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DNA damage after intracerebroventricular injection of ouabain in rats

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ABSTRACT

There is an emerging body of data suggesting that bipolar disorder is associated with DNA damage. Intracerebroventricular (ICV) administration of ouabain in rats results in manic-like alterations. We evaluated DNA damage of peripheral blood, cerebrospinal fluid and hippocampus of rats after ICV ouabain injection. Ouabain-induced hyperlocomotion was examined in an open field. Additionally, we used single cell gel electrophoresis (comet assay) to measure early transient damage in cerebrospinal fluid (CSF), hippocampus and blood; and the micronucleus test to measure persistent damage in total blood samples of rats after ouabain administration. Our findings demonstrated that ouabain induced hyperlocomotion in rats, and this response remained up to 7 days following a single ICV injection. In addition, we observed that the persistent increase in the rat spontaneous locomotion is associated with increased hippocampal and peripheral index of early DNA damage in rats. No significant alterations were observed in the micronucleus frequency in total blood samples of the rats after the ouabain ICV injection. These results suggest that ouabain may induce peripheral and central early DNA damage, but this early damage may be repaired.

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Recent studies have demonstrated that DNA damage may play a role in the pathophysiology of BD [1,4]. Andreazza et al. [2] showed that the frequency of DNA damage was correlated with the severity of symptoms of depression and mania. In addition, recent data suggests that oxidative stress may be involved in the pathophysiology of BD [1,24,27,31,37]. Studies have shown that oxidative stress can cause DNA damage [38,39,40,13], that can include chemical and structural modifications to purine and pyrimidine bases and 2′-deoxyribose and the formation of single- and double-strand breaks. Strand breaks within DNA can occur either directly, due to damage from reactive oxygen species exposure or indirectly, due to cleavage of the DNA backbone during DNA base excision repair [10,12].

The cardiac glycoside ouabain possesses stimulant properties [11,23] attributable to its ability to inhibit potently and selectively the activity of the sodium or its enzymatic version, the Na^+K^+-ATPase. In rats, intracerebral (ICV) administration of ouabain resulted in manic-like alterations [24]. The literature suggests that a small decrease in Na pump activity may result in neuronal hyperexcitability, giving rise to manic symptoms such as hyperactivity [11].

The ICV injection of ouabain induces hyperlocomotion in rodents, a common symptom of manic patients. Mynett-Johnson et al. [28] have demonstrated that an allelic association between bipolar disorder and a specific polymorphism in the gene encoding the $\alpha 3$ isoform of Na^+K^+-ATPase α subunit. In addition, the reduced $\alpha 2$ isoform of Na^+K^+-ATPase was found in the temporal cortex of patients with bipolar disorder [33].

The available evidences suggest that the hippocampus plays an important role in the pathophysiology of BD. This disease is thought to be associated with abnormalities within discrete brain regions associated with emotional regulation, particularly in fronto-limbic-subcortical circuits. Functional imaging studies showed abnormal brain activation in the hippocampus and its closely related regions during emotion, attention, and memory tasks (see [18]).

We designed the present study to investigate transient and persistent DNA damage in peripheral and hippocampus of rats subjected to an ICV injection of ouabain.

We used adult male Wistar rats (250–300 g) obtained from our breeding colony. Animals were housed five per cage with food and water available *ad libitum* and were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.) at a temperature of 22 ± 1 °C. All experimental procedures were performed in accordance with the approval of the local Ethics Committee.

Animals were anesthetized with ketamine (30 mg/kg) and xylasine (4 mg/kg) and the surgery was done according to a method

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reported previously [8]. Animals were tested on the third day following surgery. A 30 gauge cannula was fitted into the guide cannula and connected by a polyethylene tube to a microsyringe. The tip of the infusion cannula protruded 1.0 mm beyond the guide cannula aiming the right lateral brain ventricle.

Each animal was administered 5 μL of either vehicle (aCSF) or ouabain (10^{-3} M; Sigma Chemical, Saint Louis, USA; dissolved in aCSF) over 30 s and immediately after the ICV injection, the animals were placed into the open field.

We used the open field task to assess locomotor activity and crossings were counted for 5 min. The task was performed according Andreazza et al. [3].

Animals were observed in the open field for four sessions: (Session 1) before the cannula implantation (0 h); (Session 2) on the third day following surgery (72 h); (Session 3) on the fourth day following surgery (96 h), immediately after ICV injection of ouabain or saline; and (Session 4) 7 days after ICV injection of ouabain or saline, in agreement with the literature, that showed stimulatory effect of ouabain in rats immediately after the injection [24] and also nine days after a single injection of ouabain at 10^{-3} M [35].

