Depression is among the most common affective disorders in epileptic patients and occurs mainly before the seizure and during interseizure intervals. Using antidepressant drugs together with antiepileptics brings about the risk of interactions and exacerbates side effects. Lamotrigine (LAM) is a novel anticonvulsant mood-stabilizing drug that has shown efficacy in the treatment of bipolar depression and resistant major depressive episodes.

The aim of the study was to investigate the antidepressant and anticonvulsant effects as well as the side effects of LAM in rats.

Male Wistar rats weighing 180–250 g were used in the study. LAM (10 mg/kg) was administered intraperitoneally 30 min before the test. The animals were subjected to Porsolt’s test to measure the antidepressant activity [Porsolt et al., Nature, 1977]. Motor coordination was measured in the “chimney test” [Boissier et al., J Med Exper, 1960; Braszko et al., J Physiol, 2003] and the anticonvulsant effect (ED_{50} dose) was defined using Swinyard’s test [Swinyard et al., J Pharmacol Exp Ther, 1952].

LAM was administered in a single dose and after seven days of treatment did not show any antidepressant effect. A statistically significant antidepressant effect has been shown only after prolonged administration (14 and 21 days). Lamotrigine impaired motor coordination only after chronic treatment (after 14 and 21 days). ED_{50} was calculated as 19.99 mg/kg, ip (probit model).

In conclusion, LAM allows both for control of epileptic seizures and alleviation of depressive symptoms with negligible side effects in the course of epilepsy. New data suggest that the antidepressant effect of LAM can be associated with increased adrenergic and serotoninergic transmission as well as with stimulation of alpha-1 and alpha-2 receptors [Consoni et al., Eur Neuropsychopharmacol, 2006; Kaster et al., Eur J Pharmacol, 2007]. Antidepressant activity is also supposed to be connected with LAM’s anti-glutaminergic and neuroprotective properties.
The role of hippocampal BDNF in antidepressant and precognitive effects of venlafaxine, olanzapine and nicotine

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BDNF (brain-derived neurotrophic factor) is one of the key neurotrophic factors in the brain that promotes cell survival, regulates dendrites and induces plastic changes in the brain. The latest publications report the important role of BDNF in etiopathogenesis and in the pharmacotherapy of mental diseases such as depression [Duman and Monteggia, Biol. Psychiatry, 2006] or schizophrenia [Shoval and Weizman, Eur Neuropsychopharmacol, 2005].

It has been shown in recent years that cognitive processes such as learning and memory, frequently disturbed in mental diseases and modified with the pharmacotherapy used, are of vital importance for the level of marked BDNF. BDNF expression in the hippocampus, a structure of key importance in the processing of memory, is affected or regulated by the neurotransmitter systems. BDNF plays a vital role in mechanisms of brain plasticity related to learning and memory processes, affecting for instance the long-term potentiation process [Yamada, Life Sci, 2002]. Our previous papers [Nowakowska et al., Pol J Pharmacol, 1999; Nowakowska et al., Pharmacol Rep, 2008] have shown that the use of the new-generation antidepressant drugs (olanzapine and venlafaxine) have both an antidepressant and a precognitive effect in animals, and the combination of nicotine with the studied drugs enhanced this effect.

Our study aimed at explaining the role of BDNF in the antidepressant and procognitive effect of OLA and VEN and in their combined application with NIC.

Male Wistar rats weighing 180–250 g were used in the study. The animals were housed in standard laboratory conditions under a 12-hour light/dark cycle. The study groups were given venlafaxine (VEN) 20 mg/kg, po, olanzapine (OLA) 0.5 mg/kg, ip and nicotine (NIC) 0.2 mg/kg, sc. – all substances were administered twice a day. The control groups were given oral carboxymethylcellulose solution (0.5% CMC) according to the same schedule. The rats were decapitated 24 hours following the last administration of medication, and their hippocampi were sampled for determination of BDNF levels using the ELISA method.

VEN, OLA and NIC administered to rats twice daily for five weeks resulted in a statistically significant increase in the amount of BDNF protein. Combined administration of VEN+NIC and OLA + NIC, however, did not have a statistically significant effect on the level of BDNF protein in the rats’ hippocampi. The results support the hypothesis that BDNF contributes to VEN, OLA and NIC antidepressant activity by modulation of synaptic function and plasticity. BDNF expression in the hippocampus after combined administration of VEN+NIC and OLA+NIC is regulated by numerous neurotransmitter systems sensitive to nicotine.

The influence of enriched environment on lamotrigine’s procognitive effect in rats

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Patients with epilepsy are at great risk of experiencing cognitive deficits as a result of both the etiology of the disease and its applied pharmacotherapy. Antiepileptic drugs may have a negative effect on memory processes by reducing the responsiveness of neurons and increasing inhibitory neurotransmission [Meador et
The enriched environment intensifies exploration of the new area behavior, which may have a positive impact on spatial memory in rats. Lamotrigine (LAM) is a novel anticonvulsant mood-stabilizing drug that has been shown to have a favorable cognitive profile in comparison with the old generation of antiepileptic drugs [Hunt et al., Prog Neuropsychopharmacol Biol Psych, 2008; Zaccara et al., Seizure, 2008].

The aim of the study was to investigate the effect of LAM on spatial memory functions in rats in normal and enriched environmental conditions. Male Wistar rats weighing 180–250 g were used in the study. LAM (10 mg/kg) was administered intraperitoneally 30 min before the test. The animals were subjected to the Morris test [Morris, J Neurosci Methods, 1984] to test their spatial memory.

In a standard environment, LAM administered in a single dose did not significantly decrease escape latencies or crossed quadrants rate, although it produced a non-significant tendency in memory improvement. LAM given for a period of 7, 14 and 21 days lowered the values of escape latencies, indicating memory improvement. However, in an enriched environment, the cognitive improvement was noted only after 14 and 21 days of treatment, but it was significantly better than in the standard environment. In conclusion, treating epilepsy with novel drugs such as LAM allows improvement of impaired cognitive functions in the course of the disease. Memory improvement in animals may result from reduced glutaminergic activity, the blockade of neuronal voltage-dependent channels, and probably from non-specific monoamine inhibitory activity. The enriched environment increases the memory improving effect of LAM, which may be a result of the amplification of neuronal connections in rat brains.

