The action of cobra venom on the vestibular compensation and its protective effect after administration of GABA in conditions of unilateral labyrinthectomy

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Abstract

Background & Aims: It is known that unilateral labyrinthectomy (UL) causes a syndrome of oculomotor, postural and autonomic system 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 associate with recovery of vestibular nucleus activity on the injured side. However its central mechanisms are not completely uncovered yet. In the unilaterally labyrinthectomized albino rats the effect of cobra venom on activity of lateral vestibular neurons and its protective action in combination with GABA was studied.

Materials and methods: Experiments were carried out on six groups of rats: normal, unilaterally labyrinthectomized (UL), UL with administration of Naja Naja Oxiana (NOX) venom (5% LD50, 21μg/kg, im), normal with combination of GABA (2 mg/kg, im), UL with combination of GABA and UL with administration of NOX venom and GABA injection. Electrophysiological patterns of lateral vestibular nucleus neurons at 9th day after the UL in response to stimulation of paraventricular and supraoptic nuclei of hypothalamus in all groups were studied.

Results: The increasing of inhibitory and excitatory reactions of Deiters' neurons at early stage of vestibular compensation following NOX injection and reaching the norm at late stage was revealed. Administration of GABA resulted in depression of activity in normal and UL groups.

Conclusion: The cobra venom not only causes acceleration of vestibular compensation but also prevents the depressive effects of GABA on Deiters' neurons.

Keywords: Unilateral Labyrinthectomy, Vestibular Compensation, Gamma Amino Butyric Acid (Gaba), Deiters' Neurons, Paraventricular Nucleus Of Hypothalamus, Supraoptic Nucleus Of Hypothalamus, Naja Naja Oxiana (NOX) venom

Received 17 May 2016, accepted for publication 25 July 2016

Introduction

It is known that vestibular compensation (VC) after peripheral vestibular damage in terms of deprivation of afferent inputs on the example of unilateral labyrinthectomy (UL) is a process of behavioral recovery of visual, motor and postural reflexes. The biochemical and molecular mechanisms that mediate the VC still have not been adequately studied (1). It is of interest the new physiological data on mechanisms of VC after the UL. In old systems, including vestibular, when restoring the function after injury certain aspects of cellular development and plasticity may be reproduced, in particular, the study of membrane own ("intrinsic") properties of vestibular neurons (VN) of vertebrates (2). In addition, the vestibular system is favorable for determining the early steps in the appearance of action potentials and synaptic transmission in the vestibular reflex paths. It was studied their spontaneous synaptic activity during development and recovery of electrical excitability after injury to the

peripheral vestibular organs (3). In turn, the central vestibular neurons play an important role in the transformation of multisensory signals of body to the motor commands to control visual orientation and posture. After the UL on the injured side neuronal activity actually disappears, which after a week is completely restores. (4). As one of the important factors in the recovery of spontaneous pacemaker activity on the injured side, almost 3-fold increase in low-threshold calcium current was discovered (4). In many studies after UL the synaptic and/or neurochemical changes in the vestibular nuclei, as well as related central structures during vestibular compensation were observed (5, 6, 13, 14, 26, 32). The neurochemical study of molecular mechanisms of neural and synaptic plasticity in vestibular neurons, involved in the VC after the UL revealed that its development reduces the imbalance in the levels of release of various amino acids (aspartate, glutamate, glycine, taurine, alanine), except glutamine (6). Gamma – amino butyric acid (GABA) is the main inhibitory neurotransmitter in the vertebrate brain (7). It has been known that this major inhibitory chemical neurotransmitter (8) and amino acid neurotransmitter is distributed widely throughout the mammalian central nervous system (9). Electrophysiological, pharmacological and biochemical data indicate that GABA is mainly involved in the functional recovery after acute injury of the vestibular labyrinth (10). Recent studies have suggested that after unilateral labyrinthectomy, there have been obvious changes in the functional efficacy of GABA receptor on the deafferented medial vestibular nucleus (MVN) neurons (gain of depression) (11).

In brain slices, suppression of cell firing due to $GABA_B$ activation is reduced on the damaged side, a few hours following hemilabyrinthectomy and this reduction is believed contribute to static compensation (12). Following unilateral vestibular damage, GABA levels become asymmetric (13). Metabotropic GABA type B ($GABA_B$) receptors are present in all subdivisions of the vestibular nuclei (14) and have been proposed to contribute to compensation (15).

During last decade biomedical research in which venom components are being investigated for their potential as novel therapeutic agents has emerged as an interesting option (16, 17). Snake venoms contain a large amount of proteins and peptides that exert their biological effects by affecting neural system, cardiovascular system, blood coagulation system (18), chemotherapy for membrane fusion and selective killing of certain type of tumor cells (19), antinociceptive (20), and delivery system (21). Venom has been used in the treatment of a variety of pathophysiological condition in Ayurveda, homeopathy and folk medicine and empirical observation. With the advent of biotechnology, the efficacy of such treatment has been substantiated by purifying components of venom and delineating their therapeutic properties (22, 23, 20).

In our previous study, we used a mix of some neurotransmitters and assessed their effects to each other (24). The main purpose of this work was the study of protective effect of cobra venom Naja Naja Oxiana on the VC and comparative analysis of the significance of inhibitory action of GABA in normal, UL and UL with combination of NOX groups.

