradually transformed to thickened dendrite due to which evaluation of borders between body and dendrite was impeded. In some neurons a minor swelling of the nucleus was registered at the site centrally disposed nucleolus was clearly seen (Picture 2 A). The bodies of large cells, as well as dendrites at some distance on the site of dichotomy division had moderate AP activity. In the latter case a dendrite became rather expanded forming a "platform", which was significantly wider than common dendrite trunk. After this expanding, the dividing of the dendrite occurred (Picture 2 C).

At the 9th day on injured side an abrupt intensification of AP activity was recorded. Nucleus and cytoplasm borders could not be pursued; the shape of cells was preserved and in most of the cells processes reacted at big distance from the body (Picture 2 E). On uninjured side nucleus transfered to the cell center and the nerve cell having picnomorph behavior was more darken in comparison with those of intact rats neurons (Picture 2 F). As a whole, it should be mentioned that in these time constraints abrupt differences in AP activity on ipsi- and contralateral sides were not observed, i.e. there was a tendency of ferment activity balancing. The only distinction was as follows: in the neurons of intact side the light nucleus might be distinguished; this latter was absent in cells of the injured side.

At the 17th day activity of the AP in the ipsi- and contralateral Deriters' nuclei became equal. Ferment activity of both nuclei was somewhat low, pericellular edema granules in the interstices, around pericaryon and along the edges of dendrites vanished. Among the neurons of intact side, cells with normal morphology (Picture 3 C) might

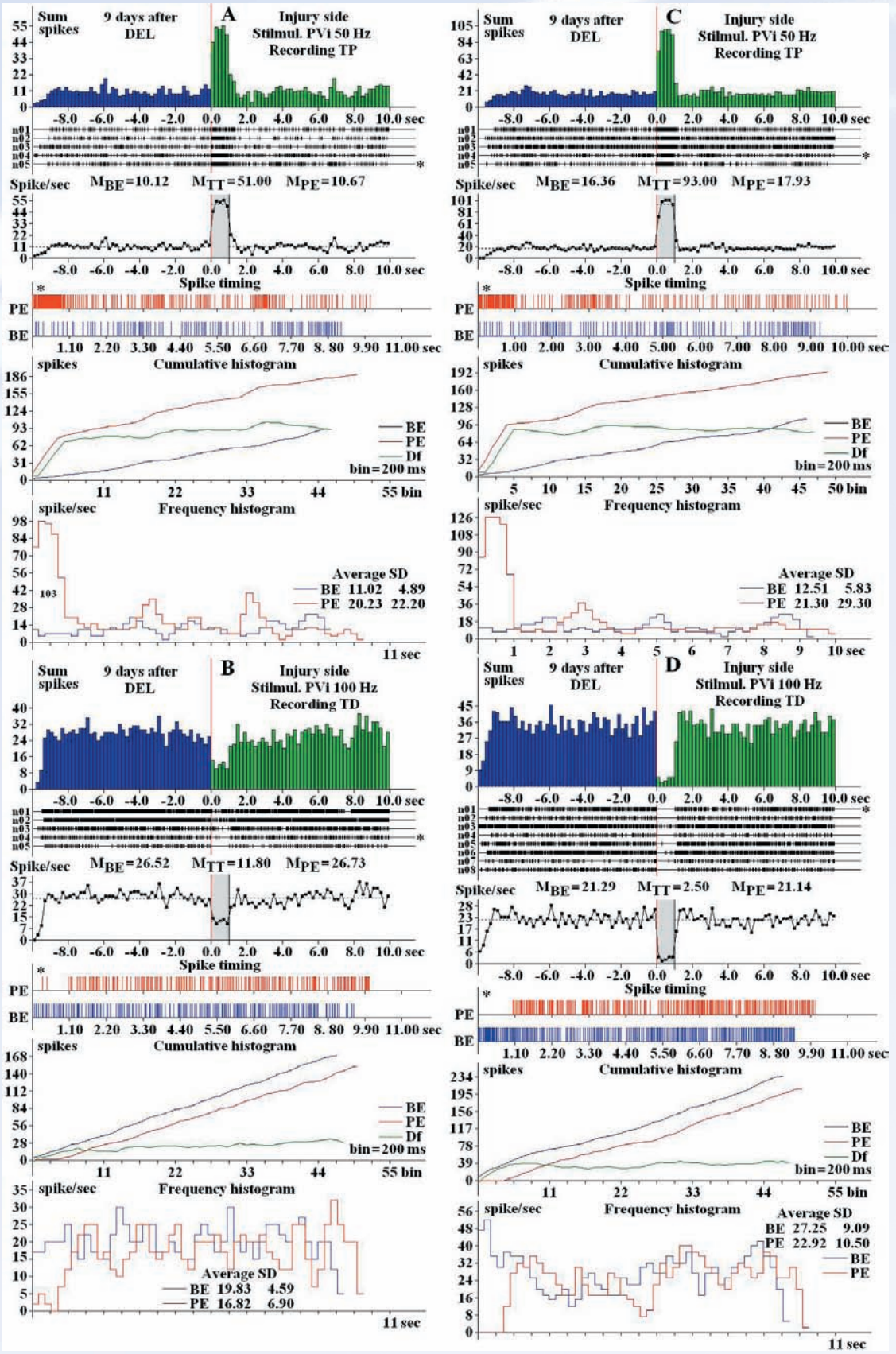


Figure 6. A-D – *peristimulus histograms of sum spikes (from above), constructed on the basis of raster of pre- and poststimulus excitatory – TP (A, C), depressor – TD (B, D) effects and manifestations of spike activity of LVN single neurons under HFS 50 (A, B)) and 100 (C, D) Hz (during 1 sec) PVi at the 9th day after UL with the use of NOX; below – diagrams of frequency of spikes, presented in raster, with average meanings and detailed analysis (Spike timing, Cumulative and Frequency histograms) of occasionally selected single neurons (shown by* in raster).*

