ISSN 0015-5497, e-ISSN 1734-9168
Folia Biologica (Kraków), vol. 64 (2016), No 3
doi:10.3409/fb64_3.189

Antioxidant Enzymes in Brain Cortex of Rats Exposed to Acute, Chronic and Combined Stress*

Snežana PEJIĆ, Vesna STOJILJKOVIĆ, Ana TODOROVIĆ, Ljubica GAVRILOVIĆ, Ivan PAVLOVIĆ, Nataša POPOVIĆ, and Snežana B. PAJOVIĆ

Accepted July 07, 2016 *Published October 2016*

PEJIĆ S., STOJILJKOVIĆ V., TODOROVIĆ A., GAVRILOVIĆ L., PAVLOVIĆ I., POPOVIĆ N., PAJOVIĆ S.B. 2016. Antioxidant enzymes in brain cortex of rats exposed to acute, chronic and combined stress. Folia Biologica (Kraków) **64**: 189-195.

The study deals with manganese superoxide dismutase, copper, zinc superoxide dismutase, and catalase activities in brain cortex of Wistar rats exposed to acute stress (immobilization or cold for 2 h), chronic stress (long-term isolation or long-term forced swimming for 21 days), or to combined chronic/acute stress. We observed that i) single episodes of acute stress by immobilization increased activity of both superoxide dismutases; ii) both types of chronic stresses significantly elevated activities of all examined enzymes; iii) chronic social isolation was a much stronger stressor than physical stress by swimming; iv) in animals pre-exposed to chronic isolation, additional stress by immobilization or cold significantly decreased previously elevated activities of all enzymes, while after chronic swimming, acute immobilization lowered only catalase activity. The obtained results indicate that stress conditions most probably altered the cell redox equilibrium, thus influencing the antioxidant response in brain cortex. Further investigation of neuronal prooxidant/antioxidant cellular conditions is needed to improve the prevention and treatment of various stress induced diseases.

Key words: Antioxidant enzymes, reactive oxygen species, acute stress, chronic stress, brain cortex.

Snežana PEJIĆ, Vesna STOJILJKOVIĆ, Ana TODOROVIĆ, Ljubica GAVRILOVIĆ, Ivan PAVLOVIĆ, Nataša POPOVIĆ, Snežana B. PAJOVIĆ, Laboratory of Molecular Biology and Endocrinology, "Vinča" Institute of Nuclear Sciences, Mike Petrovića Alasa 12-14, P.O. Box 522, 11001 Belgrade, Serbia.
E-mail: snezana@vinca.rs

Stress exposure influences body homeostasis, leading to different physiological responses but may also cause development of various pathological conditions. Physical and psychological stress is already known to induce sympathetic activity and the hypothalamic-pituitary-adrenal (HPA) axis, which activates synthesis of neuropeptides and catecholamine (SAITO *et al.* 2005). Catecholamine metabolism leads to production of reactive oxygen species (ROS) that may influence cellular redox homeostasis and oxygen radical toxicity (MEISER *et al.* 2013). Since ROS production underlies stress mechanisms, understanding the cellular and molecular basis of these processes is important to improve therapeutic approaches to stress induced diseases. This is particularly significant in CNS tissues due to high oxygen consumption in oxidative phosphorylation and deficiency of antioxidant enzyme (AOE) in nerve cells (HALLIWELL 2006).

ROS, which can oxidize cellular macromolecules, are neutralized by the antioxidant (AO) defense system. The first line of defense includes antioxidant enzymes such as copper, zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), catalase (CAT) and glutathione peroxidase (GPx), which directly remove ROS. The second line of defense consists of enzymes such as glutathione reductase (GR) and glucose-6-phosphate dehydrogenase which maintain the intracellular pool of reduced glutathione (GSH) and NADPH, respectively (RAHAL *et al.* 2014).