Materials and Methods

All the procedures utilizing rats were performed according to the “principles of laboratory animal care” (NIH publication № 85-23 revised 1985), as well as the specific rules provided by the animal care and use committee of national medical and health service. The experiments were carried out on adult, male Albino rats (230 ± 30 g). Animals were kept on standard laboratory house in a 12 light/ dark period (light on at 08:00 and off at 20:00) with temperature 20- 22°C, and supplied with dry rats food and drinking water ad libitum.

Twenty four adult male albino rats were randomly divided into six groups: normal group; without unilaterally labyrinthectomized (UL), GABA and NOX injection, UL group, UL with administration of NOX

venom (5% LD_{50}, 21 μg/kg or 0.12ml, im, three days successively. The LD_{50} of NOX was 430 μg/kg), normal with combination of GABA(2mg/kg, im) during experiment, UL with combination of GABA(2mg/k,, im) during experiment, and UL with administration of NOX venom and GABA injection group. Labyrinthectomy was performed under the anesthesia with intra peritoneal injection of pentobarbiton (40mg/k). On the anaesthetized animals, unilateral labyrinthectomy was performed by electro coagulation apparatus that composed two electrodes (cathode and anode), the cathode was inserted into inner ear and the anode was connected to skin with 20 m AM voltage for 1 minute (25).

The NOX venom was provided by Laboratory of purification, certification and standardization of physiologically active substances of L.Orbeli Inst. of Physiology, NAS, RA and GABA was provided from Sigma-Aldrich Co. LLC.

The venom (1mg) was dissolved in 8 ml of normal saline 0.9% and used for three days. In the NOX groups, snake venom was injected 24 h after UL. Then electrophysiological experiment was carried out at 9^{th} day after UL.

In the GABA groups, gamma amino butyric acid was injected (2mg/kg, im) first minute of during electrophysiological recording and also the GABA was repeated at 30 minute after the first injection.

In electrophysiological studies on Albino rat's extracellular recording of Deiters' lateral vestibular nucleus (LVN) single neurons spike activity to high-frequency stimulation (HFS, 100 Hz) of paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus were performed.

In acute experiments animals were immobilized by 1% dithylinum (25 mg/kg, ip) and local anesthetic by 5% Novocain (2ml SC place of section) under artificial ventilation the section of SC at T_1-T_3 level (with ultrasound scalpel) was carried out to achieve *encephale isole* preparation. In stereotaxic apparatus the trepanation of the skull was realized from *bregma* till *lambda* and *dura mater* was removed. Stereotaxic orientated glass electrodes of 1-2 μM tip diameter were filled with 2M 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 100 Hz during 1s). Stimulating and recording electrodes were inserted according to stereotaxic coordinates of the rat atlas (26): SON (AP – 1.3, L ±1.8, DV+ 9.4 *mm*); PVN (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). At the end of experiments the localization of stimulating and recording electrodes were verified histologically.

In the first stage, 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 realized 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. For selected comparable groups of neuronal spiking the similar complex averaged were constructed. On average, during each record up to 10-15 post stimulus trials were carried out. This special mathematical program (developer: V.S. Kamenetski) 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 take into consideration strictly permanent tetanic effects in comparison with less stable posttetanic ones too. To determine the statistical significance of differences in duration of interspike intervals before and after the

stimulus used nonparametric criterion for testing homogeneity of two independent samples - Two Sample Wilcoxon - Mann-Whitney' criterion (Wilcoxon-Mann-Whitney test). Since the number of recorded spikes was large enough (up to several hundreds of spikes in 10 second interval after the stimulus), used the variety of this test, taking into account its asymptotic normality - z-test. Comparison of critical values with the tabulated values of the normal distribution at a significance level of 0.05, 0.01 and 0.001 (for different trials), shows that as a result of HFS for most samples of neuronal activity spiking has a statistically significant change with a minimum significance level of 0.05.

The data of second stage experiment on the basic of diagram was performed by Excel program.

Results

The study on impulse activity flow of single LVN neurons evoked by bilateral stimulation of PVN and SON was carried out in Albino rats in normal, UL and UL with administration of NOX, normal with use of GABA, UL with use of GABA and UL with administration of NOX and GABA. In this experiment 143 neurons of 24 rats were recorded.

In the normal group is presented histograms of the results of averaged electrical activity of LVN neurons, evoked by stimulation of PVN and SON with HFS (100 HZ) (fig.1). As a whole to bilateral stimulation of PVN and SON the excitatory, as well as inhibitory effects were revealed.

Stimulation of the ipsilatral paraventricular nucleus (PVN_i) and contralatral supraoptic nucleus (SON_c) (PV_i and SO_c, respectively) at frequency of 100Hz evoked post stimulus effects on peristimule histograms of sum spikes and diagram in the form of tetanic potentiation (TP- part A of PV_i, part C of SO_c) and depression (TD – part B of PV_i, part D of SO_c) which for almost three times exceeded the level of background activity in the case of TP (fig.1 A,C) and for five times lower than of background activity in the case of TD (fig.1 B,D) per second. Also there was symmetry between LVN neuronal activity of left and right sides (before and after excitation of LVN neurons).

In UL group at 9th day after the UL, TP and TD was the same as in the normal group. In this group, HFS (100 Hz) of PVN and SON during 1 second produced TP and TD in LVN neurons (fig2.A-D). Evoked TP and TD were more than 6 fold bigger compared to primary excitation (fig2. A-D) and the level of spikes were upper than the magnitude in normal group after stimulation (fig. 2. A, C). In comparison with the norm mean value of excitatory and inhibitory effects in LVN neurons indicate the activity asymmetry between left and right sides.