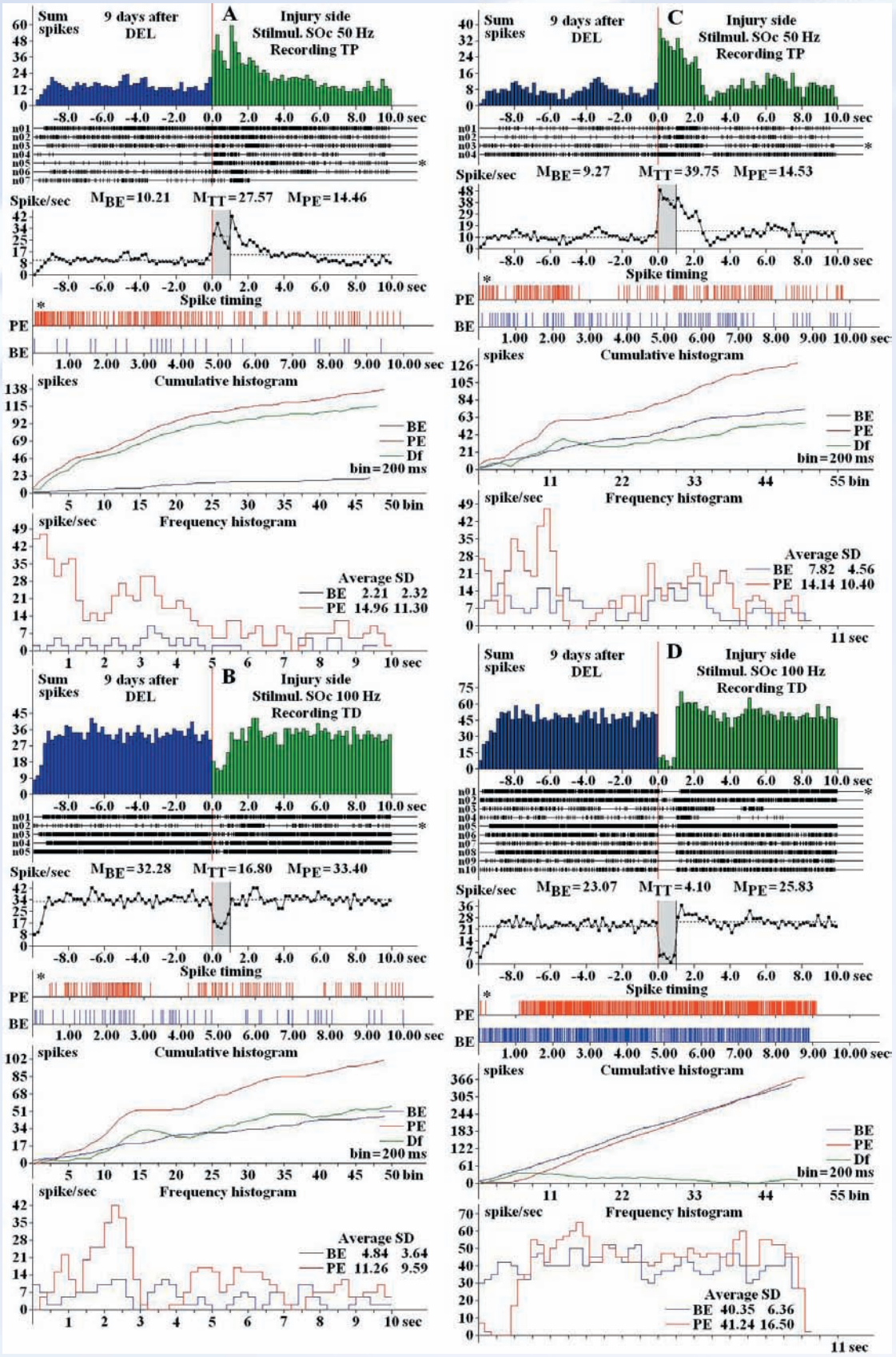
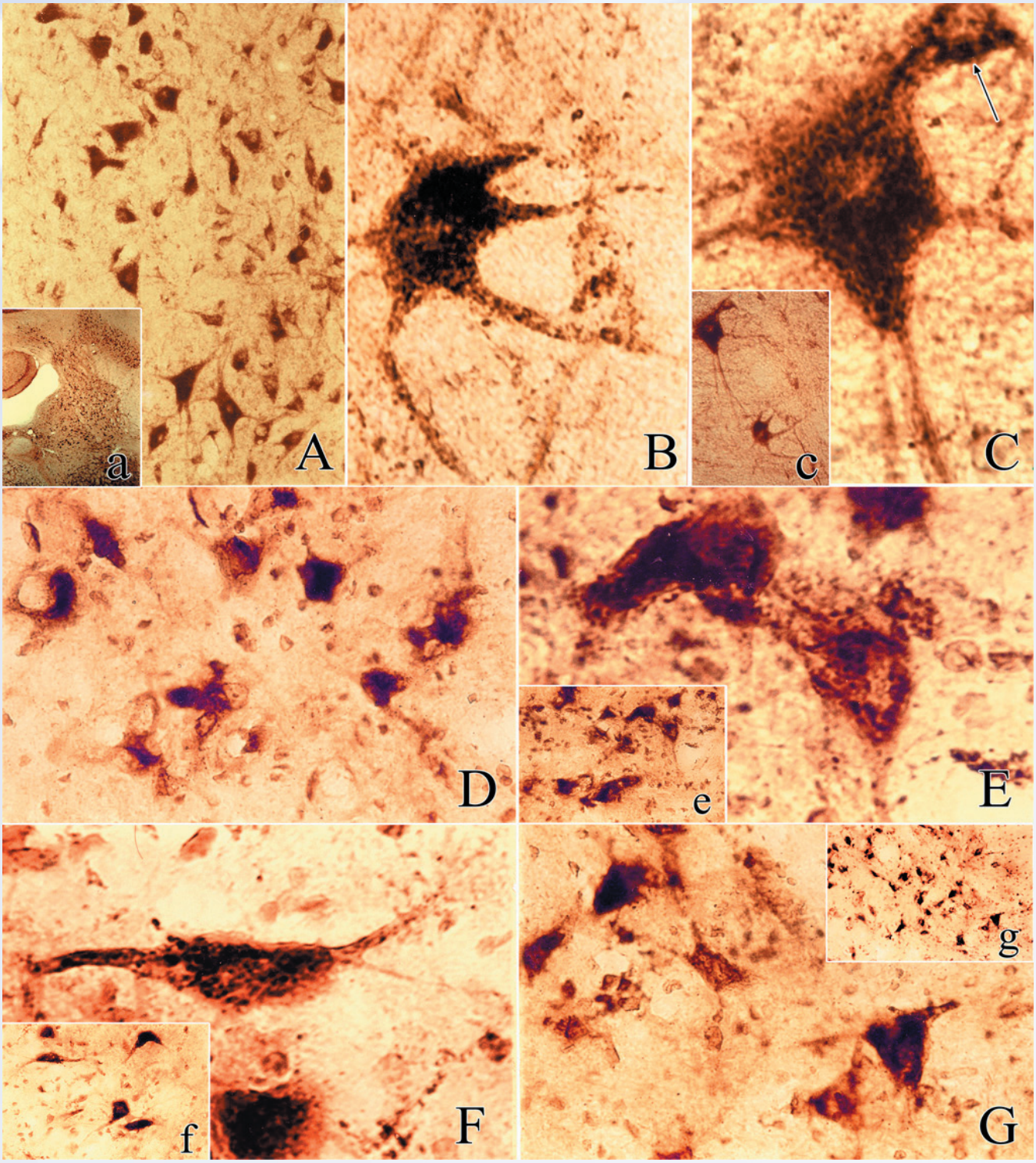