Studies have shown that different types of acute and chronic stress exposure induce alterations in

*Supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Grants 41027 and 41022).

antioxidant status. For instance, exposure to immobilization (restraint) stress decreased the brain levels of glutathione (GSH), SOD, glutathione-S-transferase (GST), and CAT, with an increase in thiobarbituric acid reactive substance (TBARS) levels (ZAIDI *et al.* 2014). Previously, ŞAHIN and GÜMÜŞLÜ (2004) showed that immobilization stress, cold stress exposure, and combined immobilization–cold stress increased brain TBARS levels and Cu,Zn-SOD and CAT activities, along with decreased brain GSH concentrations. Chronic stress by long term isolation was shown to induce TBARS levels in rat brain cells. At the same time, activity of SOD and CAT was also increased (MENABDE *et al*. 2011). Besides muscle contractions (skeletal and heart), exercise stress induces inflammatory processes associated with increased ROS production and increased release of catecholamine (GOMES *et al.* 2012).

Also, oxidative stress has been defined as a principle pathological cause of neurodegeneration. Neuronal proteins and structural components become modified due to OS in different neurological disorders leading to neuro-inflammation and loss of cognitive function in Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS) and amyolotrophic lateral sclerosis (ALS) (UTTARA *et al.* 2009). Antioxidants seem to limit neurodegeneration induced by ROS through inhibition of early or late proinflammatory events or by exerting neuroprotective properties (CHIURCHIÙ *et al.* 2016).

In our earlier study, we showed that various models of acute, chronic and combined stress exerted different catecholamine levels and AO response in rat hippocampus (PAJOVIC *et al.* 2006). Since damage to the mitochondrial electron transport chain is considered as an important factor in the etiology of neurodegenerative disorders (HROUDOVÁ *et al.* 2014), in the present work we focused on the AO enzyme activity in the brain cortex of rats exposed to acute stress by immobilization or cold, chronic stress by long term isolation or long term physical stress, and combined chronic/acute stress.

Material and Methods

Animals and stress models

Experiments were carried out on male Wistar rats, aged three months and weighing 330-400 g. They were housed in open colony cages (four per cage) under controlled conditions of temperature (21±2ºC) and illumination (lights on between 07:00 and 19:00 h), and had free access to laboratory chow and tap water. The Guiding Principles for the Care and Use of Animals based upon the Helsinki Declaration (1964) and Protocol of the "Vinča" Institute on Care and Treatment of Laboratory Animals were strictly followed. Due to ethical issues associated with non-excessive sacrifice of animals, the experiment was designed to use the respective controls for different types of stress (PEJIC *et al.* 2006).

The experiment consisted of two parts. Part I. Rats were exposed to acute stress by immobilization (IMMO) or cold (COLD) for 2 h, as well as to chronic stress by individual housing (long-term isolation, LTI) for 21 days or long-term forced swimming (LTS) every day for 15 min in water heated to 32°C during 21 days. The untreated animals served as controls (C). Part II. Rats exposed to either type of chronic stress were subjected to immobilization or cold for 2 h. Immobilization stress was induced as described by KVETNANSKY and MIKULAJ (1970). The animals exposed to cold were initially kept at ambient temperature and then transferred into a cold chamber at 4°C. Each experimental and control group had 6 animals, which may be considered as a limitation of the study. However, due to ethical reasons, it was not possible to sacrifice more animals.

Preparation of brain cortex homogenates

Animals were sacrificed by decapitation with a guillotine (Harvard-Apparatus, USA), and brain cortex of all animals were excised and kept frozen (–70°C). After thawing (+4°C), they were weighed and homogenized (1:6 w/v, Potter-Elvehjem teflon-glass homogenizer) in 0.25 M sucrose buffer containing 0.05 M Tris-HCl and 1 mM EDTA, pH 7.4. The homogenates were vortexed for 15 s three times, with intermittent cooling on ice, and left frozen at –70ºC for 20 h in order to disrupt the membranes and release MnSOD from mitochondria into crude homogenates. They were defrosted at room temperature, vortexed 1 min and centrifuged (Eppendorf centrifuge 5417R, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) at 7500 rcf for 15 min at 4°C, and the supernatants were collected.