In UL with NOX group at 9th day after UL with combination of NOX venom (fig. 3) demonstrated the results of post stimulus excitatory TP (fig.3. A, C) and inhibitory TD (fig.3.B,D) changes of Dieters' nucleus neurons activity evoked by HFS (100 Hz) of hypothalamic PVN and SON. In this group compared with the norm TD were approached to the normal level. There was symmetry between left and right sides similar to the normal group (fig.3.A-D).

In rats the plastic reorganization of Dieters' nucleus neurons in the dynamics of changes after UL, HFS of hypothalamic nuclei, and systemic injection of GABA and use of snake venom NOX as a protector was studied (fig. 4-6).

In intact animals GABA suppressed activity of LVN neurons evoked by HFS of hypothalamic PVN and SON during tetanic time (fig.4 A and B). In the normal group (without UL, GABA and NOX injection), in LVN neurons there was only TD in response to stimulation of hypothalamic paraventricular and supraoptic nuclei (Fig.4. A, B) and activity of some Dieters' neurons was suppressed after stimulation (fig4. A.n02, B.n03, n04, n05).

The results of UL at 9th day after UL with combination of GABA demonstrated that GABA suppressed all

pre- and post stimulus activity of LVN neurons evoked by HFS (100Hz) of hypothalamic PVN and SON (fig.5 A and B).

At 9th day after UL with combination of NOX and systemic injection of GABA during experiment revealed that NOX has protective effect on LVN neurons evoked by HFS of PVN and SON (fig 6.A, B).

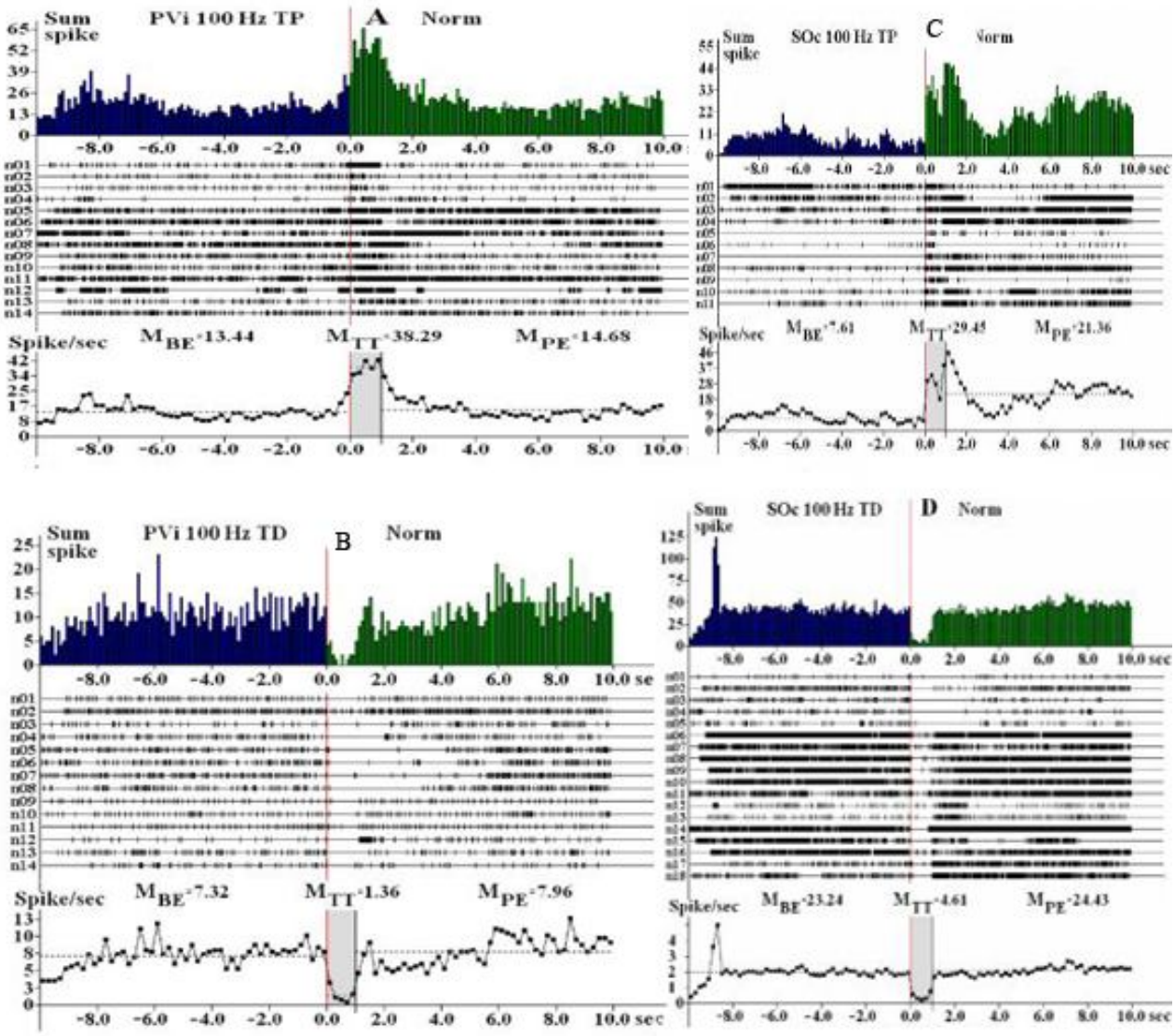

Fig.1. A-D- peristimule histograms of sum spikes (from above), constructed of raster pre - and post stimule excitatory – TP(A,C) and depressor – TD(B,D) effects and manifestations of spike activity of single neurons of LVN under HFS 100 Hz (during 1 sec) PVi (A,B) SOc (C,D) in the real time(10 sec before and after) Stimulation, at normal group.

in the bottom – diagram of neurons spikes summarized frequency, presented in the raster and in the real time, indicating average digital values of 10 sec before(M_{BE}) and 10 sec after (M_{PE}) stimulation and during 1 sec tetanic stimulation(M_{TT}).