Wistar rats were anesthetized with ketamine (80 mg/kg) and xylasine (20 mg/kg) after the least open-field session. The peripheral blood was collected by cardiac puncture, and cerebrospinal fluid (CSF) was drawn, by direct puncture of the cisterna magna with an insulin syringe (27 gauge 31/20 length). Individual samples of CSF that presented visible blood contamination were discarded [16]. Immediately after, rats were sacrificed by decapitation and the brain transferred within 1 min to ice-cold isolation buffer (0.23 M mannitol, 0.07 M sucrose, 10 mM Tris–HCl, and 1 mM EDTA, pH 7.4). The hippocampus was dissected in ice-cold buffer in a Petri dish. Peripheral blood, CSF and the hippocampus were submitted for genotoxic analyses.

A standard protocol for Comet assay preparation and analysis was adopted from Tice et al. [36]. The slides were prepared by mixing 10 μL of whole blood, CSF or hippocampus cells (washed and minced in cold PBS solution) with 90 μL of low-melting-point agarose (0.75%). The mixture (cells/agarose) was added to a fully frosted microscope slide coated with a layer of 500 μL of normally melting agarose (1%). After solidification, the coverslip was gently removed, and the slides were placed in lysis solution (2.5 M NaCl, 100 mM EDTA, and 10 mM Tris, pH 10.0–10.5, with freshly added 1% Triton X-100 and 10% DMSO) for 1 day. Subsequently, the slides were incubated in freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH > 13) for 10 min. The DNA was electrophoresed for 20 min at 25 V (0.90 V/cm) and 300 mA. After the electrophoresis slides were neutralized in 400 mM Tris (pH 7.5) and fixed (15%, w/v trichloroacetic acid, 5%, w/v zinc sulfate, 5% glycerol), washed in distilled water and over night dried. The gels were re-hydrated for 5 min in distilled water, and then stained for 15 min (37 °C) with a solution containing the following sequence: 34 mL of Solution B (0.2%, w/v ammonium nitrate, 0.2%, w/v silver nitrate, 0.5%, w/v tungstosilicic acid, 0.15%, v/v formaldehyde, 5%, w/v sodium carbonate) and 66 mL of Solution A (5% sodium carbonate). The staining was stopped with 1% acetic acid and the gels were air-dried [38].

Cells were scored from 0 (undamaged) to 4 (maximally damaged) according to the tail intensity (size and shape), resulting in a single DNA damage score (damage index) for each sample and, consequently, for each group. Thus, a damage index (DI) of the group could range from 0 (completely undamaged) 100 cells × 0) to 400 (maximum damage) 100 cells (×4) [5]. The percentage damage frequency (DF) was calculated for each sample on the basis of the number of cells with a tail versus with no tail.

The micronucleus (MN) assay for peripheral blood was carried out following standard protocols [22]. Smears for the MN assay were made with the same blood samples used for the Comet assay. Two whole blood smears from each animal were made on precleaned microscope slides, air-dried at room temperature, and stained in 5% Giemsa (Merck) in phosphate buffer (pH 5.8) for 10 min. The slides were coded, and the cells were blindly scored by light microscope at 1000× magnification. For each individual, the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) was determined in a total of 1000 erythrocytes per animal to evaluate cytotoxicity, and the frequency of micronucleated polychromatic erythrocytes in 2000 erythrocytes per rat was recorded to assess genotoxicity.

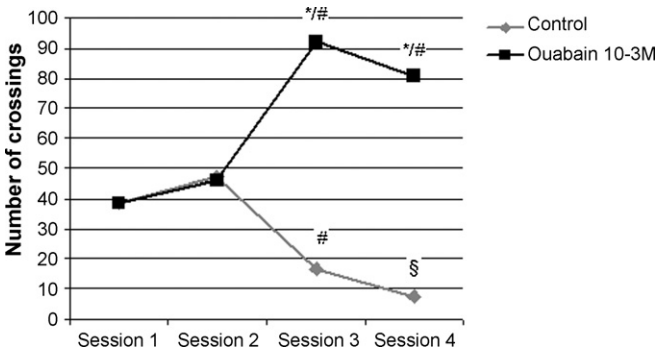


Fig. 1. Effects of the ICV injection of ouabain or saline on number of crossings in rats subjected to the open field test, for 5 min. Locomotor activity was assessed in the open-field test four times: before surgery (Session 1); (Session 2) on the third day following surgery; (Session 3) immediately after ICV injection of ouabain or saline, on the fourth day following surgery and (Session 4) 7 days after ICV injection. $^{*}p < 0.01$ vs. saline group, according to Student's t test. $^{\#}p < 0.05$ compared with Session 2, analyzed by the paired Student's t test. $^{\S}p < 0.05$ compared with Session 3, analyzed by the paired Student's t test.

