

# Workshop 6: Neurology

P6.01

## Autonomic modulation via slow deep breathing and attention

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**Introduction:** The autonomic nervous system (ANS) is important in pain recruitment, processing and perception. Slow deep breathing (SDB) increases the parasympathetic tone, measured by cardiac vagal tone (CVT) and protects against oesophageal hyperalgesia (Botha et al, Gut 2014). However, the contribution of cognitive factors to the ANS effects of SDB are unknown.

**Aim:** To study the effect of SDB and attention on the ANS.

**Methods:** 21 healthy volunteers (age  $23.9 \pm 2.57$ , 9 female), were randomised in a crossover design with 1 week washout period to: 1. 30 min SDB exercise (1-minute cycles of SDB at 0.1 Hz intercepted by 4 minutes of spontaneously paced breathing). 2. Attention task (One Back Task - Cogstate Ltd-USA). Autonomic variables were measured continuously (using Neuroscope™, Medifit Instruments, UK) at baseline, during interventions and 5 minutes after. Primary outcomes: CVT during interventions. Secondary outcomes: Cardiac sympathetic index (CSI), heart rate (HR), systolic blood pressure (SBP). CVT is presented as Median (IQR), other variables as Mean (SD).

**Results:** SDB increased CVT from baseline, 9 (7.25–15.5) vs. 8.5 (5.25–13.25),  $P < 0.01$  and returned immediately to baseline at the end of the intervention. SDB also increased CSI,  $\Delta$ CSI = 0.58, CI 0.56–1.1,  $P < 0.03$  but had no effect on SBP or HR. Attention task did not affect CVT, however, after the intervention, CVT significantly increased compared to baseline 11 (8.25–12) vs. 9.5 (5.25–11.75),  $P < 0.03$ . Attention increased both HR and SBP, ( $\Delta = 2.9$ , CI 0.35–5.3,  $P < 0.02$ ), ( $\Delta = 10.7$ , CI 5.2–16.2,  $P = 0.001$ ) respectively, but had no effect on CSI.

**Conclusion:** SDB and not attention activated sympathetic and parasympathetic systems. CVT increase post-attention task may be due to relaxation and relief. Hence, attention contribution to ANS modulation during SDB task is minimal although the potential for post task increase in CVT being affected by cognitive factors should be considered in future studies.

P6.02

## Nitric oxide has direct effect on electrical characteristics of neurons

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Discovery of the ability of mammalian cells to synthesize the free radical nitric oxide (NO) has stimulated significant efforts of researchers to study the role of NO in all areas of biology and medicine. It is found that NO is involved in behavioral programs, more and more data is accumulating that in the nervous system NO is involved in development, maturation and aging of the brain, in the processes of learning and memory. The aim of

this work was to study the effects of NO level on the membrane potential of the premotor interneurons of snail. We used an application of sodium nitroprusside (SNP), a donor of NO (at a concentration of  $10^{-4}$  mol/l) and L-NAME, inhibitor of NO-synthase (at a concentration of  $10^{-4}$  mol/l), into the solution that washing the preparation of intact snails, on the membrane potential ( $V_m$ ) of premotor interneurons.

In the experiments it is found that application of NO donor SNP in a solution that washing the preparation of intact snails, caused the hyperpolarization on 3.5 mV for 10 minutes. In that time, inhibitor of NO-synthase L-NAME caused the depolarization on 4 mV. Thus, we have demonstrated that NO can cause hyperpolarization of membrane in certain neurons. It is assumed that the response of the neuron to NO depends on the location of it in the neural network.

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P6.03

## Effects of serotonin depletion by p-chlorophenylalanine on reconsolidation of contextual memory

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It was found that long-term memory becomes variable after the reactivation, namely, after the remembering. The specific contextual learning and memory was also found in invertebrates. We conducted a study of the role of serotonin (5-HT) in context conditioning and its reconsolidation using inhibitor of 5-HT synthesis p-chlorophenylalanine (p-CPA). We elaborated the context learning in terrestrial snails, when the animals could distinguish the test signals used in different contexts (on ball and a flat surface). The next day, after testing confirming context learning, snails were placed for 20 minutes on the ball that served as a reminder, and then blocked the protein biosynthesis by injection of anisomycin. On the next day the preservation of context learning was tested. Disruption of memory demonstrated the process of reconsolidation.