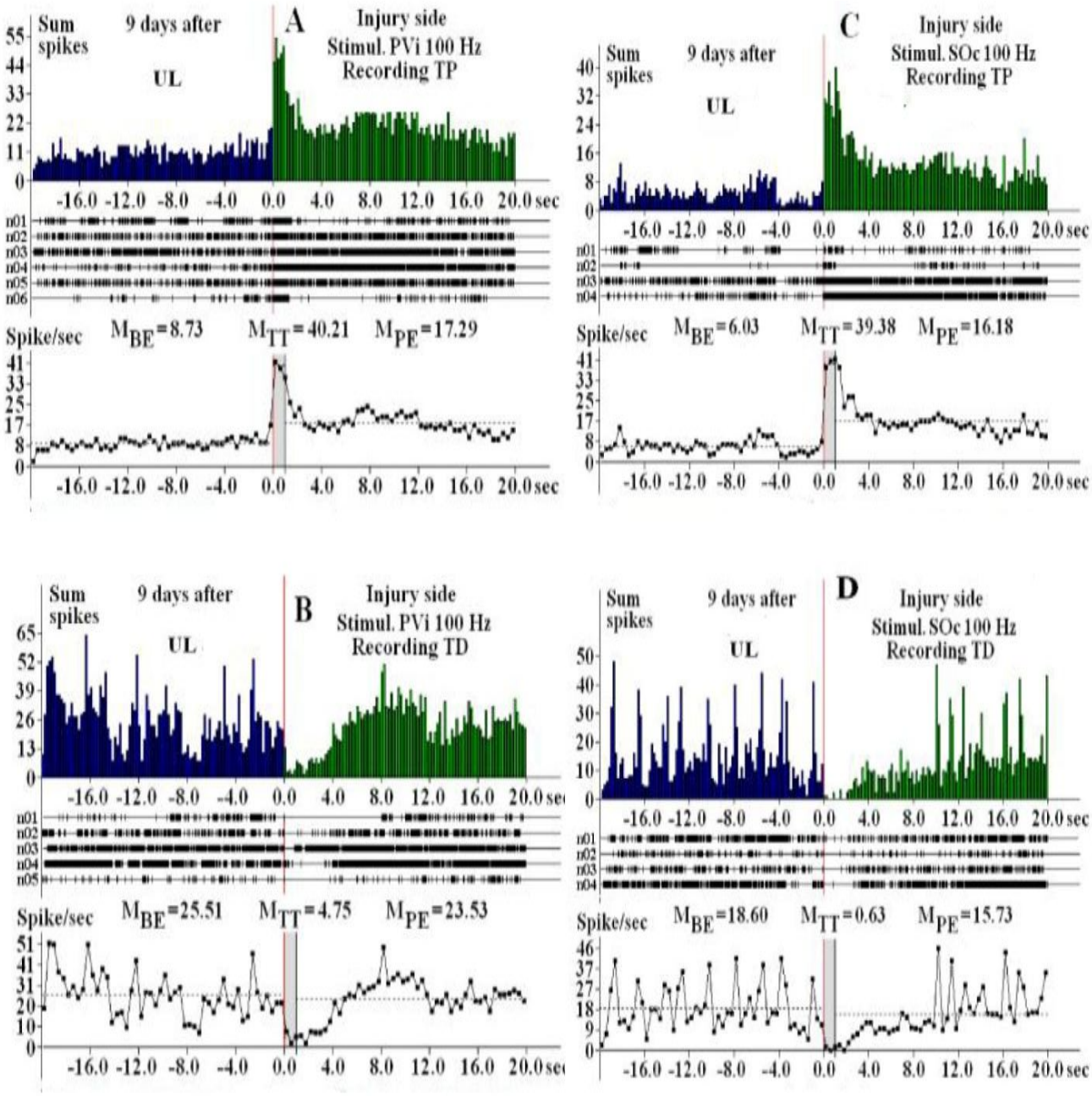

Fig.2. A-D- peristimule histograms of sum spikes (from above), constructed of raster pre - and post stimule excitatory – TP(A,C) and depressor – TD(B,D) effects and manifestations of spike activity of single neurons of LVN under HFS 100 Hz (during 1 sec) PVi (A,B) SOc (C,D) in the real time(20 sec before and after) Stimulation, at 9 days after UL.

in the bottom – diagram of neurons spikes summarized frequency, presented in the raster and in the real time, indicating average digital values of 20 sec before(M_{BE}) and 20 sec after (M_{PE}) stimulation and during 1 sec tetanic stimulation(M_{TT}).