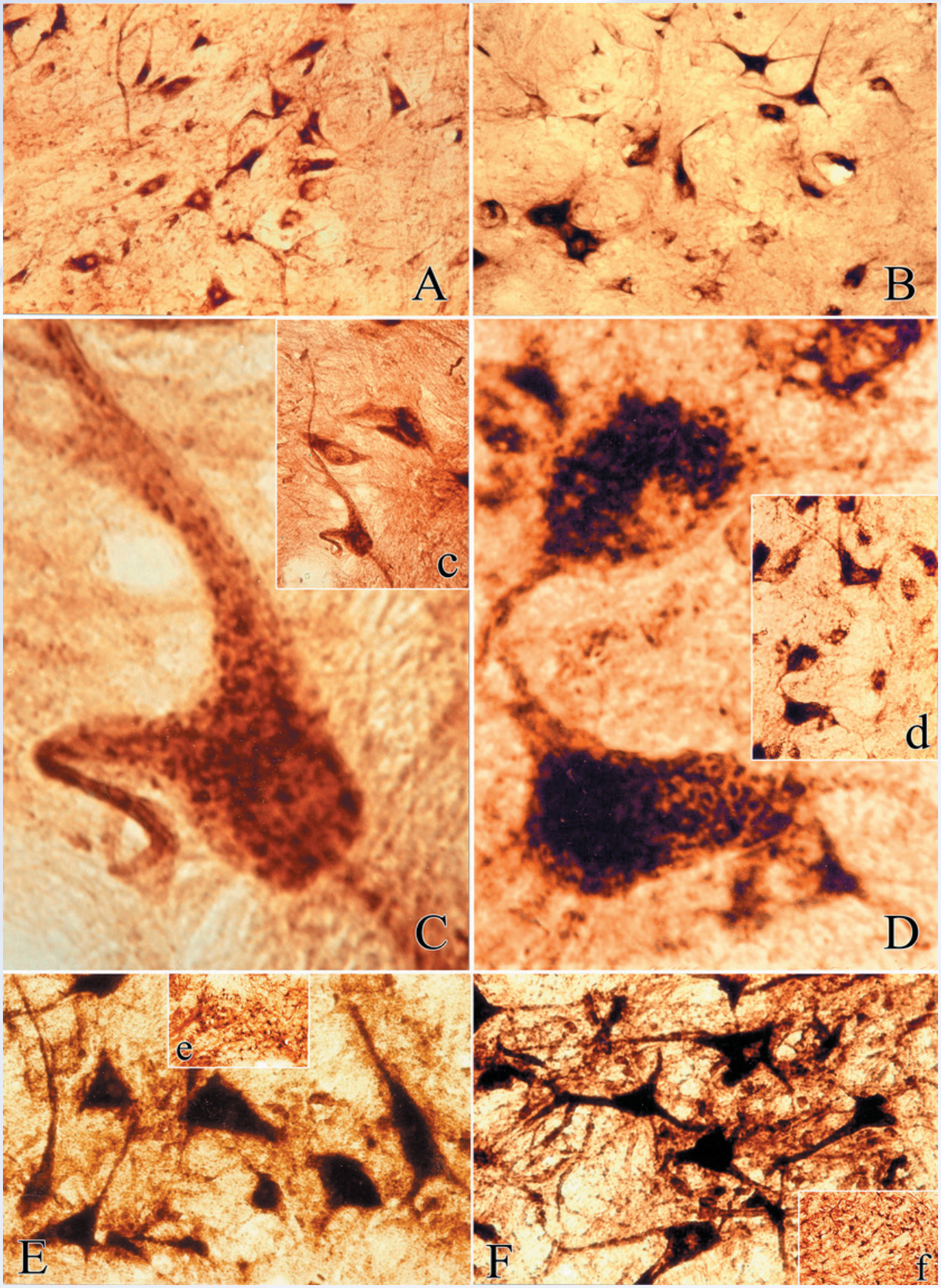