Memory storage testing showed that anisomycin without reminders didn't disrupt the contextual memory, however it caused disrupting of memory if anisomycin injected simultaneously with reminder. Thus, we observe here the disruption of reconsolidation. But p-CPA injected 3 days before anisomycin (depletion of 5-HT) led to decrease of the behavioral reactions in response to tactile stimulation in 2 times on the second day after a reminder. The obtained results point to the necessity of 5-HT for the process of reconsolidation of memory.

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## P6.04

**Restriction of motor activity in rats: dynamics of nitric oxide production in the heart after recovery**

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Hypokinesia (restricted of physical activity) is one of the most pressing medical and social problems caused by lifestyle, occupational activity, prolonged bed rest, and a range of diseases, etc. Today an important area of physiological research is to study the role of nitric oxide (NO) in the cardiovascular, nervous and other body systems. It has been found that NO impairs the progress of myocardial infarction, but is also an opposite point of view, according to which an excess of NO is a compensatory factor. The objective of the study was to investigate the role of NO in the processes under recovery after hypokinesia in heart tissues of rats growing under restricted physical activity.

The experimental group was divided into 3 sub-groups: 1) animals, kept under hypokinetic conditions for 30 days, 2 and 3) animals, kept for 1 and 2 week under recovering conditions after 30-day hypokinesia. NO content in the rat organs was determined by technique which uses spin trapping method. We have discovered that the hypokinetic regime leads to an increase in NO production in the tissues by two times. We have found that during the subsequent recovery from hypokinesia there is further increase in NO production in the heart tissues. This result indicates that the recovery from hypokinesia is also a significant immobilization stress for animals.

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## P6.05

**Opposite responses of interneurons of naive and learned animals to application of serotonin**

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Serotonergic system plays an important role in the modulation of stress-induced excitability (arousal) and defensive behavior. It was shown that long-term facilitation of connections between sensory and motor neurons of gills withdrawal reflex was mediated by serotonin (5-HT) and this form of synaptic plasticity was found to be a critical cellular mechanism of behavioral sensitization. A lot of experiments were conducted using application of 5-HT for elaboration of cellular analogues of learning. These findings have induced us to investigate the role of 5-HT in mechanisms of learning by the analysis of changes of excitability of premotor interneurons to serotonin application in naive snails and animals after learning.

The decrease of interneuron's membrane potential was found to be caused by 5-HT application in isolated preparation from both naive and trained snails. At the same time, the application of 5-HT to preparation from trained snails caused the increase of threshold potential in opposite to naive snails where no changes

were revealed in the threshold potential. This effect means that the appearance of extracellular 5-HT, which could be emitted, for example, from modulator serotonin-containing neurons leads to a decrease in interneurons excitability which was increased after training procedures.

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## P6.06

**Connectionist model of the pattern generator of one muscle's specific activity profile**

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The main goal of the project is to find common rules for building for creation of neural systems (composed from simple subsystems) with scalable cognitive qualities on the basis of simple formal neurons with spike activity. On the basis of the original software the variants of the neural structures configurations have been tested. These networks provide plasticity on the level of conditioning the signals coming from the neural blocks of different functions, which allows the implementation of the principle of Hebbian plasticity.

It was found a principal architecture of dynamic stochastic artificial neural network, which provides facilitation of the execution of the motor program which managed "outside". It was shown forming of the pattern generator of muscle activity with the activity profile of the single muscle of any specified waveform. The interconnections between the elements, which lead to the sequential activation of elements during the formation of the motor activity patterns present in proposed model. These interconnections allow for explaining the modulation polysynaptic responses observed in the electrophysiological experiments after applying of frequency stimulation. It is associated with the one of the main issues, namely with the recorded dynamic of changes of the motor responses of the spinal cord in the functional tests procedure, as well as after recovering of the complex changes of the structure of the nervous system, such as, for example, after a spinal cord injury. The described scheme also explains the necessity of optimal sensory input in restoring pre-existing motor programs, including motor programs after injuries, as well as the necessity of the additional activation or loading for improving of the motor response.