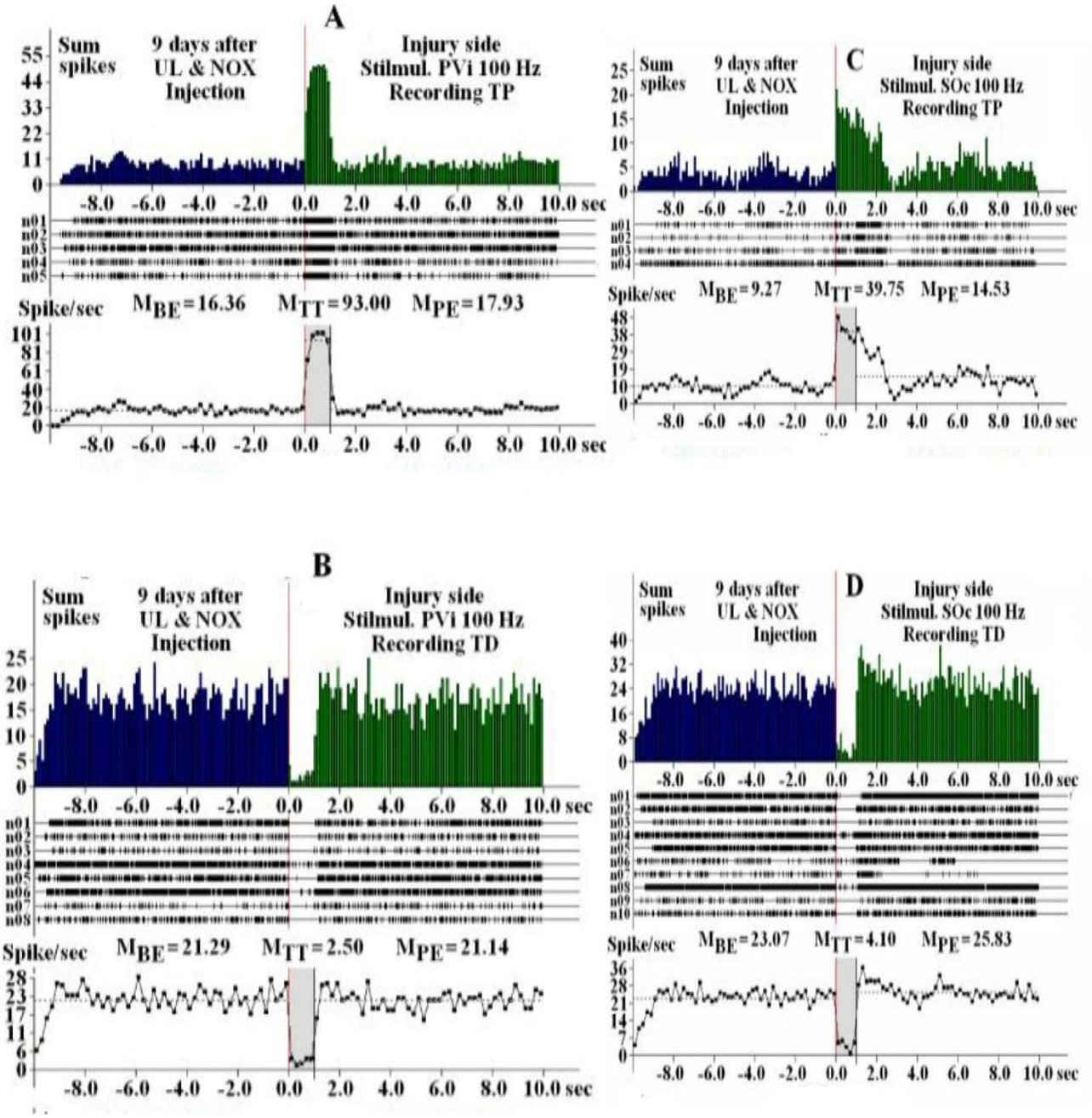

Fig.3. A-D- peristimule histograms of sum spikes (from above), constructed of raster pre - and post stimule excitatory – TP(A,C) and depressor – TD(B,D) effects and manifestations of spike activity of single neurons of LVN under HFS 100 Hz (during 1 sec) PVi (A,B) SOc (C,D) in the real time(10 sec before and after) Stimulation, at 9 days after UL with administration of NOX venom.

in the bottom – diagram of neurons spikes summarized frequency, presented in the raster and in the real time, indicating average digital values of 10 sec before(M_{BE}) and 10 sec after (M_{PE}) stimulation and during 1 sec tetanic stimulation(M_{TT}).