Immunosuppressants attenuate HMGB1 expression and release from primary astrocyte cultures exposed to combined oxygen-glucose deprivation

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The protective potential of immunosuppressants has been reported in many experimental models of ischemia both in vivo and in vitro suggesting novel therapeutic application of these drugs. On account of the fact that the high mobility group box 1 (HMGB1) protein has recently been reported to be involved in ischemic brain injury, the purpose of the present study was to determine whether treatment with immunosuppressants could decrease HMGB1 expression and release in astrocytes exposed to ischemia-simulating conditions (combined oxygen-glucose deprivation, OGD). We also studied the influence of these drugs on expression of NFκB, inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2). In addition, we investigated whether the immunosuppressants could attenuate the necrosis of astrocyte cultures exposed to OGD. Cells were treated with cyclosporine A, FK506 and rapamycin (all drugs at concentrations of 0.1, 1 and 10 mM). Our study has provided evidence that immunosuppressants decrease the expression and release of HMGB1 in ischemic astrocytes. The present results provide further information about the cytoprotective mechanisms of immunosuppressants in ischemic astrocytes in relation to the pathophysiology of ischemic brain injury. It appears that protective effects stimulated by the immunosuppressants could be mediated in part by suppression of HMGB1 expression and release in astrocytes, which leads to attenuation of ischemia-induced necrosis and neuroinflammation.
Changes in gene expression in the rat hippocampus and amygdala after administration of desipramine or citalopram – RT-PCR array study

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The onset of action of antidepressant drugs (ADs) usually takes weeks. However, the question about the sequence of molecular events during the time necessary for therapeutic effects after drug administration still remains open. Previous studies have shown that a delayed onset of ADs’ action (e.g., changes in receptor density) was induced not only by different types of chronic treatment but also single-dose antidepressant treatment as well [Antelman et al., Mol Psychiatry, 2000; Kuśmider et al., Behav Pharmacol, 2006].

The aim of the present study was to check whether a single dose of a drug followed by drug-free period induces changes similar to these observed after chronic ADs.

Based on PubMed databases, 96 genes of interest were selected – believed to be crucial in ADs action – encoding receptors (e.g., Adra, Adrb, Ar, Cnr, Drd, GalR, Gria, Opr), transcription factors (e.g., Aes, Creb, Crem, Egr, Ets, Fos, Gata2, Jun, Jund, Pax6, Stat, Sp1, Yy1), genes involved in inflammatory responses (e.g., Il10r, Il1r, Il6r, Tnf) and neuronal apoptosis and synaptic plasticity (e.g., Bcl2, Bdnf, Caena, Cdk, Ntf, Vgf), as well as some other genes hitherto unlinked directly to the action of ADs. Male Wistar rats (250–310 g at the beginning of experiments) were used. Desipramine (DMI) and citalopram (CIT) were administered in doses of 15 mg/kg/2 ml ip daily. All animals during the 21 days of the experiment were injected once a day. The control groups received vehicle (saline) and four groups were treated chronically with the appropriate drug for 21, 14 and 7 days or 72 h before the end of experiment. There were also additional groups treated with single dose of ADs and sacrificed at the same time points afterwards.

The brains (removed 24 h after the last injection, rapidly frozen in a heptane/dry ice mixture and kept in −80°C until preparation) were cut into 50 µm slices, and the dentate gyrus of hippocampus and nuclei of amygdala were cut off with a laser microdissector. Isolated RNA was reverse transcribed to complementary cDNA and kept in −20°C frozen. Real-time PCR experiments were performed using TaqMan custom array with ABI Prism 7900 Sequence Detection System, and levels of expressed genes were measured by the relative quantitative method [Pfaffl, Nucleic Acids Res, 2001], followed by statistical analyses.

Interestingly, and significant for the further understanding of ADs’ action, considerable periodic changes in the expression level of many genes have been observed correlating to different time-points in ADs treatment. The study reveals possible correlations between alterations in groups of genes and interconnected brain regions since gene expression levels were measured in two regions of the same brain. Structure-specific and time-dependent changes in genes’ expression levels are very interesting, especially since the methodology employed to detect them was very subtle (laser microdissection of tissue and RT-PCR arrays). This delayed response suggests that antidepressant’s action is a complex multicellular process requiring the orchestration of many biochemical events, including changes in transcription of genes not simply involved directly in the neurotransmitters signaling cascade. The obtained results suggest that antidepressant’s action is a complex multicellular process requiring the orchestration of many biochemical events, including changes in transcription of genes not simply involved directly in the neurotransmitters signaling cascade.

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Blockade of dopamine d1 but not d2 receptors attenuates the effect of clomipramine on the 8-OH-DPAT-induced deficit in spontaneous alternation behavior in rats

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Obsessive-compulsive disorder (OCD) is a complex psychiatric disorder with a lifetime prevalence of up to 3% in the general population. According to DSM-IV, criteria for OCD include obsessions (defined as recurrent and persistent thoughts, impulses or images) and compulsions (defined as recurrent and persistent repetitive behaviors or mental acts) that cause marked distress, are time consuming, and significantly interfere with normal everyday activity. Despite a great number of preclinical and clinical studies conducted in recent decades, the etiology and pathophysiology of OCD is still far from clear. The treatment of OCD is also viewed as difficult and unsatisfactory – almost half of OCD patients do not respond to pharmacotherapies established thus far. Based mainly on the clinical efficacy of SSRIs it has been hypothesized that OCD may be related to functions of the brain serotonin system. However, recently a growing amount of evidence including results of our earlier studies suggest that the mesolimbic dopamine system may also be involved in the mechanisms of OCD and its treatment.

The present study was designed to evaluate the role of the dopamine D1 and D2 receptors in the mechanism of action of clomipramine in the spontaneous alternation (SA) model. The SA model is based on the natural tendency of rats to explore two arms of the T-maze sequentially and in succession. Thus, the paradigm consists of a choice situation that, if disturbed under specific pharmacological conditions, leads to preservative responses, and therefore it is believed to model some aspects of human OCD, namely, indecisiveness and perseverance.