Data were analyzed by Student's t test and are expressed as mean ± standard deviation. All analyses were performed using the Statistical Package for the Social Science (SPSS) software. In the open-field, the differences between sessions were analyzed by the paired Student's t test. In all experiments, the level of significance was $p < 0.05$.

As illustrated in Fig. 1, the spontaneous locomotion of rats submitted to the open field apparatus during the first and second sessions was not different among groups. However, as illustrated in Fig. 1 (Session 3), the ICV administration of ouabain increased rat spontaneous locomotion compared to control group immediately after injection and hyperlocomotion was still observed 7 days following a single ICV injection of ouabain ($p < 0.001$). In addition, in the open-field apparatus, aCSF-treated rats displayed reduced number of crossings when re-exposed 24 h later to the apparatus (Session 3), thus indicating habituation to the environment. However, rats ICV injected with ouabain immediately before testing displayed increased number of crossings (Session 3) compared to the crossings displayed in the Session 2, which indicates that acute ICV administration of ouabain induces an immediate motoric hyperactivity in the rat. aCSF-treated rats displayed reduced number of crossings when re-exposed 7 days latter, in Session 3 (Session 4), indicating that habituation was still observed. Importantly, rats ICV injected with ouabain did not present significant differences in locomotor activity among Session 3 and Session 4, indicating that hyperactivity was still observed.

As shown in Fig. 2, ICV injection of ouabain showed no difference of DNA damage in CSF when compared with the control group. However, the administration of ouabain significantly increased DNA damage in the hippocampus and blood ($p < 0.001$) compared to control group. Results for Micronucleus test are shown in Fig. 3; ouabain did not alter the frequency of micronucleus in comparison to the control group in blood of rats.

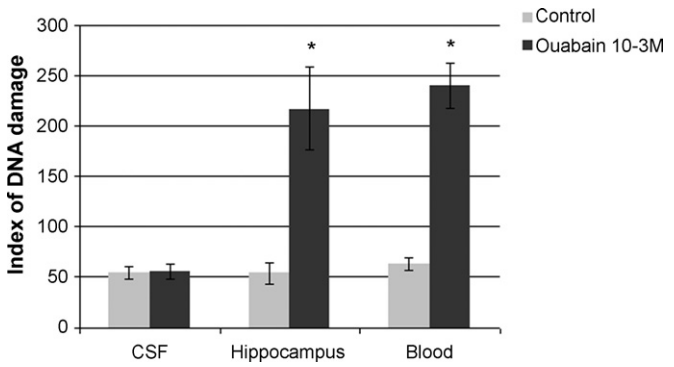


Fig. 2. Effects of the ICV injection of 5 μL of vehicle (aCSF) or ouabain (10^{-3} M; dissolved in aCSF) on index of damage in blood, CSF and hippocampus of rats evaluated with the Comet assay. Values are expressed as means and standard deviation. $^{*}p < 0.01$ vs. saline group, according to Student's *t* test.

Previous studies showed that immediately administration of ICV ouabain in rats may produce manic-like behavioral [9,12,34]. Ruktanonchai et al. [34] demonstrated that hyperactivity induced by ouabain remains about 1 week, and suggested that this persistent increase in locomotor activity is associated with increased hippocampal sodium pump units (as reflected by increase in [^{3}H] ouabain binding) and a decreased in sodium pump activity. The inhibition of Na–K ATPase induces depolarization-inducing neuronal excitation [27], which might be related to the increased locomotor activity. In the present study we demonstrated that ICV administration of ouabain increased locomotor activity in rats, and this hyperactivity remains 7 days after a single ICV injection, replicating previous studies.

Recently, Jornada et al. [20] showed that ouabain-induced hyperlocomotion can be prevented and reversed with classical mood stabilizers drugs such as lithium and valproate. In addition, these authors demonstrated changes in brain-derived neurotrophic factor (BDNF) expression in this animal model of mania, indicating a good face, predictive and construct validity.