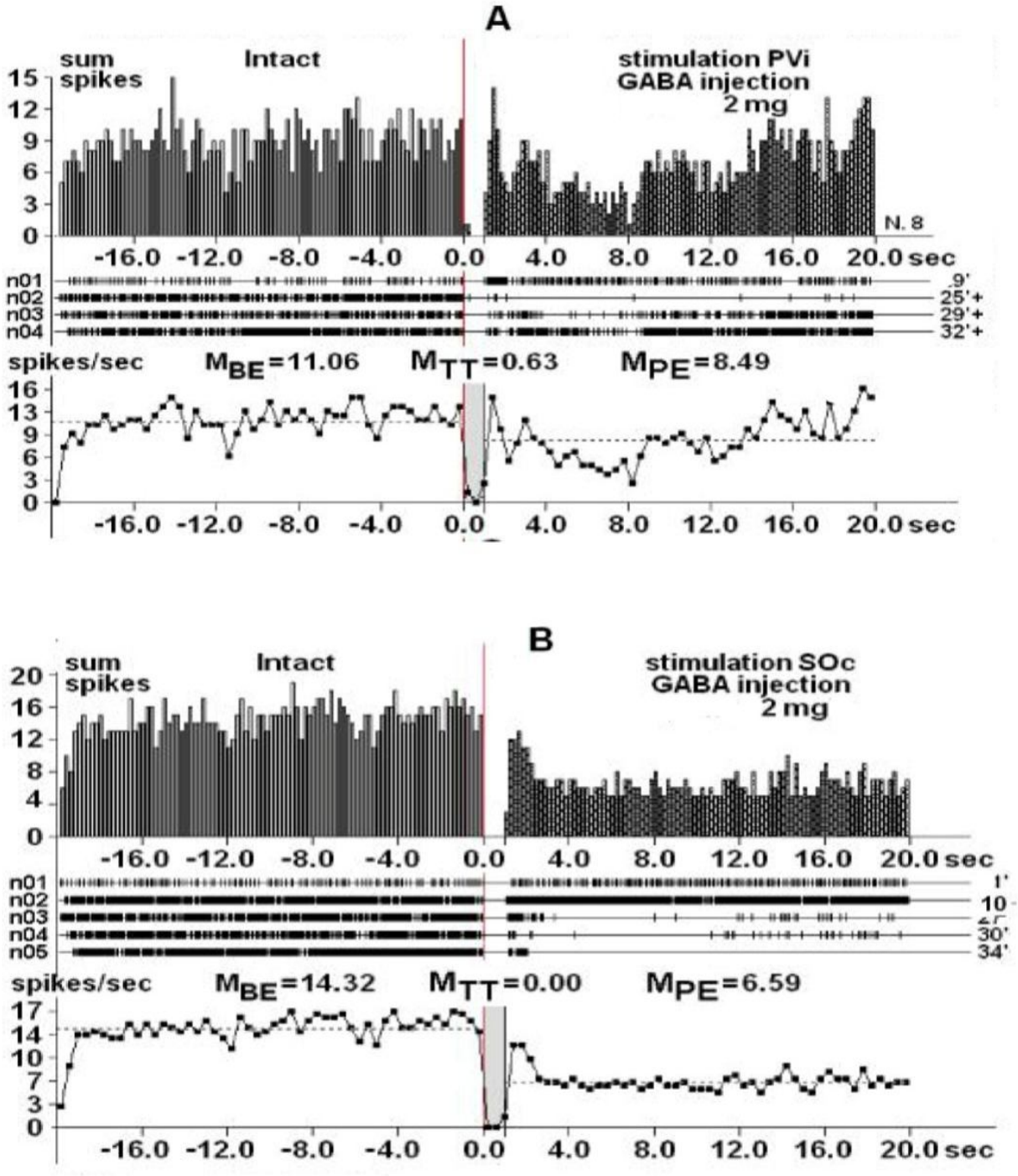

Fig.4. A-B - peristimule histograms of sum spikes (from above), constructed of raster pre - and post stimule depressor – TD(A,B) effects and manifestations of spike activity of single neurons of LVN under HFS 100 Hz (during 1 sec) PVi (A) SOc (B) in the real time(20 sec before and after) Stimulation, at normal group with combination of GABA. in the bottom – diagram of neurons spikes summarized frequency, presented in the raster and in the real time, indicating average digital values of 20 sec before(MBE) and 20 sec after (MPE) stimulation and during 1 sec tetanic stimulation(MTT).