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P6.07

### Influence of inhibitor of NO-synthase L-NAME on nitric oxide production in the rat hippocampus in acute phase of ischemic and hemorrhagic insult

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It is known that the prolonged deficit of oxygen leads to hypoxia of the brain, which is under certain conditions accompanied by the development of tissue ischemia. According to this study of the pathogenesis, the methods of correction and the mechanisms of stroke is important both from the theoretical and practical points of view. Nitric oxide (NO) is an important signaling molecule that is widely used in the nervous system. Our investigation's main purpose is to study the processes of NO-synthase involvement in the control of NO levels in the hippocampus of rats after modeling both ischemic and hemorrhagic stroke.

By spectroscopy of electron paramagnetic resonance it was shown that in 5 h after the onset of symptoms ischemic and hemorrhagic stroke the level of NO in the hippocampus was reduced by 2-3 times and this reduction was maintained for 24 and 72 h. It has indicated that nonselective NO-synthase blocker L-NAME reduced the low level of NO production in 3 times by its administration in 72 h after post-ischemic and hemorrhagic stroke. But it was discovered that L-NAME returns the level of NO production to baseline (control) by its administration in 5 h after ischemia.

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P6.08

### Management of acute ischaemic stroke at IRCCS San Martino in Genoa: from epidemiology to therapy

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**Background:** Thrombolysis improves functional outcome after acute ischemic stroke (AIS), but in Italy only the 12.5% of AIS patients undergo this treatment. Here, we studied the management of consecutive patients with suspect of AIS in order to identify factors associated with possible underutilization of thrombolysis.

**Materials and methods:** Among individuals ( $n = 816$ ) arrived at the Emergency Room (ER) with a suspect of AIS from 1 January 2016 to 31 December 2016, we reviewed 458 records (264 females and 194 males) in which the diagnosis of AIS was confirmed.

**Results:** 49% of patients arrived at the ER within 4.5 h of symptoms onset and 49% of these were treated with systemic thrombolysis. In the remaining 51%, main reasons for NOT undergoing iv thrombolysis were: minor stroke or stroke in

rapid improvement, ongoing anticoagulant therapy, high risk of bleeding due to recent hemorrhage/ trauma or surgery, severe comorbidities or advanced age and epilepsy at onset. 17% patients had a wake-up stroke and for this reason were excluded from thrombolytic treatment. 67% patients who arrived at the ER in therapeutic window, underwent intracranial ANGIO-TC which revealed an occlusion of a proximal cerebral artery in 55% of cases. Among these patients who were eligible for endovascular revascularization, 57% underwent mechanical thrombectomy. TICI 2b/3 was obtained in 60% of patients who performed rescue thrombectomy. According to the SIST MOST definition, SICH rate was 1.8%. The mortality rate was 12%. Mean duration of hospitalization was 10 days. At discharge, 49% of patients returned home, 17% were transferred to Rehabilitation Centre and 12% in RSA.

**Conclusions:** A late patient presentation is a barrier for using thrombolysis in AIS. Intra-venous thrombolysis was utilised in a higher rate of patients than expected. These data demonstrate how at the IRCCS San Martino the new ISO-SPREAD guidelines have been implemented.

P6.09

### Sustained disease remission in aggressive multiple sclerosis after autologous haematopoietic stem cell transplantation

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**Background:** Despite the advent of new highly-active therapies for multiple sclerosis (MS), long-term disease remission remains elusive and only a small percentage of patients achieves the so-called no evidence of disease activity (NEDA) status. Against this scenario, autologous haematopoietic stem cell transplantation (AHSCT) has recently demonstrated the potential to maintain disease remission in aggressive MS patients.