The single-cell gel-electrophoresis technique (comet assay) is used to detect transient DNA single- and double-strand breaks [30] whereas the DNA damage that persists after the action of different repair systems can be assessed using the micronucleus test [15,26]. The understanding of the transient and permanent consequences of oxidative stress to DNA integrity has turned into central issues in the study of psychiatric disorders, which include BD [3,4,29,17]. Here, we demonstrated that ouabain injection induced a significant increase in transient DNA damage in the hippocampus and blood of rats, but not in the CSF. Interestingly, we have previously demonstrated an increased in transient DNA damage in hippocampus and blood samples, in a dopaminergic animal model of mania induced by amphetamine [3]. Andreazza et al. [2] have shown that DNA damage was markedly increased in BD patients and, this DNA damage was correlated with the severity of symptoms of depression and mania. In this context, studies showed that oxidative stress was increased in both BD patients [21,23,25,32] and animal model of mania [19,38].

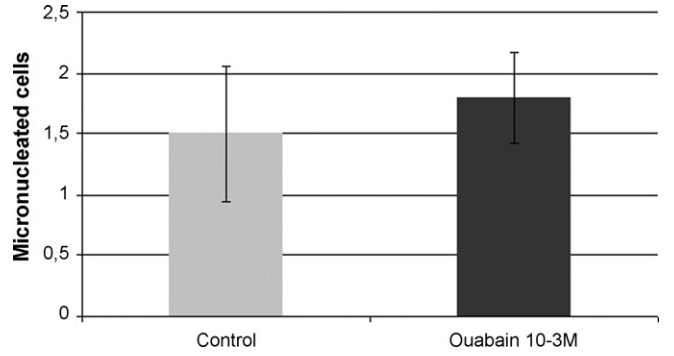


Fig. 3. Effects of the ICV injection of 5 μL of vehicle (aCSF) or ouabain (10^{-3} M; dissolved in aCSF) on frequency of micronucleus in rats. We carried out the micronucleus analysis in erythrocytes. Values are expressed as means and standard deviation. $^{*}p < 0.05$ vs. saline group, according to Student's *t* test.

According Plata-Salamán [31] the nervous system, through the production of neuroregulators (neurotransmitters, neuromodulators and neuropeptides), can regulate specific immune system functions, while the immune system, through the production of immunoregulators (immunomodulators and immunopeptides). The presence of immunoregulators in the brain and cerebrospinal fluid is the result of local synthesis, by intrinsic and blood-derived macrophages, activated T-lymphocytes that cross the blood–brain barrier, endothelial cells of the cerebrovasculature, microglia, astrocytes, and neuronal components, and/or uptake from the peripheral blood through the blood–brain barrier (in specific cases) and circumventricular organs. Acute and chronic pathological processes stimulate the synthesis and release of immunoregulators in various cell systems. These immunoregulators have pivotal roles in the coordination of the host defense mechanisms and repair, and induce a series of immunological, endocrinological, metabolical and neurological responses. This may explain at least in part the lack of damage in the liquor.

Xie et al. [41] have demonstrated that partial inhibition of Na^+K^+-ATPase by ouabain increased reactive oxygen species (ROS) production. ROS formation is a constant element of a cell's oxygen metabolism. They are its normal products and in physiological concentrations they play important roles in a variety of cell processes. Disturbances in the balance between ROS formation and the efficiency of antioxidant mechanisms lead to oxidative stress, that causes damage to important macromolecules, such as DNA, proteins, and lipids (see [35]).

In the present study, we did not find change in the frequency of micronucleus; these results suggest that ouabain induces only transient damage to DNA. It is known that there are multiples systems to repair early damage and prevent lesion removal [6,7,14]. This suggests that ouabain may have transient genotoxic effects; however ouabain does not appear to be mutagenic. In addition, is important to emphasize that the DNA damage detected with the comet assay could give rise not only to cytogenetic damage, but also to point mutations that are not detectable by micronucleus test. The response of the cell to DNA damage and its ability to maintain genomic stability by DNA repair is very important for the elucidation of the physiological mechanisms involved in the BD pathogenesis, facilitating the identification of targets in order to design drugs for the treatment of this disease.

The persistent increase in the rat spontaneous locomotion induced by ouabain is associated with increased in transient DNA damage in hippocampus and blood samples. However, we did not observe an increase in micronucleus frequency, suggesting that such transient DNA damage might have been repaired by endogenous repairing systems. Thus, ouabain-induced mania-like behavior may provide a useful model to test the hypothesis of transient DNA damage to bipolar disorder. Studies addressing the effect of mood stabilizers and antipsychotics on this animal model may provide new insights about the pathophysiology of this complex psychiatric disorder.