Measurement of enzyme activities

The SOD activity was measured by the method of MISRA and FRIDOVICH (1972). The reaction of auto-oxidation of adrenaline to adrenochrome was performed in 3 ml of incubation mixture containing 0.05 M Na_2CO_3, 0.1 mM EDTA, pH=10.2, followed by the addition of 100 µl of sample and 100 µl of 3×10^{-4} M adrenaline (Sigma Chemicals Co.). The inhibition of auto-oxidation by SOD contained in the samples was monitored spectrophotometrically at 480 nm, 26ºC, 4 minutes (Cecil CE 2040 spectrophotometer, Cecil Instruments Ltd., Cam-

bridge, UK). After assaying the total SOD activity, the samples were treated with 8 mM KCN in order to inhibit CuZnSOD (GELLER & WINGE 1983), and subjected again to enzyme assay described above. The obtained values and the differences between the two measurements were considered as MnSOD and CuZnSOD activities, respectively. The results were expressed as specific activity of the enzyme in units per mg protein (U/mg protein). One unit of SOD is defined as the amount of protein which causes 50% inhibition of the conversion rate of adrenaline to adrenochrome between the 3rd and 4th minute under specified conditions.

Catalase activity was measured by the method of BEUTLER (1984). The method is based on the rate of H_2O_2 degradation by CAT contained in the examined samples. The reaction was performed by adding 20 µl of the catalase sample in an incubation mixture containing 50 µl of a buffer (1 M Tris-HCl, 5 mM EDTA, pH 8.0), 900 µl of a substrate (10 mM H_2O_2) and 30 µl of distilled water. Degradation of hydrogen peroxide was monitored spectrophotometrically for 3 minutes at 230 nm, 37ºC. One unit of CAT is defined as the amount of protein which degrades 1 µmol H_2O_2/min under specified conditions. The extinction coefficient for H_2O_2 at 230 nm was 0.071 $mM^{-1}cm^{-1}$.

Total protein concentration (mg/ml) was measured according to the method of LOWRY *et al.* (1951).

Data analysis

Data were analyzed using GraphPad Prism software. The Kolmogorov-Smirnov test was used for testing the normality distribution of the small sample size. Since data were normally distributed, no data transformation was employed and the results are reported as means±SEM. The level of statistical significance was set to 5%. Differences of antioxidant enzymes activity were analyzed by one-way ANOVA, followed by the Dunnett *post hoc* test Part I. The effects of acute stress (IMMO, COLD) and chronic stress (LTI, LTS) were compared to the untreated controls (C). Part II.A. The effects of acute stress (IMMO, COLD) were compared to the groups pre-treated with LTI or LTS stress, which were used instead of controls. Part II.B The effects of combined stress treatment were analyzed by a two-way ANOVA to test for the two main effects (chronic and acute stress) and for the interaction between them.

Results

One way ANOVA analysis showed significant variation of MnSOD ($F_{4,25}$=116.4, P<0.001) among the examined groups (Fig. 1). Compared with control values, exposure to acute IMMO significantly increased MnSOD activity (P<0.001) while exposure to cold had no effect on this enzyme. Both chronic stresses, LTI and LTS, significantly elevated MnSOD activity (P<0.001). When additional acute stress by IMMO or COLD was applied after pretreatment with chronic stresses which were used for comparison (Fig 4.), significant variation of MnSOD activity was again recorded only in the LTI-pretreated group ($F_{2,15}$=28.24, P<0.001). Also, both types of acute stress, IMMO and COLD, significantly lowered the enzyme activity (P<0.01), (Fig 4.A).

A significant difference in CuZnSOD activity was observed among the groups (one way ANOVA, $F_{4,25}$=167.5, P<0.001). In comparison to the control values, the exposure to acute IMMO, and to both types of long-term stress, LTI and LTS, induced a significant increase of CuZnSOD activity ((P<0.01), P<0.001, respectively) (Fig. 2). There was also significant variation of CuZnSOD activity ($F_{2,15}$=68.08, P<0.001) among the groups pre-treated with LTI stress and additional acute stress (Fig. 4A). Both types of acute stress significantly lowered the CuZnSOD activity (P<0.01).