**Materials and methods:** We analyzed data from 35 consecutive patients with aggressive MS (77% relapsing-remitting MS, 23% active secondary progressive MS) treated with AHSCT at Neurologic Departments of Genoa and Turin between 1998 and 2015. All patients underwent the same transplant protocol made of cyclophosphamide followed by carmustine-cytarabine-etoposide-melphalan (BEAM) plus anti-thymocyte globulin. NEDA status (a composite of absence of relapses, no sustained disability progression, and no new T2 or T1 gadolinium-enhancing lesions on MRI), disability scores and reports of adverse events were collected.

**Results:** NEDA status was achieved by 33 of 35 patients (94%) at 1 year, 21/25 (84%) at 3 years and 11/14 (79%) at 5 years. Improvement was noted in neurologic disability from a median pre-transplant disability score of 6.5 to 6 at 5 years. Adverse events were consistent with expected toxic effects associated with AHSCT and no treatment related mortality was reported.

**Conclusions:** Our data demonstrate that AHSCT is extremely effective for inducing long-term disease remission in aggressive RRMS patients and it is associated with improvements in neurologic functions.

## Workshop 8: Neurology

O8.01

### The National Institutes of Health Stroke Scale is a strong predictor of mortality and disability in patients with primary intracerebral haemorrhage

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**Introduction:** Primary intracerebral hemorrhage (ICH) has a worse prognosis than ischemic stroke. The National Institutes of Health Stroke Scale (NIHSS), attained a very good prognostic value in ischemic stroke, but it is not clear whether this is true in ICH, too.

**Aim:** Objective of our study is to investigate the accuracy of NIHSS in predicting fatality rate and dependency degree in an unselected group of patients with spontaneous ICH.

**Materials and methods:** We included in the study all consecutive patients admitted for ICH to our Stroke Unit since 1st August 2011 to 31st January 2016. NIHSS was evaluated in all subjects within 24 hours from the onset. We systematically followed up all patients and we used modified Rankin Scale (mRS) to evaluate functional outcome at discharge and after three-months. Spearman's Rank Correlation Coefficient analysis was used for statistics. Sensitivity, Specificity, Predictive positive (PPV), Negative predictive (NPV) values, Global Accuracy (GA), and ROC curve were also computed, using the median score 7 as NIHSS cut-off and the score 4 as mRS cut-off.

**Results:** We included 156 subjects. Mean NIHSS ( $\pm$ SD) at admission was 10.82 ( $\pm$ 8.27). Thirty-two patients (20.5%) died within 30 days and other 10 (29.9%) within 3 months. Mean mRS (score 6 for patients who died) at three months was 3.38 ( $\pm$ 2.42). We found a highly statistically significant correlation coefficient between initial NIHSS and mRS after 30 days (0.74) and three months (0.66,  $P < 0.01$ ). Sensitivity was 88.7 and 80%, Specificity 83.5 and 79.01%, PPV 81.8 and 77.9%; NPV 89.9 and 81.1%, GA 85.9 and 79.48%, respectively at 1 and 3 months. The ROC curve showed a fitted area as 0.914.

**Conclusions:** NIHSS correlates very well with the 1 and the 3-months mortality and functional outcome in patients with ICH, showing good levels of Sensitivity, Specificity, and Global Accuracy.

O8.02

### Ischemic preconditioning in rats: effects of nitric oxide and activation and inhibition of K-ATP - channels

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Activation of K<sup>+</sup><sub>ATP</sub>-channels is considered to be a main component of response in preconditioning models. The role of NO in

the development of ischemic cell damage is equally important. The properties of NO action depend on the intensity of its production and the state of the surrounding tissue. NO hyperproduction in stroke causes damage to structural and regulatory components of cells. Moderate activation of NO during preconditioning may exert a neuroprotective effect, activating antioxidant enzymes, triggering anti-apoptotic mechanisms, and increasing cerebral blood flow. The present study is focused on the relationship between K<sup>+</sup><sub>ATP</sub>-channels and NO, as well as on the investigation of mitochondrial mechanisms in cerebral ischemia.