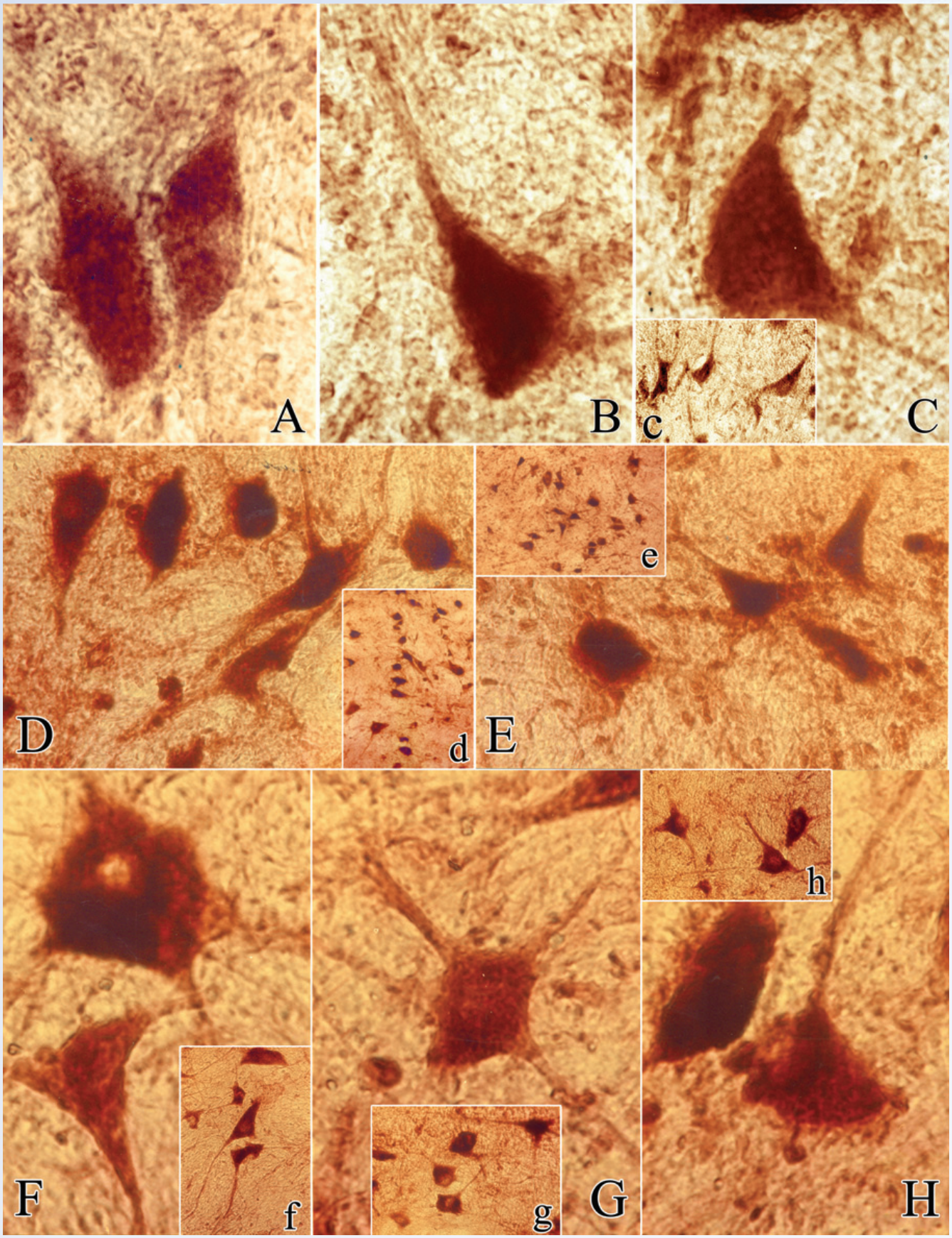
Figure 7. A-D – *peristimulus histograms of sum spikes (from above), constructed on the basis of raster of pre- and post-stimulus excitatory – TP (A, C), depressor – TD (B, D) effects and manifestations of spike activity of LVN single neurons under HFS 50 (A, B) and 100 (C, D) Hz (during 1 sec) SOc at the 9th day after UL with use of NOX; below – diagrams of frequency of spikes, presented in raster, with average meanings and detailed analysis (Spike timing, Cumulative and Frequency histograms) of occasionally selected single neurons (shown by* in raster).*