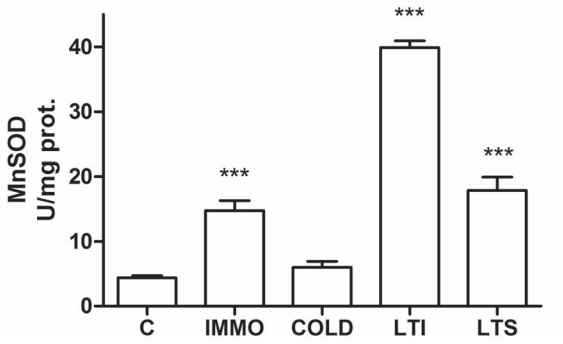

Fig. 1. The activity of MnSOD (U/mg protein) in brain cortex of untreated rats (C) and rats exposed to stress by immobilization (IMMO), cold (COLD), long-term isolation (LTI) and long-term forced swimming (LTS). The values are mean±SEM of 6 animals per group. Symbol: ***P<0.001 compared to C (Dunnett test).

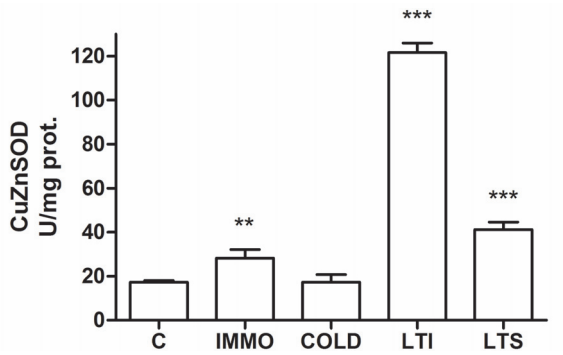

Fig. 2. The activity of CuZnSOD (U/mg protein) in brain cortex of untreated rats (C) and rats exposed to stress by immobilization (IMMO), cold (COLD), long-term isolation (LTI) and long-term forced swimming (LTS). The values are mean±SEM of 6 animals per group. Symbol: **P<0.01, ***P<0.001 compared to C (Dunnett test)

Similarly to both SOD enzymes, there was a significant difference in CAT activity (Fig. 3) among the groups (one way ANOVA, $F_{4,25}$=108.4, P<0.001), and the LTI and LTS chronic stress significantly elevated enzyme activity (P.001) compared to control values. Additional acute stress by IMMO or COLD after pretreatment with either type of chronic stress induced significant variations of CAT in both examined groups (LTI: one-way ANOVA $F_{2,15}$=84.31, P<0.001, Fig. 4A), (LTS: one-way ANOVA $F_{2,15}$=17,43, P<0.001, Fig. 4B). Additional acute stress by IMMO induced a significant decrease of CAT activity in both LTI (Fig. 4A) and LTS (Fig. 4B) pre-treated groups (P<0.01), while acute cold stress lowered CAT activity only in the LTI pre-treated group (P<0.01, Fig. 4A).

Two-way ANOVA analysis (Table 1) of combined stress treatments (LTI/IMMO, COLD;

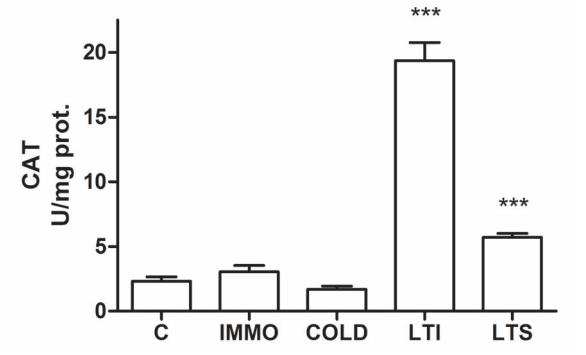

Fig. 3. The activity of CAT (U/mg protein) in brain cortex of untreated rats (C) and rats exposed to stress by immobilization (IMMO), cold (COLD), long-term isolation (LTI) and long-term forced swimming (LTS). The values are mean±SEM of 6 animals per group. Symbol: ***P<0.001 compared to C (Dunnett test).