Sub-acute (three-time) injections of clomipramine (10 mg/kg, ip) effectively reversed the reduction of the SA behavior produced by 8-OH-DPAT (1 mg/kg, ip). The drug had no effects on the SA behavior in animals not challenged with 8-OH-DPAT. Similar sub-acute injections of SCH23390 (0.00625–0.01 mg/kg, ip) and Raclopride (0.05–0.1 mg/kg) had no effect on the SA responses and did not affect the decrease in SA induced by 8-OH-DPAT. When administered 15 min before each clomipramine injection, SCH23390 (0.01 mg/kg) reversed the action of clomipramine and Raclopride (0.075 mg/kg) had no effect.

These results are consistent with recent developments in the clinical pharmacotherapy of OCD and provide further support for the usefulness of the SA procedure as an experimental tool for investigating the physiological and neurochemical mechanisms underlying OCD and its treatment. They also indicate the involvement of dopamine D1 receptors in the therapeutic mechanism of action of clomipramine in the SA model. The role of D2 receptors remains unclear and requires further studies.
Effect of chronically administrated carbamazepine and gabapentin on kynurenic acid in rats

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Kynurenic acid (KYNA) is an endogenous brain constituent that inhibits the activity of all three ionotropic excitatory amino acid receptors. KYNA acts most potently at the glycine site of the NMDA receptors complex. The cerebral synthesis of KYNA from its bioprecursor L-kynurenine is catalyzed primarily by kynurenine aminotransferase II (KAT II). Disturbances in KYNA production have been linked to the occurrence of epilepsy, Huntington’s disease, Alzheimer’s disease, schizophrenia, AIDS-related dementia and others. The anticonvulsant and neuroprotective role of KYNA in vivo and in vitro is well documented.

In the present study, we investigated the influence of chronic treatment with the antiepileptic drugs carbamazepine and gabapentin on (1) KAT II expression in rat brain cortices, and (2) on the KYNA level in rat brain cortices and sera.

Male Wistar rats were administered carbamazepine or gabapentin intraperitoneally (10 mg/kg and 100 mg/kg, respectively) for 14 days. Brain cortex and serum were collected 24 h after the last injection of a drug. KAT II expression was measured by qualitative reverse transcription polymerase chain reaction (RT-PCR) and the level of KYNA was quantified using the HPLC system with fluorometric detector.

Chronic treatment of rats with carbamazepine or gabapentin did not change KAT II expression. Carbamazepine increased KYNA levels in the brain cortex from 2.90 to 13.27 pmol/g wet tissue (p < 0.001) and in serum from 6.95 to 21.13 pmol/ml (p < 0.001). Gabapentin enhanced KYNA levels in serum from 6.95 to 16.65 pmol/ml (p < 0.001) and did not affect KYNA levels in the brain cortex.

The obtained data suggest that chronic antiepileptic treatment with carbamazepine and gabapentin produced an increase of KYNA that was not accompanied by change in KAT II expression.

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Effects of combined administration of the proteasome inhibitor lactacystin and the pesticide rotenone on striatal dopamine metabolism and cell viability in vivo and in vitro

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Epidemiological studies have shown a correlation between exposure to pesticides and Parkinson’s disease (PD). The pesticide rotenone, which is a mitochondrial complex I inhibitor, has recently been used as a model substance evoking the majority of symptoms of sporadic PD. On the other hand, genetic and biochemical studies have demonstrated a critical role of ubiquitin-proteasome system impairment in the pathogenesis of PD. Based on the multifactorial etiology of PD, the present study was aimed at determining how administration of rotenone influenced striatal dopamine (DA) metabolism under conditions of
lactacystin-induced proteasome inhibition in rats, and whether combined treatment would potentiate the lactacystine-induced cell damage in vitro. 

In vivo studies. Male Wistar rats were used for the study. The animals received systemic injections of rotenone (1 mg/kg, sc for 15 days) and unilateral injections of single doses of lactacystin (0.5, 1 or 5 µg/2 µl) into the substantia nigra pars compacta (SNc), alone or in combination. On day 15 of rotenone treatment, the animals were killed by decapitation and their striata and SNc were dissected on an ice-chilled plate. The levels of DA and its metabolites were assayed in striatal homogenates using the HPLC method. No differences were observed in the levels of striatal DA in rats treated with rotenone alone; on the other hand, a significant increase was observed in DOPAC and HVA levels, as well as in the DOPAC/DA and HVA/DA ratios, but a decrease was seen in 3-MT levels and in the 3-MT/DA ratio. Lactacystin alone caused a dose-dependent decrease in the content of DA and its metabolites. With regard to DA catabolism, lactacystin (1 and 5 µg/2 µl) evoked a huge acceleration in MAO-dependent oxidative DA deamination, COMT-dependent O-methylation and total DA catabolism in the left striatum. After combined treatment, no further significant decreases in DA content were observed in the left striatum compared to lactacystin-treated groups. However, the metabolic ratios of DOPAC/DA and HVA/DA were markedly higher in those groups than in groups receiving lactacystin or rotenone alone.

In vitro studies. The experiment was conducted on primary cultures of mouse cortical neurons and human neuroblastoma SH-SY5Y cells, differentiated for seven days with retinoic acid. In order to estimate cell death, the level of lactate dehydrogenase (LDH) released from damaged cells into the culture media was measured. Cell viability assessments were done using a tetrazolium salt colorimetric assay with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). In cortical and SH-SY5Y cells, lactacystin (2.5 or 10 g/ml, respectively) reduced cell viability to about 50% of the control group value after 48 h. Concomitant administration of rotenone (1 µM) and lactacystin evoked a statistically significant increase in cortical cell death compared to cells treated with a single agent. Also, in SH-SY5Y cells, combined treatment with rotenone (50 and 100 µM) and lactacystin evoked a similar significant increase in LDH release after 48 h when compared to cells treated with one agent only.