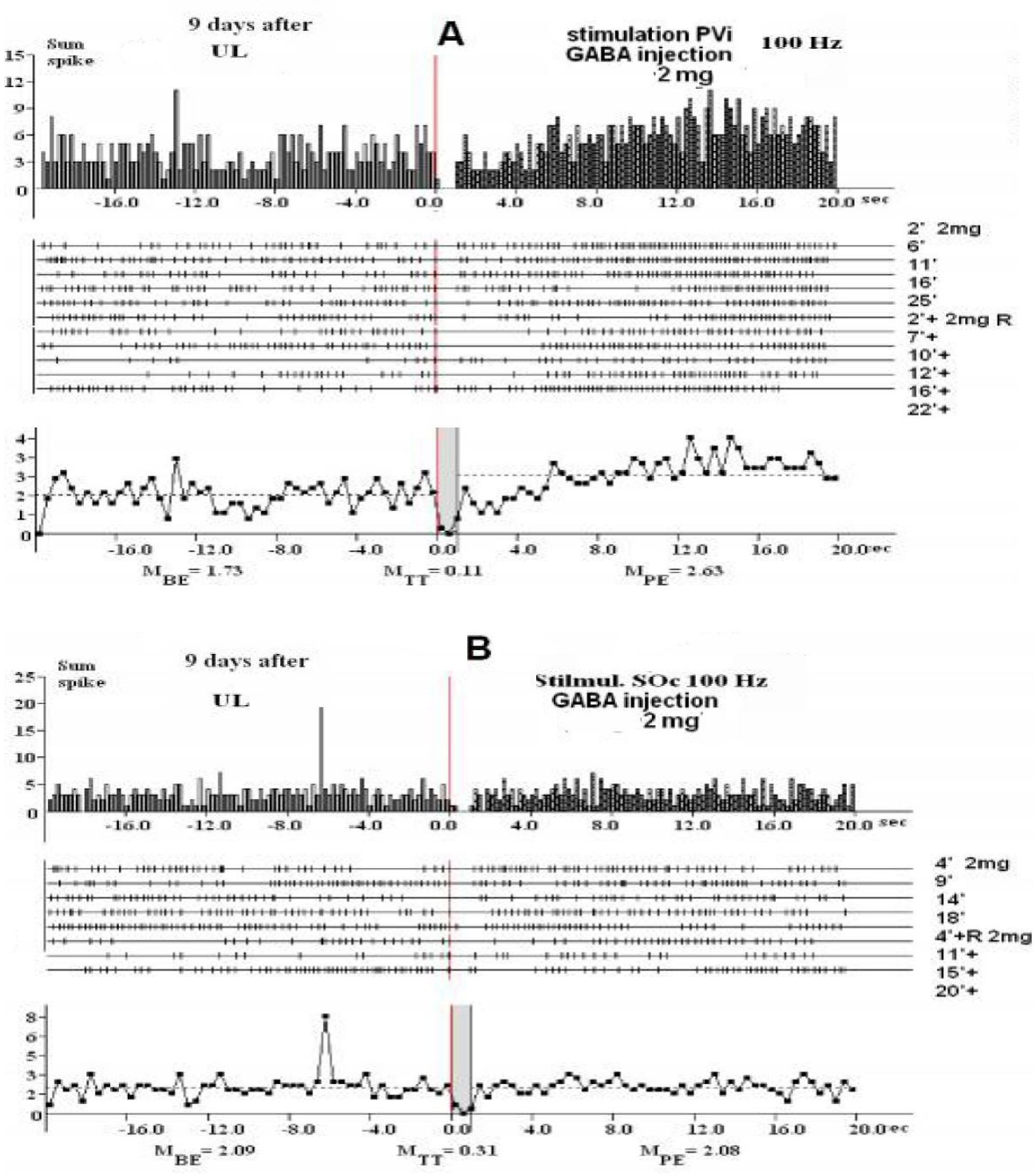

Fig.5. A-B- peristimule histograms of sum spikes (from above), constructed of raster pre - and post stimule depressor – TD(A,B) effects and manifestations of spike activity of single neurons of LVN under HFS 100 Hz (during 1 sec) PVi (A) SOc (B) in the real time(20 sec before and after) stimulation, at UL with combination of GABA.

in the bottom – diagram of neurons spikes summarized frequency, presented in the raster and in the real time, indicating average digital values of 20 sec before(M_{BE}) and 20 sec after (M_{PE}) stimulation and during 1 sec tetanic stimulation(M_{TT}).