Table 1

Antioxidant enzyme activity of rats exposed to combined acute and chronic stress

	MnSOD (U/mg protein)		CuZnSOD (U/mg protein)		CAT (U/mg protein)	
	LTI	LTS	LTI	LTS	LTI	LTS
	40.0+1.0	17.8+2.0	121.5+4.3	41.1+3.3	19.2+1.4	5.6+0.2
IMMO	19.7+4.1***	17.1+1.6***	68.5+8.2***	35.5+2.2	5.3+0.6***	1.6+0.1***
COLD	14.74+0.7	18.2+1.8	31.0+2.2***	34.51+4.2	2.7+0.5	4.1+0.7

Values are means+-SEM. Two-way ANOVA: a significant main effect of chronic stress, acute stress and interaction effect (*** P<0.001)

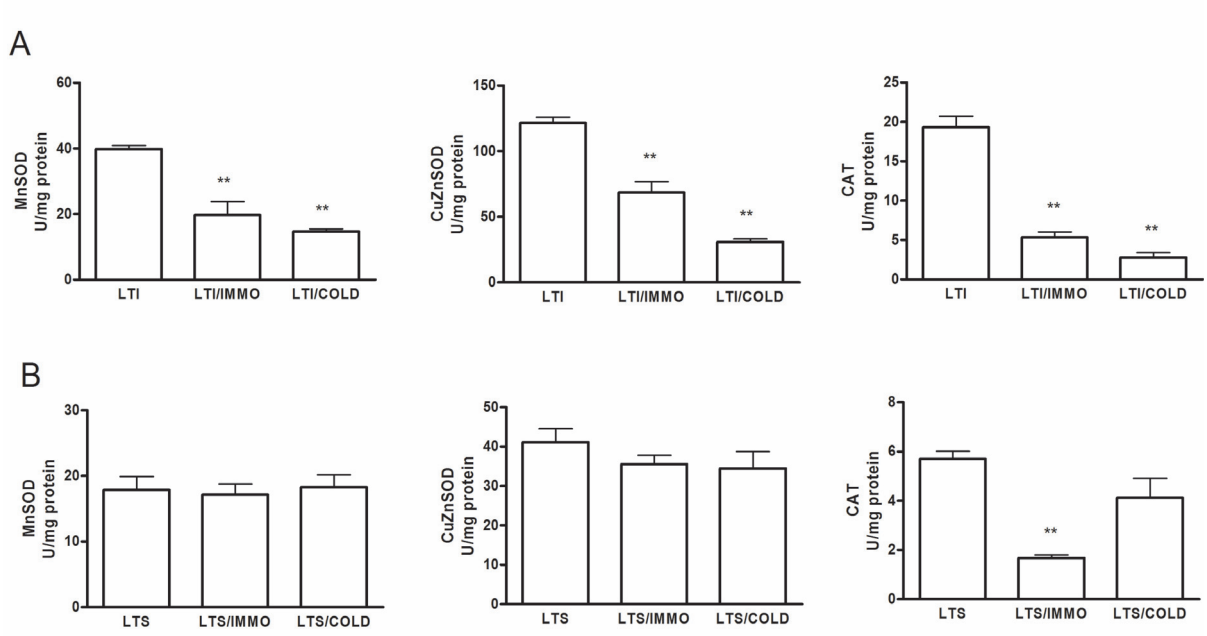

Fig. 4. The activities of MnSOD, CuZnSOD and CAT activities in brain cortex of rats pretreated with long-term isolation (LTI) or long-term forced swimming (LTS)), taken as control values, and exposed to combined chronic/acute stress by immobilization (IMMO) or cold (COLD). The values are mean±SEM of 6 animals per group. Symbol: **P<0.01 compared to the LTI (panel A), or the LTS (panel B) group, (Dunnett test).

LTS/IMMO, COLD) showed a significant main effect of chronic stress (F=15.13), acute stress (F=18.16), and the interaction effect (F=18.36) on MnSOD activity (P<0.001). Long-term stress participated in ~13%, acute stress in ~31% and interaction of these two factors in ~32% of total variation. For CuZnSOD activity, a significant main effect of chronic stress (F=95.35), acute stress (F=56.84), and interaction effect (F=41.99) was also recorded (P<0.001). Chronic stress participated in ~30%, acute stress in ~35% and interaction of these two factors in ~26% of total variation. For CAT activity, there was again a significant main effect of both types of stress; chronic stress (F=69.40), acute stress (F=89.41), and significant interaction effect (F=47.16), (P<0.001). Long-term stress participated in ~19%, acute stress in ~48% and interaction of these two factors in ~26% of total variation of enzyme activity.