The obtained results show that although combined treatment with the proteasome inhibitor lactacystin and the pesticide rotenone does not potentiate the lactacystin-evoked decrease in DA content, it markedly increases DA catabolism when compared to groups receiving lactacystin or rotenone alone. Moreover, our in vitro studies demonstrate that rotenone potentiates the lactacystin-induced cell death. The obtained data seem to support the multifactorial hypothesis of Parkinson’s disease.

The influence of the acute and chronic administration of LPS on α-synuclein expression in rat brains

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Chronic inflammation is involved in the etiology of Parkinson’s disease (PD). Examination of postmortem PD brains revealed activated microglia and astrogliosis. The release of potent inflammatory mediators such as TNF, interleukins, and interferon species may be important for aggravating the death of dopaminergic cells. Furthermore, it was demonstrated that frequent users of anti-inflammatory drugs such as aspirin had lower incidence of the disease. One of the most important markers of PD is Lewy bodies and its constituent α-synuclein that has been shown to aggregate in cell bodies and neurites. Animal models of Parkinson’s disease involve the acute and chronic administration of LPS on α-synuclein expression in rat brains.
son’s disease employing toxins such as MPTP or paraquat (PQ) also induce inflammation and alter the α-synuclein expression.

The goal of this research was to establish the potential role of inflammation in increased formation of α-synuclein aggregates.

In the present study we examined the long-term effects of both single and four doses of lipopolysaccharide (LPS, 10 µg/kg, ip once a week for four weeks) in rats. All animals were decapitated seven days after the last treatment.

The induction of systemic inflammation was monitored by the body temperature. Six hours after a single LPS injection the body temperature increased significantly. Moreover, chronic LPS administration increased the body temperature systematically and for a prolonged time – up to seven days after the injection. The greatest increase was observed after three weeks of treatment compared to the rats’ original temperatures before the experiment. These results confirmed the development of chronic inflammation in rats.

None of the dopaminergic parameters measured by HPLC in the tissue homogenates of striata changed after the single treatment with LPS, but chronic treatment elicited decreases in the levels of dopamine and its metabolites DOPAC and HVA.

The same analysis revealed also that the single dose of LPS increased levels of the serotonin metabolite 5-HIAA in the striata. However, after four doses of LPS the levels of serotonin and 5-HIAA decreased.

Interestingly, the densitometric analysis of immunohistochemical staining for α-synuclein in the substantia nigra showed decreased signal for this protein after chronic LPS treatment.

The above results suggest that chronic inflammation alone can change the expression of α-synuclein protein, supporting the importance of such a state both in PD and in the synucleinopathies. Moreover, decreased levels of dopamine and its metabolites in the striata confirm the deleterious influence of inflammation especially on the nigrostriatal system.

Changes in serotonergic transmission after both acute and chronic LPS treatments seem to be interrupted during the inflammation in the brain.

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Co-localization of c-Fos and glucocorticoid receptor immunoreactivity-expressing cells in the brain structures of low and high anxiety rats

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We sought to determine the co-localization of c-Fos (a marker of neuronal activation) and glucocorticoid receptor immunoreactivity-expressing cells (GRs-ir) in the dorsomedial prefrontal cortex (M2), the dentate gyrus of the hippocampus (DG), and the basolateral nucleus of the amygdala (BLA) in low and high anxiety rats (i.e., rats with duration of a freezing response in the conditioned fear test one standard error or more below or above the mean value: low responders (LR) and high responders (HR), respectively). It was found that 1.5 h after a testing session of the conditioned fear test, the LR animals had higher activity in the cortical M2 area and DG (c-Fos), a higher expression of GRs-ir, as well as an increased number of cells co-expressing c-Fos and GRs-ir in the same brain areas. In the case of HR rats, they had similar expression of...
c-Fos in the BLA, but a significantly higher concentration of GRs-ir and c-Fos/GR co-localized neurons in the same amygdala nucleus. These data extend our previous findings to the neurobiological correlates of the two examined populations. We have previously found that LR rats had a higher activity of the dorsomedial section of the prefrontal cortex area (M2), the dentate gyrus of the hippocampus, and median raphe nucleus (c-Fos expression) in comparison to the high responders. These animals also had stronger 5-HT and CRF-related immunostaining in the M2 and in the paraventricular nucleus of the hypothalamus (parvicellular part, pPVN) and increased concentration of GABA in the basolateral nucleus of the amygdala (in vivo microdialysis). The low responders group vocalized more during the test session in the aversive band (22 kHz) and had higher serum levels of corticosterone in comparison to the HR rats. The neurochemical profiles of both groups of animals were different in a qualitative way, with different brain structures and neurotransmitter systems involved in the organization of distinct response strategies to conditioned aversive stimuli.

The present data add to the arguments for the neurobiological background of differences in individual responses to aversive conditioned stimuli.

Celastrol potentiates striatal dopamine metabolism and increases cell death under conditions of proteasome inhibition: in vivo and in vitro studies

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Several studies have suggested that a failure in the ubiquitin-proteasome system may be an important factor in the development of Parkinson’s disease (PD). Dopaminergic cell death in PD has also been linked to excessive oxidative stress. Celastrol is a potent antioxidant compound extracted from Tripterygium wilfordii. Recently, it has been shown that this compound prevents degeneration of nigrostriatal neurons and a loss of striatal dopamine (DA) in an MPTP model of PD in mice. The aim of our study was to determine whether celastrol may exert a neuroprotective effect in a model of lactacystin-induced proteasome inhibition in vivo and in vitro.

In vivo studies. The experiment was carried out on four groups of male Wistar rats injected unilaterally with 5 g/2 l of lactacystin or solvent into the left substantia nigra pars compacta (SNc), alone or in combination with celastrol, 3 mg/kg, ip, given subchronically (four days). On day 8 after lactacystin administration, the animals were killed by decapitation. The levels of DA and its metabolites were determined in striatal homogenates using the HPLC method. One week after intranigral lactacystin administration, a significant decrease in the levels of DA and its metabolites was observed in the ipsilateral striatum. With regard to DA catabolism, lactacystin evoked acceleration of total (HVA/DA) DA catabolism, MAO-dependent oxidative DA deamination (DOPAC/DA) and COMT-dependent O-methylation (3-MT/DA). Celastrol did not change the level of striatal DA; however, there was a significant increase in DOPAC and HVA levels, but a decrease in the 3-MT level. Combined treatment with lactacystin and celastrol did not prevent the lactacystin-induced loss of striatal DA and its metabolites; the decrease in the level of DA metabolites in that group was even more pronounced than in the lactacystin-treated group. Moreover, after combined treatment, the metabolic ratios of DOPAC/DA and HVA/DA were markedly higher than in the lactacystin-treated group.