Picture 1. *Intact rats brain LVN neurons (A-C) and those at the 7th day after UL (D-G); C- small cell (black arrow), closely adjoining with process of the large cell. D, E, G – injured side (reaction of glial cells, shortening of cell processes); F – uninjured side. Magnification: 25 (a); 160 (A, g); 400 (c, D, e, f, G); 1000 (B, C, E, F).*



Picture 2. *Deiters' neurons at the 3rd (A-D) and 9th day after UL with administration of PRP-1 (E, F); A, C – injured side (thickening of processes, moderate ferment activity and minor swelling of the cells nuclei, detection of fine blood vessels); B, D – uninjured side (close convergence of neurons, ectopia of the nucleus, high AP activity); E – injured side; F – uninjured side. Magnification: 100 (e, f); 160 (A, B); 400 (c, d, E, F); 1000 (C, D).*



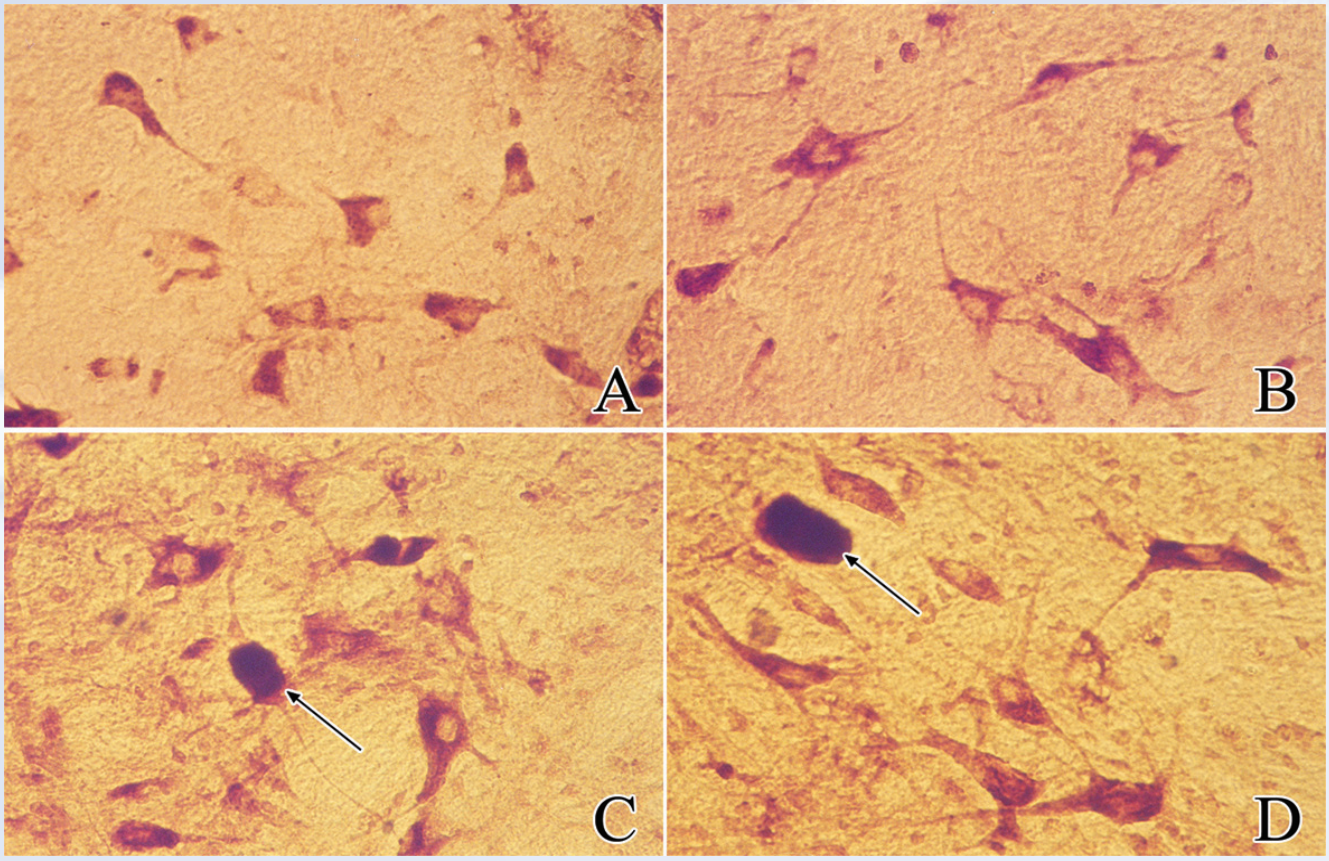
Picture 3. *Deiters' neurons at the 17th (A-C) (equalizing of the ferment activity of ipsi- and contralateral nuclei) and the 19th day after UL with administration of PRP-1(D-H) (misbalance of the ferment activity of ipsi- and contralateral nuclei); A, B, F, G, H – injured side; C, D, E – uninjured side. Magnification: 160 (d, e); 400 (c, D, E, f, g, h); 1000 (A, B, C, F, G, H).*

be found very often. At the injured side there were isolated and closely contacting neurons (Picture 3 A); the reaction of the processes was preserved (Picture 3 B). However, unlike the LVN neurons of intact rats there was no granule suspend but homogeneous staining was recorded. From the 19th day a misbalance in ferment activity of ipsi- and contralateral Deiters' neurons was observed, in particular, in LVN neurons of intact side an abrupt intensification of AP occurred (Picture 3 D, E), whereas on injured side neurons preserved the 17th day morphological pattern (Picture 3 F-H). In other words, on intact side PRP-1 led to intensification of phosphorylation, which is more typical for neurons surviving deep stress [*Humar M. et al., 2007*].

On the injured side under NOX effect at the 3rd day after UL the morphological pattern reminded a slight form of nerve cells acute swelling (Picture 4 A, B), which regards to rather accepted form of cellular pathology. Different pathological effects of exo- and endogen origin might be the reason of this process. The shape of neurons from the view of their structure was the morphological argument of their metabolism disorders. Probably, due to development of the pericaryon metabolic events, the cells breathing process was disturbed and there took place the decrease of AP activity in the neurons suspends granules distributed irregularly. In some cells granular suspend was intensively gathered around the nuclear membrane, in some others – in the site of process output and even over the periphery of the cell. In other parts of the cells suspend had dust-like character. Nuclei of the cells being in the stage of swelling also look swollen, surrounded by dark ring of suspend granules. However, the important criterion for evaluation of cell lesion degree was the degree of the nucleus lesion. Our data indicated that basically nuclei of the neurons had central location or intended to keep vital activity by means of displacement to the periphery of the cell. The nuclei of satellite glia were absent. Similar pattern was recorded on uninjured side, though ferment activity was somewhat higher and there were isolated dark coloured shapeless formations typical for hyper phosphorylated condition of stressed neurons (Picture 4 C, D).

At the 9th day after UL with NOX injection on injured side, an abrupt gain of AP activity was recorded. Basically, nuclei of the neurons might not be revealed and the edge between nucleus and cytoplasm was impossible to transact (Picture 5 A-G). Due to the gain of ferment activity a distinct granulation of suspend could not be pursued and neurons looked like more homogeneously stained. Granules were also seen along the edges of short processes, on the cellular membrane and even by surface tangent. This process evoked the dissimilation in the interstitials (Picture 5 C, D). Probably, appearance of paths around the pericaryons and processes and granulation restricted the focus of membrane over excitation and promote the preservation of its structure [*Bicov V. et al., 2006*]. At uninjured side there existed neuroglial reaction in form of the proliferate process (Picture 5 H-J). Neuroglia was extremely sensitive to lesions of nerve fiber and its response to pathogenic stimuli was stereotypic. However, in case of UL with NOX administration there was no tendency of neuroglial cells to progressive forms of mitosis and regressive changes. In cytoplasm of the large cells the activity of AP was expressed moderately. The cell processes could be pursued at long distance. LVN neuron had oval shape and seemed to be swelled. Ferment activity was intensified (Picture 5 I). LVN neurons on uninjured side morphologically had the same pattern with those of intact rats' brain but in most cells high ferment activity was registered in that half of the cell body, where the nucleus was situated (Picture 5 H). Data obtained testified to the beginning of proliferation process as one of the four basic stress-responsible transcriptional regulators of the FOS that is different from others [*Head M. et al., 1996*].