Discussion

The results show that acute stress by immobilization elevated activities of both SOD enzymes in brain cortex. This observation is in accordance with the findings of ŞAHIN and GÜMÜŞLÜ (2004) and it differs from our previous study of acute stress effects in hippocampus. In that brain region, we found that both acute stresses, IMMO and COLD, decreased activities of CuZnSOD and CAT (PAJOVIC *et al.* 2006). Elevation of SOD activity, observed in this study, may indicate that this type of stress shifted brain cell-redox state towards a pro-oxidant direction. Immobilization was already shown to be a strong stressor that activates the sympathoneural and adrenomedular system thus increasing the plasma catecholamine level (KVETNANSKY *et al.* 2009). Since under stress conditions reaction of catecholamine hydroxyl residue with highly reactive superoxide radicals and quinones (HAQUE *et al.* 2003) have deleterious effects, swift elevation of SOD activity after acute IMMO may also reflect a preventive response against stress neurotoxicity.

Both chronic stresses, isolation and swimming, elevated activities of all examined AO enzymes in brain cortex, but regarding their effect, psychosocial stress of isolation seemed to be a stressor of higher intensity than physical stress of swimming. In our earlier study on the hippocampus region, we found a similar effect of both stress types on AOE activity, including the observed difference in AO response intensity (PAJOVIC *et al.* 2006). Social isolation of rodents is compared with psychological stressors in humans and different data regarding the AO response in brain regions have been obtained in studies to date. In rat cortico-striatum, chronic social isolation for 8 weeks increased SOD activity, along with increased lipid peroxidation and decreased oxidized/reduced GSH ratio, causing redox disturbances (MÖLLER *et al.* 2011). The recent study of SHAO *et al.* (2015) indicated opposite effects of social isolation after 8 weeks, in which SOD, CAT, GPx activities and total AO capacity decreased and H_2O_2 level increased, particularly in cortex and hippocampus. The increase of enzyme activity as a response to isolation stress found in our study may be a consequence of shorter observational period in which AO defense is still induced by increased radical production. This finding is supported by the study of MENABDE *et al.* (2011) in which 20 days of prolonged isolation increased SOD and CAT activity. However, after 40 days of social isolation, the activity of these enzymes was drastically reduced. These data also indicated that isolation stress served as a factor for excessive generation of free radicals that, to some degree, may be neutralized by the AO enzymes. However, prolonged stress may result in some irreversible processes that can result in dysfunction of the AO system in rat brain.

Physical exercise is classified as a type of stress but it also protects against deleterious effects of inflammation and oxidative stress in the brain by various molecular mechanisms. One of these mechanisms includes exercise-induced modulation of ROS levels and AO enzymes (GERECKE *et al.* 2013). Although exercise can induce ROS formation that may be detrimental to cellular functions, it was suggested that regular exercise causes strengthening of cellular antioxidant capabilities by a significant increase of AO enzyme activities, thus increasing resistance to oxidative stress and reducing cellular oxidative damage (MOGHADDASI *et al.* 2014). Increased activity of SOD and CAT recorded in our study after long term swimming is in accordance with some previous findings showing that swimming induced an increase in SOD activity after a 4-month swimming exercise (DEVI & KIRAN 2004), as well as GPx activity and lipid peroxidation (HARA *et al.* 1997). However, studies also show that AO status depends on many factors such as brain region, the type of physical exercise, its intensity and duration, and even the strain of rats (RADAK *et al.* 2013).

In rats chronically pretreated with isolation, additional stress by acute immobilization or cold markedly decreased activities of all examined AO enzymes in brain cortex but their activity still remained elevated, compared with control. After long term swimming, only additional stress by immobilization reduced CAT activity while both SOD activities also remained increased. The observed changes in AOE activities indicate that any stress type, acute, chronic or combined stress, may

alter the redox equilibrium and induce an adaptive response of the AO system in rat brain.