In vitro studies. The experiment was conducted on primary cultures of mouse cortical neurons and hu-
man neuroblastoma SH-SY5Y cells differentiated for seven days with retinoic acid. In order to estimate cell death, the level of lactate dehydrogenase (LDH) released from damaged cells into the culture media was measured. Cell viability assessments were carried out using a tetrazolium salt colorimetric assay with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). In cortical and SH-SY5Y cells, lactacystin (2.5 or 10 g/ml, respectively) reduced cell viability by about 40–50% of the control group value after 48 h. Celastrol (5 and 10 mM) induced primary cortical cell death in a concentration-dependent manner at 48 h after treatment. Concomitant administration of a subtoxic concentration of celastrol (1 mM) and lactacystin increased cell death dose-dependently compared to cells treated with lactacystin alone. In SH-SY5Y cells, celastrol (1 and 2.5 mM) induced LDH release in a concentration-dependent manner. Combined treatment with toxic concentrations of celastrol (1 µM) and lactacystin evoked a significant increase in LDH release after 48 h compared to cells treated with one agent only.

Our study showed that celastrol did not attenuate the loss of DA in lactacystin-treated rats. Moreover, celastrol itself induced cell death and potentiated the lactacystin-evoked cell death in vitro. The obtained results did not confirm the neuroprotective effect of celastrol previously observed in the MPTP model of PD.

Systemic administration of 1,2,3,4-tetrahydroisoquinoline prevents the loss of striatal dopamine in rats injected intranigraly with the selective proteasome inhibitor lactacystin

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1,2,3,4-Tetrahydroisoquinoline (TIQ) is an endogenous amine that occurs naturally in mammalian organisms, in particular in the brain of humans and rodents. It is also widely spread over the environment, present in numerous plants and foodstuffs. It has been found that TIQ readily penetrates into the brain, as it is actively transported across the blood-brain barrier by an organic cation transporter system (OCT) and is quickly eliminated from it by P-glycoprotein. Our earlier rat studies demonstrated that TIQ decreased the oxidative MAO-dependent catabolism of dopamine (DA), which indicated its antioxidant mode of action. In cell cultures, TIQ prevented the DAT-mediated toxicity of MPP⁺, and in a rat model it protected striatal dopaminergic terminals against malonate-induced neurotoxicity. Furthermore, it was found that TIQ increased glutathione (GSH) and nitric oxide (NO) levels in both the striatum and the substantia nigra (SN), lowered the level of reactive oxygen species in the SN, and inhibited the generation of hydroxyl radical in the abiotic system. All those investigations suggest that TIQ can be regarded as a potential neuroprotective agent.

The ubiquitin-proteasome system (UPS) is a major multicatalytic proteinase complex responsible for the intracellular degradation of misfolded and abnormal proteins. Impairment of the UPS has been reported in Parkinson’s disease (PD). The postmortem studies of parkinsonian brains have shown selective damage of 20/26S proteasomal activities and reduced levels of proteasome subunits and their activators (19S/PA700, PA28) in the substantia nigra pars compacta (SNc). Since the progressive loss of nigrostriatal dopaminergic neurons accompanied by the formation of intracytoplasmic proteinaceous inclusions (known as Lewy bodies) is one of the cardinal pathological features of PD, UPS dysfunction has been proposed as a potential etiopathogenic factor of the disease. Recently, selective proteasome inhibitors have been used as new model substances producing a parkinsonian-like syndrome in animals. However, since systemic administration of these compounds seems to be controver-
sial, a proteasome inhibitor was applied intrastructurally in our study.

The aim of the present study was to determine whether sub-chronic, systemic administration of TIQ can prevent the loss of striatal dopamine in rats injected intranigrally with a single dose of the selective proteasome inhibitor lactacystin. The experiment was conducted on four groups of male Wistar rats that were injected unilaterally with 1 µg/2 µl of lactacystin or solvent into the left SNc. Two experimental groups received TIQ (50 mg/kg) intraperitoneally for seven days, while two others were given solvent. The animals were killed by decapitation 2 h after the last dose of TIQ or solvent, and their striata were dissected on an ice-chilled plate. Concentrations of dopamine (DA) and its metabolites were determined in striatal homogenates using the HPLC method with coulochemical detection. One week after unilateral intranigral lactacystin administration, a significant decrease in the levels of DA and its metabolites was observed in the ipsilateral striatum. With regard to DA catabolism, lactacystin evoked acceleration of the total (HVA/DA) and MAO-dependent oxidative (DOPAC/DA) DA catabolism in the ipsilateral striatum. Sub-chronic TIQ administration prevented the loss of striatal DA and attenuated the lactacystin-enhanced DA catabolism in the ipsilateral striatum.

The obtained results suggest that TIQ can act as a neuroprotective agent. Putative mechanisms of the neuroprotective activities of TIQ are discussed in the context of PD.

What is the influence of cycloheximide, a protein synthesis inhibitor, on the development of PTZ kindled seizures in rats?

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Epilepsy is a phenotypically diverse, neurological disorder characterized by recurrent hypersynchronous discharges of neurons, followed by spontaneous seizures. Epileptogenesis can be viewed as a form of neuroplasticity subjected to the general principles, e.g., it is a continuous process dependent on de novo protein syntheses. Epilepsy can be modeled using the kindling phenomenon, whereby the consecutive administration of initially subconvulsive electrical or chemical (e.g., pentylenetetrazol, PTZ) stimuli leads to generalized seizures. Besides the epilepsy, kindling is widely accepted as a model for interictal behavioral disturbances that accompany seizures and also as a model of neuroplasticity. Taking all these facts together it seemed interesting to check whether the neuroplastic processes underlying seizures and epilepsy are subjected to mechanisms ruling the phenomenon of reconsolidation. To this aim, the effect of CHX administered immediately after PTZ-evoked seizures in fully kindled rats was assessed. The changes in the seizure score were the primary end-points of observation. CHX (icv) injected for a period of time is crucial for reconsolidation of memory traces, and had no significant effects on the intensity of the seizures. Bearing in mind that there is a time window during which reconsolidation can occur, we also decided to administer CHX 0.5 hour before PTZ injection followed by tonic-clonic seizures. Such an administration scheme resulted in a drastic decrease in the total score of seizures, but it was completely reversed after CHX withdrawal. These observations pointed out that CHX displayed acute anti-seizures activity.