We found that 24 hours after ischemic preconditioning in rats, there is a two-fold decrease in expression of mitochondrial K<sup>+</sup><sub>ATP</sub>-channels in nervous tissue, a comparable increase in expression of cytochrome c oxidase, and a decrease in intensity of protein S-nitrosylation and nitration. Pharmacological preconditioning with the K<sup>+</sup><sub>ATP</sub>-channels opener diazoxide led to a 25–41% reduction of free NO concentration, statistically significant 9 and 72 hours after ischemic stroke simulation. We attribute this result to the restructuring of tissue energy metabolism, namely to the provision of catalytic sites in mitochondria and increased elimination of NO, which prevents a decrease in cell sensitivity to oxygen during subsequent period of severe ischemia.

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O8.03

### System of antioxidant protection in rat after spinal cord injury and production of nitric oxide

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The high frequency of spinal injury (SCI) combined with the complexity of the pathogenesis, and the lack at present adequate methods of treatment and rehabilitation of patients with consequences of spinal cord (SC) render this problem beyond the purely medical aspects. Great interest attracts the participation in the mechanisms of development of various pathological conditions of the body free radical compounds - nitric oxide (NO). It is known that major damaging factor during the development of processes of apoptosis is the peroxynitrite (ONOO<sup>-</sup>), which is formed when NO interacted with superoxide (O<sub>2</sub><sup>-</sup>). Dismutation of superoxide by cytosolic enzyme Cu,Zn-COD (superoxide dismutase) is the primary and primary protection against free-radical oxidation processes. During modeling of SCI it is studied the content of copper, which is an indicator of activity of COD, and also the production of NO in the SC. There was applied the method of EPR spectroscopy with the examination of ratio of complexes (DETC)<sub>2</sub>-Cu and (DETC)<sub>2</sub>-Fe<sup>2+</sup> -NO (NO/Cu). Ratio NO/Cu in the SC of intact rats is averaged 1:80, which is, apparently, helps prevent the formation of peroxynitrite. In the end, the ratio of the NO/Cu in early posttraumatic period amounted

to an average of 1:50, and in late - 1:3. Changing of this ratio shows the impairment of the antioxidant protection of the spinal cord in chronic posttraumatic period.

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#### O8.04

##### Sensory deprivation and delirium after stroke

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**Background:** Delirium is a common syndrome especially in elderly people and also in acute phase of stroke.

There are 3 types of delirium, in accordance to clinical presentation: hyper kinetic (with psychomotor agitation and hallucination), hypo kinetic (with lethargy and apathy) or the mixed form.

Among risk factors for post stroke delirium, sensorial disturbances are common, in particular visual and hearing deficits.

Only one clinical study [Oldenbeuving et al., 2011] evaluated the association between post stroke delirium and sensorial deprivation, reporting a weak association between delirium and previous visual disturbance (OR 1.5, but  $P = 0.75$ ).

Complex visual or auditory hallucinations may appear in many organic neurological disorders such as cortical or subcortical lesions and/or degenerative diseases.

**Methods:** We studied 100 patients with acute stroke admitted to Stroke Unit of San Martino Hospital in Genova; we evaluated delirium with DSM-V criteria and we screened delirium with 4AT scale and the association between delirium and sensorial disturbances.

**Results:** DSM-V criteria revealed 32% of cases of post stroke delirium in the acute phase. 4AT scale was used for delirium screening revealing a 52% of cases of delirium, the same observed by the consensus diagnosis of two senior neurologist (that was 50%).

We found a statistically significant association between delirium, hallucinations and visual disturbance; in particular between delirium and visual deficits ( $P = 0.004$  Chi Square Test) and between hallucinations and visual disturbance ( $P < 0.001$ , Chi Square Test). There is no association between hearing loss, delirium and hallucinations.