Adaptation to different stress stimuli can induce beneficial effects (GERECKE *et al.* 2013).In the brain, exercise has a preventive and therapeutic role in stroke and neurodegenerative diseases (RADAK *et al.* 2013) and it was shown to increase antioxidant gene expression in a variety of tissues, including brain (BRONIKOWSKI *et al*. 2002). Even one swimming session after immobilization stress attenuated oxidative stress markers in rat brain (RADAK *et al*. 2001). However, changes in redox equilibrium may also be a prerequisite for development of pathological processes, especially in the case of social isolation stress, flagged as a robust stressor. In humans, social stress is viewed as a major etiological factor for emotional disorders, including anxiety and depression. In animal models, beside behavior changes, social stress produces many changes in brain neuronal structure and in neurochemical transmission (BLANCHARD *et al*. 2001).

For all examined enzymes, the effects of additional acute stress exposure depended on the type of chronic stress, with social isolation being a more powerful factor than swimming. Although both types of acute stress significantly decreased AOE activity in brain cortex of the LTI- pretreated animals, acute stress by immobilization had a more profound effect on these enzymes. Taken together, the resultsof this study show that in brain cortex various types of stress affect AO enzymes to a different degree, most probably by generating an imbalance in production/elimination of ROS. Further investigation of different redox changes and their functional implications could provide more insight in stress-induced neuropsychological processes and their treatment.

References

BEUTLER E. 1984. *Catalase.* (In: Red Cell Metabolism: a Manual of Biochemical Methods. E. BEUTLER ed. Grune & Stratton Inc, Orlando):105-106.

BLANCHARD R.J., MC KITTRICK C.R., BLANCHARD D.C. 2001. Animal models of social stress: effects on behavior and brain neurochemical systems. Physiol. Behav. **73**: 261-271.

BRONIKOWSKI A.M., MORGAN T.J., GARLAND T., CARTER P.A. 2002. Antioxidant gene expression in active and sedentary house mice (*Mus domesticus*) selected for high voluntary wheel-running behavior. Genetics **161**: 1763-1769.

CHIURCHIÉ V., ORLACCHIO A., MACCARRONE M. 2016. Is Modulation of Oxidative Stress an Anwer? The State of the Art of Redox Therapeutic Actions in Neurodegenerative Diseases. Oxid. Med. Cell. Longev. **2016**, ID.7909380.

DEVI S.A., KIRAN T.R. 2004. Regional responses in antioxidant system to exercise training and dietary vitamin E in aging rat brain. Neurobiol. Aging. **25**: 501-508.

GELLER B.L., WINGE D.R. 1983. A method for distinguishing Cu, Zn-and Mn-containing superoxide dismutases. Anal. Biochem. **128**: 86-92.

GERECKE K.M., KOLOBOVA A., ALLEN S., FAWER J.L. 2013. Exercise protects against chronic restraint stress-induced oxidative stress in the cortex and hippocampus. Brain Res. **1509**: 66-78.

GOMES E.C., SILVA A.N., DE OLIVEIRA M.R. 2012. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. Oxid. Med. Cell. Longev. **2012**, ID.756132.

HALLIWELL B. 2006. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. **97**: 1634-1658.

HAQUE M.E., ASANUMA M., HIGASHI Y., MIYAZAKI I., TANAKA K., OGAWA N. 2003. Apoptosis-inducing neurotoxicity of dopamine and its metabolites via reactive quinone generation in neuroblastoma cells. Biochim. Biophys. Acta **1619**: 39-52.

HARA M., IIGO M., OHTANI-KANEKO R., NAKAMURA N., SUZUKI T., REITER R., HIRATA K. 1997. Administration of melatonin and related indoles prevents exercise-induced cellular oxidative changes in rats. Neurosignals **6**: 90-100.

HROUDOVÁ J., SINGH N., FIŠAR Z. 2014. Mitochondrial dysfunctions in neurodegenerative diseases: relevance to Alzheimer's disease. BioMed Res. Int. **2014**, ID.175062.