Obtained data indicate anti-seizure but not anti-reconsolidation-like effects of the protein synthesis inhibitor cycloheximide (CHX).
Effect of the synthetic leucopyrokinin antagonist [D-Ala⁵]-[2-8]-leucopyrokinin ([D-Ala⁵]-[2-8]-LPK) on analgesia induced in rats by selective agonists of central opioid receptors

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It has been previously demonstrated that [D-Ala⁵]-[2-8]-LPK, a synthetic analog of insect neuropeptide leucopyrokinin (LPK), inhibits LPK-induced analgesia in rats. Moreover, it was shown that naloxone, an unselective opioid antagonist, as well as some selective opioid antagonists inhibited LPK-induced analgesia in rats. These findings prompted us to evaluate the effect of [D-Ala⁵]-[2-8]-LPK on analgesia evoked in rats by selective subtypes of opioid agonists. The study was performed on adult male Wistar rats. One week before the experiment, the polyethylene cannulas were implanted into the right, lateral brain ventricle of anesthetized rats. [D-Ala⁵]-[2-8]-LPK was injected icv in the dose of 5, 10 and 100 nmols. Next, after 15 min an icv equimolar dose of 5, 10 and 100 nmols of [2-8]-LPK or of one of the opioid agonists was injected: μ, δ, and κ: DAMGO, DPDPE and GR 89696 fumarate salt, respectively. It was found that [D-Ala⁵]-[2-8]-LPK mainly inhibited antinociceptive effects evoked by DAMGO, but had no influence on the effect of DPDPE and GR 89696 fumarate. The result of the present study proved the conclusion that [D-Ala⁵]-[2-8]-LPK is an antagonist of μ-opioid receptors in the rat brain.

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Study on effect of veratridine on pain perception in rats

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Sodium channels play a key role in the generation and propagation of action potentials in excitable membranes as well as in neuronal excitability. Pharmacological effects of several drugs are due to sodium channels’ blocking effect. It was previously found that the sodium channel blocker, local anesthetic lidocaine, when applied directly into the lateral brain ventricle exerts antinociceptive effect in rats. A similar effect has been induced by phenytoin an anti-epileptic drug.

The aim of the present study was to investigate pain perception in rats after opening sodium channels. Therefore, the effect of veratridine, an agent opening voltage-dependent sodium channels and preventing their activation, was determined.

Experiments were performed on adult male Wistar rats, which had been implanted with the polyethylene cannula into the right brain ventricle (icv) under xylazine with ketamine analgesia one week prior to experiments. On the day of experiment veratridine was icv injected at the range of doses of 1–65 nmol. The antinociceptive effect was determined using a tail immersion test. It was found that veratridine exerts hyperalgesia in rats. Moreover, it was found that prior icv administration of veratridine at the dose of 20 or
65 nmols completely blocked antinociceptive effect of equimolar icv doses of lidocaine injected 10 min later. The results of the present study suggest that the model of veratridine-induced hyperalgesia may be useful in the study of the antinociceptive effect of different drugs.

Whole genome microarray analysis in the prefrontal cortex of mice lacking the noradrenaline transporter

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One of main theories of depression is closely linked with noradrenergic transmission. It is widely accepted that depression might result from the decrease in noradrenaline concentrations in the synaptic cleft. Antidepressant drugs like reboxetine or desipramine (DMI) act via blocking the noradrenaline transporter therefore patients normalize the level of that neurotransmitter. Our previous behavioral study has shown that genetically modified mice lacking the noradrenaline transporter (NAT-KO) [Xu F et al., Nature Neuroscience, 2000] display “depressive-resistant” behavior. They showed significantly shorter immobility times in both the forced swim test and the tail suspension test [Dziedzicka-Wasylewska et al., Neuropsychopharmacology, 2006].

The aim of the present work was to see how different the expression of the genes in NAT-KO mice is in comparison to wild type (WT) animals, and to determine whether genes expressed in WT mice treated chronically with DMI resemble those of NAT-KO mice. To achieve this goal the whole genome Affymetrix microarray was used.

Three groups of animals were used: WT and NAT-KO mice were injected with physiological saline for seven days, to match the treatment of another group of animals, WT, which were treated with DMI (ip; 20 mg/kg), once daily for seven days. The brains were frozen, RNA was extracted from the prefrontal cortex and was reverse transcribed into cDNA. Biotinylated cRNA were prepared according to the standard Affymetrix protocol and hybridized to Affymetrix microarray. Data were analyzed using Affymetrix GCOS software (MAS 5.0 algorithm). Comparisons between the NAT-KO group and the group after DMI injection were performed using t-test. A value of p < 0.004 was considered to be significant.