At the 15th day after UL on injured side there was morphologically recorded decreasing of phosphorylation degree. The pattern and sizes of the cells was recovered, in most of them processes began to respond, but they were basically thickened. In all the neurons a few swelled and light nuclei with central localization were clearly distinguished. Granular suspend was in the cytoplasm and processes and ferment activity was intensified (Picture 6 A-B). LVN neurons on intact side (Picture 6 C, D) morphologically looked

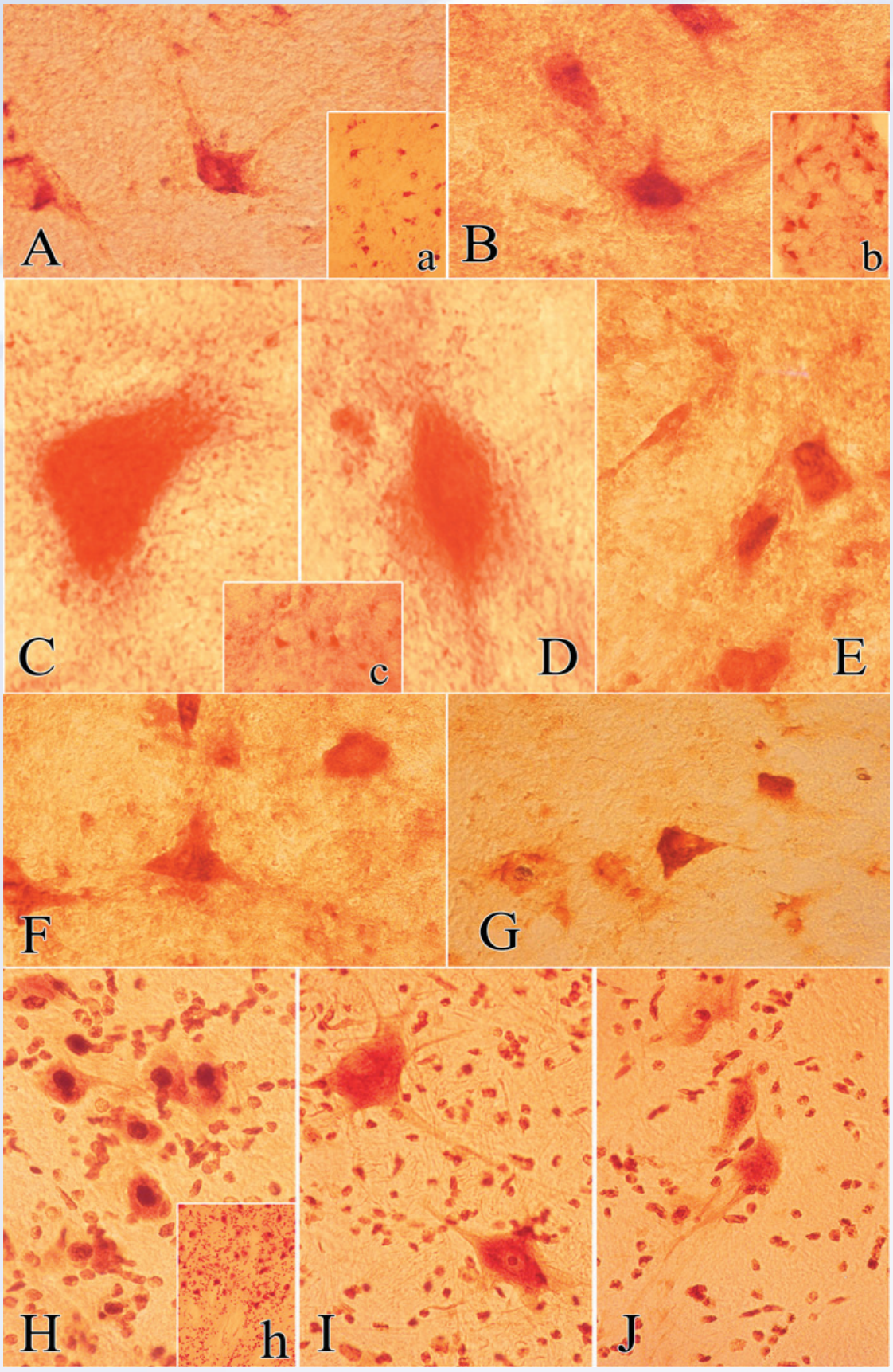


Picture 4. *Deiters' neurons at the 3rd day after UL with administration of NOX; A, B – injured side (swelling of neurons and nuclei, decreasing of ferment activity); C, D – uninjured side, where among neurons with swelled nuclei and weak AP activity there can be seen dark hyper phosphorylated formations (arrows). Magnification: 400 (A - D).*