KVETNANSKY R., MIKULAJ L. 1970. Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. Endocrinology **87**: 738-743.

KVETNANSKY R., SABBAN E.L., PALKOVITS M. 2009. Catecholaminergic systems in stress: structural and molecular genetic approaches. Physiol. Rev. **89**: 535-606.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265-275.

MEISER J., WEINDL D., HILLER K. 2013. Complexity of dopamine metabolism. Cell Commun. Signal **11**: 34.

MENABDE K., KUCHUKASHVILI Z., CHACHUA M., CHIPASHVILI M., KOSHORIDZE N. 2011. Brain Oxidation Stress Caused by Isolation and Violation of Diurnal Cycle. Bull. Georg. Natl. Acad. Sci. **5**: N2.

MISRA H.P., FRIDOVICH I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. **247**: 3170-3175.

MOGHADDASI M., JAVANMARD S.H., REISI P., TAJADINI M., TAATI M. 2014. The effect of regular exercise on antioxidant enzyme activities and lipid peroxidation levels in both hippocampi after occluding one carotid in rat. J. Physiol. Sci. **64**: 325-332.

MÖLLER M., DU PREEZ J.L., EMSLEY R., HARVEY B.H. 2011. Isolation rearing-induced deficits in sensorimotor gating and social interaction in rats are related to cortico-striatal oxidative stress, and reversed by sub-chronic clozapine administration. Eur. Neuropsychopharmacol. **21**: 471-483.

PAJOVIC S.B., PEJIC S., STOJILJKOVIC V., GAVRILOVIC L., DRONJAK S., KANAZIR D.T. 2006. Alterations in hippocampal antioxidant enzyme activities and sympatho-adrenomedullary system of rats in response to different stress models. Physiol. Res. **55**: 453-460.

PEJIC S., STOJILJKOVIĆ V., TODOROVIĆ A., PAJOVIĆ S. 2006. CuZn-Superoxide dismutase in brain of rats exposed to acute, chronic or combined stress. Biotechnol. Biotechnol. Equip. **20**: 116-122.

RADAK Z., SASVARI M., NYAKAS C., KANEKO T., TAHARA S., OHNO H., GOTO S. 2001. Single bout of exercise eliminates the immobilization-induced oxidative stress in rat brain. Neurochem. Int. **39**: 33-38.

RADAK Z., MARTON O., NAGY E., KOLTAI E., GOTO S. 2013. The complex role of physical exercise and reactive oxygen species on brain. J. Sport Health Sci. **2**: 87-93.

RAHAL A., KUMAR A., SINGH V., YADAV B., TIWARI R., CHAKRABORTY S., DHAMA K. 2014. Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed. Res. Int. **2014**, ID.761264.

ŞAHIN E., GÜMÜŞLÜ S. 2004. Alterations in brain antioxidant status, protein oxidation and lipid peroxidation in response to different stress models. Behav. Brain Res. **155**: 241-248.

SAITO T., ISHIWATA T., HASEGAWA H., NOMOTO S., OTOKAWA M., AIHARA Y. 2005. Changes in monoamines in rat hypothalamus during cold acclimation. J. Therm. Biol. **30**: 229-35.

SHAO Y., YAN G., XUAN Y., PENG H., HUANG Q.J., WU R., XU H. 2015. Chronic social isolation decreases glutamate and glutamine levels and induces oxidative stress in the rat hippocampus. Behav. Brain Res. **282**: 201-208.

UTTARA B., SINGH A.V., ZAMBONI P., MAHAJAN R.T. 2009. Oxidative stress and neurodegenerative diseases: review of upstream and downstream antioxidant therapeutic options. Curr. Neuropharmacol. **7**: 65-74.

ZAIDI S.K., HODA M.N., TABREZ S., ANSARI S.A., JAFRI M.A., SHAHNAWAZ KHAN M., HASAN S., ALQAHTANI M.H., MOHAMMED ABUZENADAH A., B N. 2014. Protective effect of *Solanum nigrum* leaves extract on immobilization stress induced changes in rat's brain. Evid. Based Complement. Alternat. Med. **2014**, ID.912450.