We selected a statistically significant group of 143 probeset (NAT-KO group vs. WT group) and 88 probeset (DMI group vs. WT group) from 45,101 total probeset for further study. These groups of genes were searched to find common genes for NAT-KO mice and mice treated with DMI. Twelve common genes have been found. Some products of these genes are located in mitochondria (Spsn1, Acsl1), cytoplasm (Atg7, Map2k7, Twf1, Ntrk3), the plasma membrane (Efnb2), Golgi apparatus (B3gat3) and the nucleus (Gadd45b). They are involved in transport (Spsn1), the process of apoptosis (Atg7, Gadd45b), magnesium ion binding (B3gat3), metabolic processes (Acsl1), nervous system development (Efnb2) or in the activation of MAPKK activity (Gadd45b). The most interesting of them seems to be Atg7, Gadd45b and Map2k7. Atg7 (autophagy-related 7) is involved in negative regulation of apoptosis, cerebral cortex development, neurological system processes, pyramidal neuron development, amino acid metabolic processes, neurite development and membrane organization. Another gene, Gadd45b (growth arrest and DNA-damage-inducible 45 beta) takes part in the activation of MAPKK activity, the regulation of cell cycle, cell differentiation and apoptosis. The product of the Map2k7 gene (mitogen-activated protein kinase kinase 7) has ATP binding activity, protein serine/threonine kinase activity, metal ion binding activity and it is involved in the response to stress including a stress-activated MAPK cascade.
All of these selected genes will be verified by RT-PCR in the prefrontal cortex and related structures of the brain. It may be concluded that microarray technique adopted for the study of genetically modified mice lacking the noradrenaline transporter allowed us to find an interesting group of genes that could be involved in the mechanism of action of antidepressant drugs.

c-Fos mapping of changes in neuronal activity during the course of PTZ kindling of seizures

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(Approximately about 1% of the total population suffers from epilepsy. Different animal models of epilepsy have been used to elucidate its mechanisms. The kindling of seizures is one of the most widely used animal models of temporal lobe epilepsy. It models a process of a progressive decrease of the threshold of seizures, leading to tonic-clonic convulsions after repeated administration of subconvulsive doses of pro-convulsive agents or sub-threshold electrical stimulation of brain structures. In the kindling induced by electrical stimulation, an increased neuronal activity is initially localized at the site of stimulation, gradually spreading to other brain areas.

In the present study, the immunocytochemistry (c-Fos expression) was used to map in a systematic way brain structures recruited during the evolution of major seizures after repeated systemic administration of pentylenetetrazol (PTZ) at the subconvulsive dose (35 mg/kg, ip) in rats. It has been found that the earliest expression of c-Fos, at stage 1,2 of kindling, appeared in the nucleus accumbens-shell, the piriform cortex, the prefrontal cortex and the striatum. On the third stage of kindling, the central amygdala nucleus, the entorhinal cortex and the lateral septal nucleus (LSV) showed an enhanced concentration of c-Fos. On the fourth stage of kindling, c-Fos was increased in the basolateral amygdala and the CA1 area of the hippocampus. Finally, c-Fos labeling was enhanced in the dentate gyrus of the hippocampus only when the stage 5 of kindling was fully developed, i.e., the tonic-clonic convulsions.

It appeared that there are important similarities in the structures recruited at the beginning and at the end of electrically and chemically-induced kindling, i.e., the piriform cortex and the hippocampal dentate gyrus, respectively. On the other hand, the differences gradually disappear at later stages of kindling, followed by the symmetrical propagation of epileptic activity from the limbic system to the neocortex during the generalized seizures.)
Effect of antiepileptic drugs and β-adrenoceptor antagonists on aminophylline-induced convulsions and toxicity in mice

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The convulsive activity of methylxanthines has been known for many years. Clinical data indicate that treatment with aminophylline may induce repetitive generalized seizures in the patients with obstructive lung diseases. Some cases of aminophylline-induced seizures may be even fatal [Schwartz and Scott, 1974; Yarnell and Chu, 1975; Zwillich et al., 1975]. Conventional antiepileptic drugs very poorly control this type of convulsions and are practically ineffective in lowering aminophylline-induced mortality [Chu, 1981; Czuczwar et al., 1987]. We evaluated the protective activity of phenobarbital and valproate combined with propranolol, atenolol, labetalol and pindolol against aminophylline-induced convulsions and mortality. The experiments were carried out on male Swiss mice weighing 20–25 g. Chemical seizures were induced by intraperitoneal (ip) injections of aminophylline and defined as clonus of the whole body with an accompanying loss of righting reflex lasting for over 3 s. Phenobarbital (up to 75 mg/kg) and valproate (up to 300 mg/kg) reduced the incidence of convulsions, but were not effective in preventing mortality.

Propranolol (up to 10 mg/kg), atenolol (up to 25 mg/kg), labetalol (up to 10 mg/kg) and pindolol (up to 15 mg/kg) were ineffective in protecting against convulsions and lethality. Phenobarbital and valproate combined with atenolol and propranolol led to significant protection against aminophylline-induced convulsions and mortality. On the contrary, antiepileptics combined with labetalol and pindolol did not protect against aminophylline-induced seizures and mortality.

The obtained results point to a novel method of sufficiently reducing convulsion and the lethal effects of an aminophylline overdose.

Evaluation of a new model of Parkinson’s disease employing chronic intraventricular infusion of MPP⁺ with Alzet osmotic minipumps to rats

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Parkinson’s disease (PD) is a progressive neurodegenerative disorder characterized by a loss of pigmented, neuromelanin-containing dopaminergic neurons in the substantia nigra (SN), which leads to a massive drop in striatal dopamine (DA) levels (80–90%) and the presence of Lewy bodies, mainly in the SN. Although the underlying mechanism of degeneration is unknown, mitochondrial dysfunction, oxidative stress and proteasomal dysfunction are likely to contribute. The animal models of PD developed so far and based on the factors proposed above include 6-hydroxydopamine, MPTP, rotenone, paraquat and proteasomal inhibition. However, all of them have certain advantages and disadvantages; hence it seems necessary to search for a new, chronic model of PD that causes selective, progressive and marked nigrostriatal neuronal degeneration along with neuropathological and neurochemical symptoms of the disease, in particular for
the purpose of studying putative neuroprotective drugs.

The experiment was performed on male Wistar rats (300–360 g) that received an infusion of MPP+ iodide (Sigma-Aldrich, Poland) or vehicle (saline-iodide) into the left cerebral ventricle using an ALZET osmotic minipump (Cupertino, CA; model 2ML4), implanted subcutaneously in the back (Yazdani et al., 2006). Cannula placement was carried out stereotaxically (A = +8.7, L = +1.4, D = –3.5 – according to Paxinos and Watson, 1986). MPP+ was administered at two doses of 0.284 and 0.428 mg/kg/day for 28 days with a drug delivery rate of 2.5 µl/h. All the animals were killed by decapitation at the end of a 28-day infusion period. Brains from the part of animals assigned for in situ hybridization were rapidly removed and frozen at –80°C. Brains from another group of rats were also dissected: forebrain structures were frozen at –80°C and processed by HPLC, whereas midbrains were prepared for immunohistochemistry (postfixed in a 4% paraformaldehyde and cryoprotected in 20% sucrose).