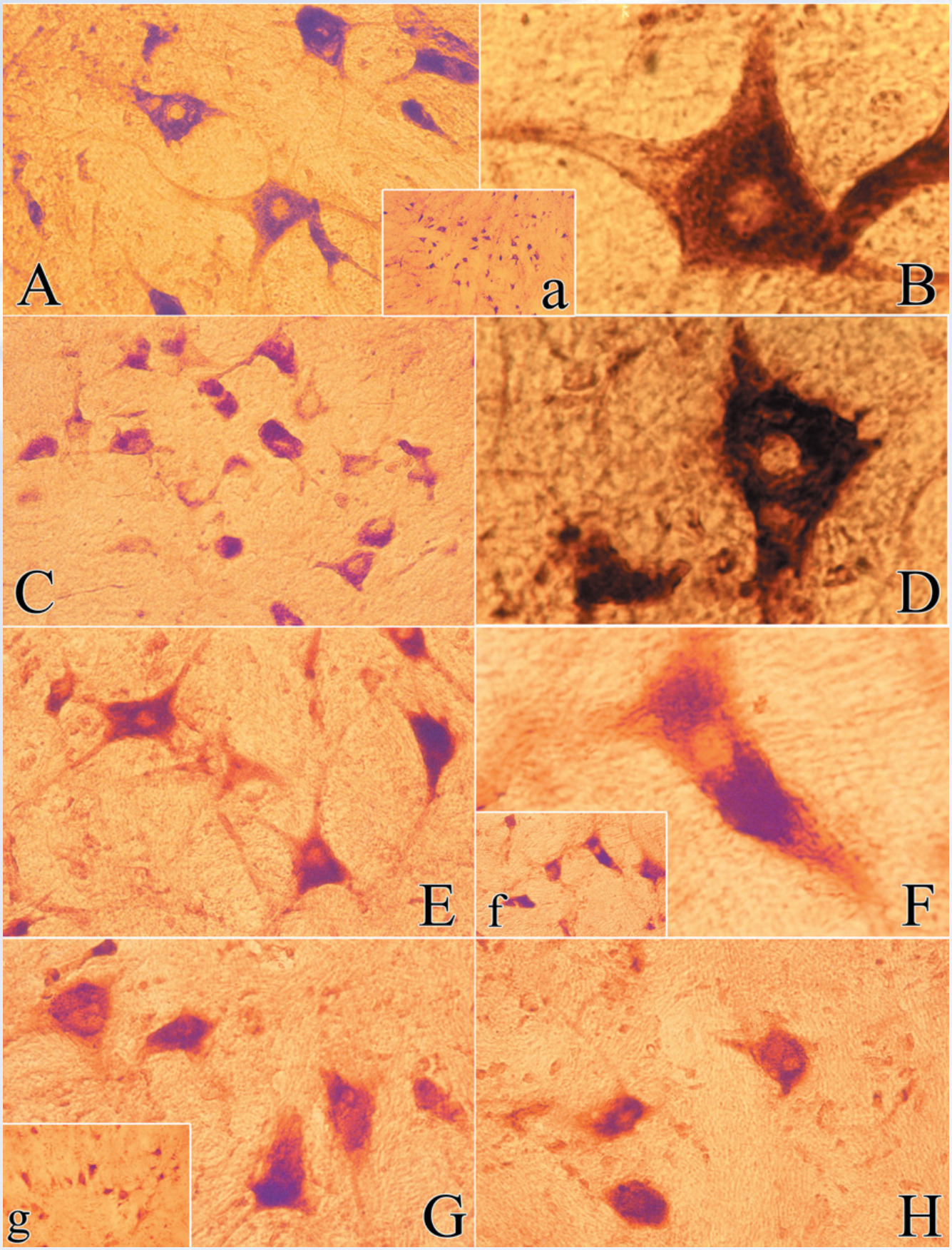
almost like those of intact rats (Picture 6 A, B). At the 35th day after UL on the injured side a gain of ferment activity in the cell cytoplasm and processes was recorded. Light nuclei of the cells were not swelled yet and localized centrally (Picture 6 E). As a result of active condition in the cytoplasm of some large cells the suspend granules of lead phosphate existed, but these granules were longer and thinner (Picture 6 F). Such Hamori-positive thread-like formations morphologically looked like flexuous neural fibers and their appearance might be probably considered as adaptive response, delaying more progressive neural fiber jumble. In comparison with intact rats at the 35th day after UL a significant reduction in LVN neurons localization density was observed. Possibly, as a result of deep metabolic disorders in nerve cells and intercellular fiber the accumulated products of catabolism were followed by cell demolishing. Probably, cells which cannot be recovered continue keeping the processes of regeneration for the long period, finally changing to degeneration. In cell death a considerable role is performed by physiologically "battered" neurons, in which changes of the metabolic processes with reduction of oxidative ability and enzymatic functions were already outlined. On the intact side, probably under the effect of NOX primary stimulated neurons are in the condition of hyperactivity in struggle with causal agents for a long time (Picture 6 G, H)

DISCUSSION

Results of the present study indicate to presence of excitatory and inhibitory post stimulus development of LVN neurons activity to stimulation of PVN and SON with different sequence, intensity, duration, and latency of onset. The fact of permanent existence in post stimulus activity of LVN neurons depressive effects or TD, as well as early and late PTP is of interest. Moreover, depressive effects and particularly tetanic ones can



Picture 5. *Deiters' neurons at the 9th day after UL with administration of NOX; A-G – injured side (abrupt intensification of AP activity, granulation around the cells bodies); H-I - uninjured side (proliferation of the neuroglial nuclei). Magnification: 160 (a, b, i); 400 (A, B, c, E, F, G, H, I, J); 1000 (C).*



Picture 6. *Deiters' neurons at the 15th (A-D) and 35th (E-H) days after UL and NOX administration; A, B, E, F – injured side (recovery of neurons' shape and sizes, gain of ferment activity); C, D, G, H – uninjured side. Magnification: 160 (a, g); 400 (A, C, E, f, G, H); 1000 (B, D, F).*

be of quite different origin: real inhibition or even disfacilitation. Earlier in a series of intracellular experiments it has been shown that stimulation of numerous brain structures (cerebellum, spinal trigeminal nucleus, lateral reticular nucleus, inferior olive, posterior hypothalamus, etc.) evoked IPSP in Deiters' neurons [*Sarkisian V., 2000*]. Moreover, LVN being permanently under the inhibitory action of different brain structures preventing predominance of the extensor tonus is in continuous default of excitatory input.