**Conclusions:** We showed that the 4AT is useful to identify delirium in stroke patients.

The presence of visual sensory deprivations is a risk factor for post stroke delirium and hallucinations; therefore it is important to identify all risk factors in order to prevent it.

#### O8.05

##### Treating tension-type headaches in schoolchildren: the efficacy of aminophenylbutyric acid hydrochloride

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The research covered 56 schoolchildren aged 14–15 with tension-type headaches (TTH): 11 boys and 23 girls with episodic tension-type headache (ETTH), and 8 boys and 24 girls with chronic tension-type headache (CTTH). All children were prescribed GABA-receptors agonist - aminophenylbutyric acid hydrochloride (Anvifen®) 250 mg 3 times a day for 3 weeks. The reduction of TTH intensity was assessed by 3-grade scale: "no change", "significantly reduced", and "completely stopped". We assessed accompanying symptoms over the last 6 months according to the Likert scale. ETTH/CTTH: (1) difficulty falling asleep, restless sleep ( $0.91 \pm 0.83/1.54 \pm 1.26$ ), (2) difficulty concentrating during the day ( $0.61 \pm 0.92/2.04 \pm 1.21$ ), (3) morning sickness ( $0.32 \pm 0.87/1.81 \pm 1.29$ ), (4) feeling ill in the morning with improvement in the second half of the day ( $0.17 \pm 0.57/1.95 \pm 0.95$ ), (5) meteosensitivity ( $0.58 \pm 1.01/1.59 \pm 1.22$ ), (6) decrease in physical capability ( $0.20 \pm 0.64/1.72 \pm 0.82$ ).

After 3 weeks, the pain in ETTH subgroup completely stopped in 27 children (79%), and significantly reduced in 7 (21%). In CTTH subgroup pain completely stopped in 9 children (41%), and significantly reduced in 13 (59%). Associated symptoms of ETTH/CTTH: (1)  $0.26 \pm 0.44$ ,  $P = 0.000093/0.45 \pm 0.80$ ,  $P = 0.000224$ . (2)  $0.17 \pm 0.38$ ,  $P = 0.003707/0.54 \pm 0.85$ ;  $P = 0.000007$ , (3)  $0.08 \pm 0.28$ ,  $P = 0.058101/0.77 \pm 1.06$ ,  $P = 0.001682$ . (4)  $0.08 \pm 0.28$ ,  $P = 0.083119/0.27 \pm 0.45$ ;  $P = 0.000000$ , (5)  $0.32 \pm 0.63$ ,  $P = 0.010304/1.36 \pm 1.09$ ,  $P = 0.021450$ , (6)  $0.058 \pm 0.23$ ;  $P = 0.133706/0.95 \pm 1.04$ ,  $P = 0.005055$ .

Aminophenylbutyric acid hydrochloride (GABA-receptors agonist) reduces the intensity of tension-type headache and has a positive effect on associated symptoms. It can be recommended for inclusion into the scheme of tension-type headache treatment in schoolchildren.

#### O8.06

##### Speech disorders in patients with vascular encephalopathy

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The authors present the results of a three-year research project on speech disorders in patients with vascular encephalopathy. The research was conducted with the use of neuroimaging and psychometric instruments designed in Kazan Federal University. An in-depth neurolinguistic testing, also developed in Kazan Federal University for patients with different types of encephalopathy, included balanced diagnostic phonetic, lexical and grammar subtests, as well as test tasks aimed at discourse analysis. Testing involved qualitative and quantitative (point-based) scores. For each subtest, experiments were held in order to identify linguistically valid diagnostic units. As a result, a database of diagnostic materials was set up. Since the dynamics of speech status must be monitored in the course of treatment, each patient every time was offered a neurolinguistic questionnaire with unique structure but featuring different linguistic material. It can be concluded that speech disorders in patients with vascular encephalopathy differ from disorders in patients with focal lesions. Revealed speech disorders mainly refer to lexical, syntactic, discourse and pragmatic levels. Phonetic and morphological