Chronic intraventricular delivery of MPP+ produced a marked, dose-dependent loss of DA and its metabolites DOPAC and HVA (50–90%) in the left striatum, ipsilateral to the infusion site. DA concentration was normal in the non-infused, right striatum. The levels of 5-HT and 5-HIAA were decreased in the left striatum after the higher dose of MPP+ only. The stereological counting of DA neurons and the measurement of their density in the SN, stained with the antibody against tyrosine hydroxylase, showed a 30–50% loss on the lesioned side. Those changes were accompanied by a diminished expression of mRNA for the dopamine transporter in the SN (by about 30%). In situ hybridization studies also indicated an enhanced expression of mRNA for both adenosine A2A and dopamine D2 receptors in the striatum. Additionally, an attenuated expression of α-synuclein mRNA in the SN was also observed, yet only after the lower dose of MPP+.

The obtained results showed that the intracerebral infusion of MPP+ with ALZET osmotic minipumps produced a number of neurochemical changes resembling PD. This chronic model of continuous toxin delivery produces a selective nigrostriatal DA cell loss via mitochondrial dysfunction, and is characterized by a very low mortality rate in rats. Hence, this model seems well suited for the neuropharmacological testing of putative neuroprotective compounds.

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The comparison of effects of the endogenous enantiomer, (R)- with the racemate (R,S)-1-methyl-1,2,3,4-tetrahydroisoquinoline on dopamine metabolism in the brain structures and its in vivo release in rat striatum

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1-Methyl-1,2,3,4-tetrahydroisoquinoline, (R,S)-1MeTIQ, the racemate present in the mammalian brain is a mixture of two enantiomers: (R)-1MeTIQ – synthesized endogenously in the brain, and the exogenous form (S)-1MeTIQ coming to the brain from the foods. Earlier experiments have shown that the racemate (R,S)-1MeTIQ demonstrates a wide and very interesting profile of action on central nervous system. Among them the neuroprotective and anti-addictive activity of this compound was the most important not only from a scientific perspective but also from practical point of view [Antkiewicz-Michaluk et al., Eur
Fear extinction, c-Fos and glucocorticoid receptor immuno-reactivity in the hippocampus of low and high anxiety rats

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The aim of the study was to compare the fear-related extinction process in animal differing in the strength of a freezing response in the conditioned fear test (context evoked freezing response). The rats were divided into two groups according to their behavioral response in the conditioned fear test: HR – (high responders, freezing time longer than the mean + SD) and LR (low responders, freezing time shorter than the mean – SD). Next, 7 and 14 days after the test, the animals were exposed again to the aversive context. Finally, on the 21st day the animals were reexamined in the conditioned freezing test. One and a half hour later the brains were collected for the assessment of expression of c-Fos (a marker of neuronal activation), glucocorticoid receptor and their co-expression in the hippocampus.
It was found that after 14 days, the extinction of the fear response was accomplished (there was no difference between conditioned and not conditioned groups). Nevertheless, both groups differed in the length of their freezing responses studied at 7 and 14 days after training. After seven days, the freezing response to aversive context was decreased by 11% in HR group, and 18% in LR group. After fourteen days, the freezing response was decreased by 58% in HR and 19% in LR group. After 21 days, during the retest, there appeared to be a significant increase in c-Fos expression in the dentate gyrus \([t = 2.11, \text{df} = 30, p < 0.05]\), with a decrease of cells co-expressing c-Fos and glucocorticoid receptor immunoreactivity in CA1 \([t = 2.12, \text{df} = 30, p < 0.05]\), and a longer freezing time in the conditioned rats as compared to the rats without conditioning. Additionally, HR animals had higher c-Fos immunoreactivity in DG \([t = 2.2, \text{df} = 17, p < 0.05]\) and longer freezing time \([t = 2.57, \text{df} = 19, p = 0.01]\) compared to LR rats.

These results showed different neurochemical profiles of high and low responding animals (HR and LR). The reported differences in c-Fos and GR-immunoreactivity in limbic structures might underlie individual differences in the emotional responses.

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N-acetyl-β-D-hexosaminidase in rat brain after experimental hypoxia

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N-acetyl-β-D-hexosaminidase (HEX) is one of the most active lysosomal exoglycosidases that catalyses the release of N-acetylglucosamine and N-acetylgalactosamine residues from the non-reducing ends of free and bound oligosaccharide chains of glycoconjugates (glycolipids, glycoproteins and proteoglycans). N-acetyl-β-D-hexosaminidase (HEX) has mainly been used in clinical diagnosis of Tay-Sachs and Sandhoff diseases. The lack of glycosaminoglycan storage observed in Tay-Sachs and Sandhoff’s diseases is due to the presence of hexosaminidase in amounts that are small but sufficient to prevent accumulation of glycosaminoglycans.

In our previous experiments, we indicated that experimental hypoxia changed behavioral activity in rats as follows: induced amnesia, inhibited locomotion and exploratory activity, impaired acquisition, consolidation and retrieval of conditioned responses and exhibited an anxiogenic effect in the elevated “plus” maze. Hypoxia was induced by anoxemia resulting from placing rats into the gas mixture (2% oxygen and 98% nitrogen) with stable flow and no pressure changes. The repeated episodes of hypoxia were induced every day (1st, 5th, 10th day of hypoxia).

The main aim of our experiment was to determine the activity of N-acetyl-β-D-hexosaminidase (HEX) in the following structures of the brain: cortex, striatum, hippocampus, cerebellum without or with episodes of hypoxia.

We found a statistically significant decrease in the activity of HEX mainly in the striatum, cortex, hippocampus, cerebellum without or with episodes of hypoxia.

We observed changes of HEX activity between the 1st, 5th and 10th days of hypoxia.

Conclusion: Our results demonstrated a probable correlation between hypoxia, the expression of N-acetyl-β-D-hexosaminidase, and the degree of injury to the brain.