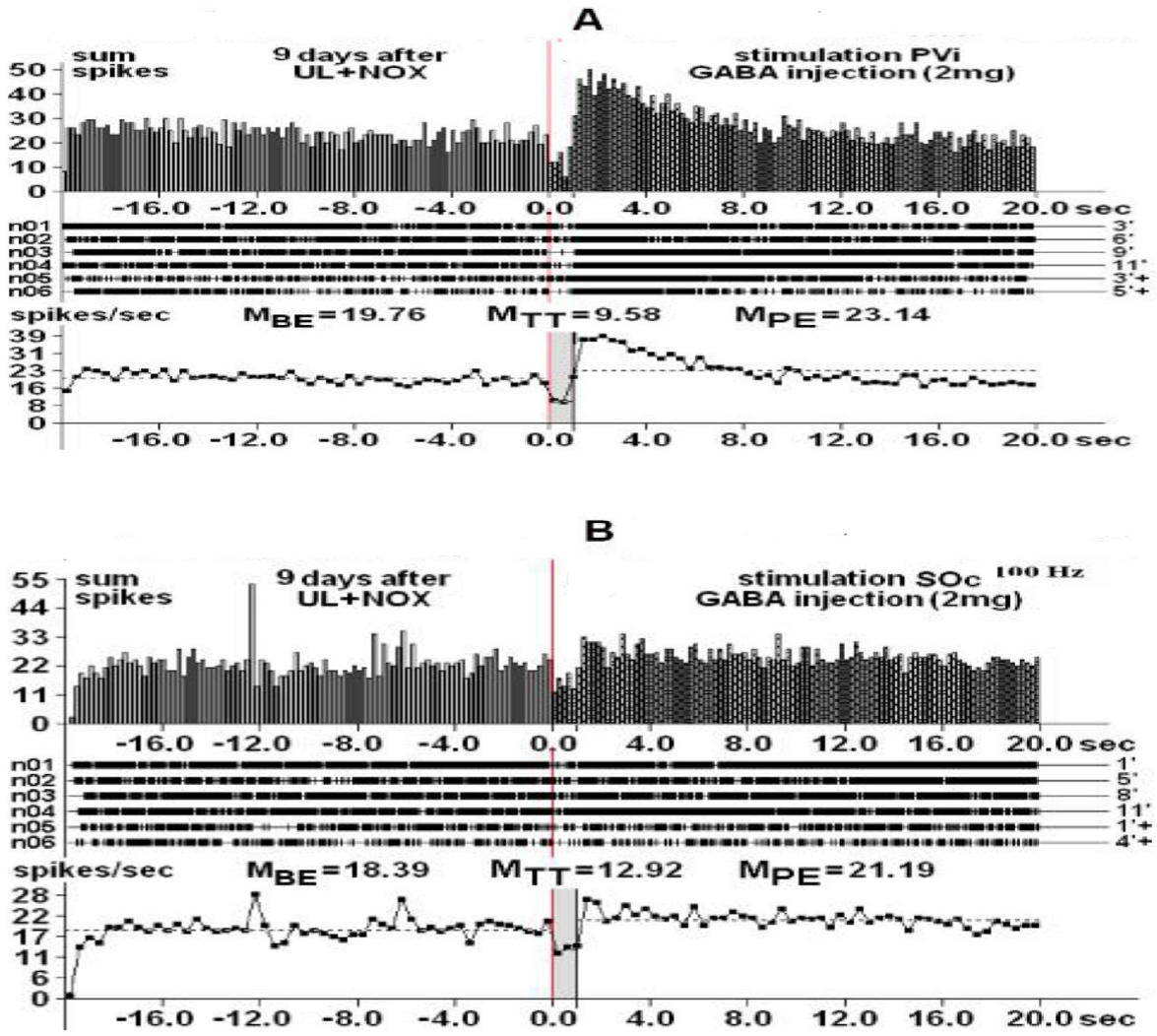

Fig.6. A-B- peristimule histograms of sum spikes (from above), constructed of raster pre - and post stimule depressor – TD(A,B) effects and manifestations of spike activity of single neurons of LVN under HFS 100 Hz (during 1 sec) PVi (A) SOc (B) in the real time(20 sec before and after) Stimulation, at 9 days after UL with administration of NOX venom and GABA.

in the bottom – diagram of neurons spikes summarized frequency, presented in the raster and in the real time, indicating average digital values of 20 sec before(M_{BE}) and 20 sec after (M_{PE}) stimulation and during 1 sec tetanic stimulation(M_{TT}).

Discussion

The results of this study at first indicate that NOX venom accelerates the vestibular compensation. It was probably mediated by GABA receptors during recovery of failed functions following vestibular damage. Explanation of the results can be obtained by reviewing the latest literature data on the UL. It should be noted that the main reason of oculomotor and postural symptoms of unilateral vestibular deficiency is misbalanced commissural inhibitory system and that its re-balance occurs in parallel with behavioral recovery during VC (27). So, after the UL increase of excitability in vestibular neurons on the injured side may be the result of down-regulation of GABA receptors, opposing the excessive commissural inhibition. It is assumed that restoration of pacemaker resting discharge in deafferented neurons is responsible for increased pacemaker excitability (28). In addition, the lack of

excitatory inputs on the injured side causes a decrease in their sensitivity to inhibitory amino acids, which should facilitate the restoration of normal bilateral balance of averaged resting discharge of vestibular neuron (29). In other words, there is a down-regulation of the effectiveness of $GABA_B$ receptors on the ipsilateral side and up-regulation - on the contralateral (30). It is also believed that the adaptive regulation of the efficiency of GABA receptors in vestibular neurons may be an important mechanism of cellular homeostasis or bilateral balance of their excitability (31). Electrophysiological, pharmacological and biochemical data indicate that GABA is mainly involved in the functional recovery after acute injury of the vestibular labyrinth (10, 32, 33). Recent studies have suggested that unilateral labyrinthectomy leads to change in the functional efficacy of GABA receptor on the deafferented MVN neurons (11, 34). Following UL GABA levels become asymmetric (13).

Earlier was shown that within 4 h after UL in the rat, marked compensatory changes take place in the functional efficacy of GABAergic inhibition in both ipsilesional and contralesional MVN neurones. These changes are in the appropriate direction to help restore resting activity in the ipsilesional MVN cells and lead to a rebalancing of the excitability of the MVN neurones on the lesioned and intact sides in vivo (31).

The results of our study showed that GABA suppresses all pre- and post stimulus activity of LVN neurons evoked by HFS of hypothalamic PVN and SON in the norm and 4 hours after UL, since GABA is the main inhibitory neurotransmitter in the vertebrate brain (7). GABA-mediated transmission has been implicated in the regulatory control of cellular properties such as neuronal excitability and the statistical likelihood of neuronal firing and of presynaptic neurotransmitter release (35, 36, 37). In normal group GABA lead to TD + PTP, whereas 4 hours after UL it evoked only TD. It can be the effect of GABA being increased after UL and synergism effect of systemic GABA injection in the vestibular nuclei. Some results indicated that immediately after the UL there is a significant increase in the release of GABA on injured side, which is not prevented by bilateral flocculectomy, indicating that the effect is (liable to)depend on hyperactivity of commissural inhibitory neurons. With the improvement of behavioral symptoms (more than 96 hours after UL) GABA levels on injured side approaches to the norm, while those on the intact side was not significantly changed in the early stages of the VC, but dropped in the later (96 hours) (38).

At 9^{th} day after UL and administration of NOX, there was not any suppresses in pre- and post stimulus activity of LVN neurons. This testifies that NOX venom together with GABA cause protective effect. The mentioned literature data were obtained in the absence of pharmacological intervention, while these studies were performed under the protective action of NOX venom. Protective effect of NOX, restoring in these conditions the profound decrease of inhibition, apparently came down to acceleration the VC through early restoration of inhibitory control of neurons on the injured side. This is consistent with high specificity and irreversibility of effects, resulting in long-term action of snake venoms (39).

Conclusion

This study indicated that NOX venom causes acceleration of vestibular compensation. In intact and UL animals GABA suppressed all pre and post stimulus activity of LVN neurons evoked by HFS of hypothalamic PVN and SON, but at 9^{th} day after UL and NOX administration there was not significant effect of GABA on the LVN neurons. Thus it may be concluded that NOX venom has protective effect on neurons of the lateral vestibular nucleus during GABA injection.

Acknowledgments

We were fortunate to have had the excellent developer of the special mathematical program by V.S.Kamenetski.

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