Stimulation of PVN and SON produced inhibitory and excitatory reactions in Deiters' neurons, which are important for prevention of possible intensification of vestibular tonus together with exclusion of flexor-extensor imbalance supervising by motor centers of the brain. This study represents data on characteristics of deafferented Deiters' neurons after treatment by PRP-1 and cobra venom NOX. First, the very fact should be mentioned that even in case of NOX venom action, again the protective effect was realized through PRP-1. This was testified by means of given venom after hemisection of SC [*Abrahamyan S. et al., 2007*], as well as the fact of existence of PRP-1-positive cells almost in all brain stem neuronal groups, including vestibular nuclei [*Abrahamyan S. et al., 2003*].

Of interest is the fact that in some systems during development of nervous system GABA acts as trophic factor influencing various events including proliferation, migration, differentiation, maturing of the synapse, cellular death, and expression of $GABA_A$ receptor [*Owens D., Kriegstein A., 2002*]. According to recent data, it is assumed that GABA and glycine may play a significant and/or perhaps varying role in developing and mature central vestibular system [*Tighilet B., Lacour M., 2001*]. Moreover, the plasticity of $GABA_A$ systems during ageing focuced on VC with necessity of pharmacological intervention [*Giardino L. et al., 2002*]. Therewith it was shown that GABA and glutamate mediate rapid neurotransmission from the suprachiasmatic nucleus to NPV in rat [*Hermes M. et al., 1996*]. Recently there was presented a new regulatory mechanism in fast CNS synapses involving L-type voltage-depended Ca^{2+} channels, which do not participate in low frequency release of transmitter but have contribution to accumulation of presynaptic Ca^{2+} during high frequency activity. This promotes the release of vesicles during tetanic stimulation, as well as intensifies the probability of transmitter releasing in post-tetanic period being completed by PTP manifestation. The results were obtained during registration of GABA-ergic IPSP from hippocampus cell culture in conditions of applying baclofen as an inhibitor of N and P/Q type voltage-depended Ca^{2+} channels leading to triple amplification of PTP. Moreover, in the basis of PTP there is accumulation of Ca^{2+} in presynaptic terminal in the period of GABA-ergic neuron tetanic stimulation, which leads to intensification of vesicles release up to 1 min [*Jensen K. et al., 1999*]. Finally, there was demonstrated the modulatory action of histaminergic system on neurotransmission in vestibular nuclei and changes in its plasticity course, particularly through inhibition of GABA releasing both by means of direct influence on presynaptic H(3) receptors (probably localized on GABA-ergic terminals) and through indirect path, involving increased releasing of glycine by activation of post-synaptic H(1 and 2) receptors (probably on glycine-ergic neurons) [*Bergquist F. et al., 2006*]. In other words, the involvement of real GABA-ergic inhibition during TD may not be excluded.

In histochemical studies following UL in combination with PRP-1 Deiters' neurons still existed at the 3rd day and looked like those of intact rats. At the 9th day after UL a dramatic intensification of AP activity occurred. Moreover, at ipsi- and contralateral sides the activity of AP was almost the same. In other words, there was balancing of ferment activity. The shape of changes at the 17-19th day was characterized by reduction of ferment activity on intact side. On the injured side there were found neurons with normal morphology and cells with hyperchromatism of nuclei indicating to hyper-phosphorylation. In comparison with the intact side a decrease of neurons was registered, however the morpho- and histological pattern of the latter allowed to assume their survival, thus indicating to successful protective effect of PRP-1.

In whole, morphological and histochemical data indicate that PRP-1 inhibits progressing central chromatolysis of LVN neurons in UL animals

which leads finally to deep neurodegenerative morphological pattern such as cellular shade up to its total disappearance. As a result, the ferment activity remains during 19 days after UL and PRP-1 exerts more favorable influence to LVN neurons of the injured side. On the intact side PRP-1 leads to intensification of phosphorylation, which is more typical for neurons surviving deep stress [*Humar M. et al., 2007*].

The morphological and histochemical data with application of NOX give evidence to assume that in some large neurons there takes place a delay of deep metabolic disorders resulting in total degeneration. The morphological and histochemical picture indicates that under the NOX action a hyper activation is observed. However, it might be also assumed that abrupt intensification of ferment activity probably promotes the purification of affected nerve tissue and tendency to adaptation. Thus, the protective effect of NOX venom after UL is obvious. However, effects are quite expressive and frequently exceed the norm level, which indicates their abnormal expression. Without diminishing the significance of NOX venom of exogenous nature, one should be convinced of natural and successful effect of PRP-1: a true endogenous biological modulator. In this work we tried to make our contribution to the problem of VC promotion.

Recently, several studies have highlighted astrocytes importance in many functions such as neurotransmission, metabolism and electrolyte homeostasis, cell signaling, inflammation, and synapse modulation. Moreover, increasing evidence is stressing the emerging role of astrocyte dysfunction in the pathophysiology of neurological disorders, including neurodegenerative diseases. For this reason, a possible goal for neuroscience researchers could be a deeper understanding of altered astrocyte–neuron interaction in order to develop therapeutic strategies able to modify the activity of glial cells, reduce their neurotoxic effects, and enhance their neuroprotective action [*Ricci G. et al., 2009*]. Finally, in the last years the neurogenesis was discovered, which is a normal phenomenon in the adult brain, and is accentuated by brain injury, but the mechanism responsible for this effect is unknown [*Greenberg D., Jin K., 2006*]. Despite stimulation of neurogenesis by an acute injury, it is not sufficient for function restoration. However, therapeutic stimulation of neurogenesis as a facility to treat neurodegeneration is still generally speculative and might contribute to functional "repair" of the adult diseased brain [*Galvan V., Bredesen D., 2007*]. In conclusion a wide spectrum and obvious effectiveness of the used protector PRP-1 does not exclude the possibility of neurogenesis promotion. The analogous effect may be proposed for NOX venom as well.

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