Acknowledgements

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References

[1] A.C. Andreazza, C. Cassini, A.R. Rosa, M.C. Leite, L.M. de Almeida, P. Nardin, A.B. Cunha, K.M. Cereser, A. Santin, C. Gottfried, M. Salvador, F. Kapczinski, C.A. Gonçalves, Serum S100B and antioxidant enzymes in bipolar patients, J. Psychiatr. Res. 41 (2007) 523–529.

[2] A.C. Andreazza, B.N. Frey, B. Erdtmann, M. Salvador, C.A. Goncalves, F. Kapczinski, DNA damage in bipolar disorder, Psychiatry Res. 153 (2007) 27–32.

[3] A.C. Andreazza, M. Kauer-Sant'Anna, B.N. Frey, L. Stertz, C. Zanotto, L. Ribeiro, K. Giasson, S.S. Valvassori, G.Z. Réus, M. Salvador, J. Quevedo, C.A. Gonçalves, F. Kapczinski, Effects of mood stabilizers on DNA damage in an animal model of mania, J. Psychiatry Neurosci. 33 (2008) 516–524.

[4] N. Buttner, S. Bhattacharyya, J. Walsh, F.M. Benes, DNA fragmentation is increased in non-GABAergic neurons in bipolar disorder but not in schizophrenia, Schizophr. Res. 93 (2007) 33–34.

[5] A.R. Collins, D.L. Mitchell, A. Zunino, J. de Wit, D. Busch, UV-sensitive rodent mutant cell lines of complementation groups 6 and 8 differ phenotypically from their human counterparts, Environ. Mol. Mutagen. 29 (1997) 152–160.

[6] M.S. Cooke, M.D. Evans, R. Dove, R. Rozalski, D. Gackowski, A. Siomek, J. Lunec, R. Olinski, DNA repair is responsible for the presence of oxidatively damaged DNA lesions in urine, Mutat. Res. 574 (2005) 58–66.

[7] C. Culmsee, M.P. Mattson, p53 in neuronal apoptosis, Biochem. Biophys. Res. Commun. 331 (2005) 761–777.

[8] J.L. Davis, D.T. Masuoka, L.K. Gerbrandt, A. Cherkin, Autoradiographic distribution of L-proline in chicks after intracerebral injection, Physiol. Behav. 22 (1979) 693–695.

[9] S. Decker, G. Grider, M. Cobb, Open field is more sensitive that automated activity monitor in documenting ouabain-induced hyperlocomotion in the development of an animal model for bipolar illness, Prog. Neuropsychopharmacol. Biol. Psychiatry 24 (2000) 455–462.

[10] S.F. El-Khamisy, K.W. Caldecott, TDP1-dependent DNA single-strand break repair and neurodegeneration, Mutagenesis 21 (2006) 219–224.

[11] R.S. El-Mallakh, The Na,K-ATPase hypothesis for manic-depression. I. General considerations, Med. Hypotheses 12 (1983) 253–268.

[12] R.S. El-Mallakh, S. Hedges, D. Casey, Digoxin encephalopathy presenting as mood disturbance, J. Clin. Psychopharmacol. 15 (1995) 82–83.

[13] I. Emerit, Reactive oxygen species, chromosome mutation, and cancer: possible role of clastogenic factors in carcinogenesis, Free Radical Biol. Med. 16 (1994) 99–109.

[14] G Evan, T. Littlewood, A matter of life and cell death, Science 281 (1998) 1317–1322.

[15] M. Fenech, A.A. Morley, Cytokinesis-block micronucleus method in human lymphocytes: effect of in vivo ageing and low dose X-irradiation, Mutat. Res. 161 (1986) 193–198.

[16] J.J. Fortunato, F.R. Agostinho, G.Z. Réus, F.C. Petronilho, F. Dal-Pizzol, J. Quevedo, Lipid peroxidative damage on malathion exposure in rats, Neurotox. Res. 9 (2006) 23–28.

[17] B.N. Frey, A.C. Andreazza, M. Kunz, F.A. Gomes, J. Quevedo, M. Salvador, C.A. Gonçalves, F. Kapczinski, Increased oxidative stress and DNA damage in bipolar disorder: a twin-case report, Prog. Neuropsychopharmacol. Biol. Psychiatry 31 (2007) 283–285.

[18] B.N. Frey, A.C. Andreazza, F.G. Nery, M.R. Martins, J. Quevedo, J.C. Soares, F. Kapczinski, The role of hippocampus in the pathophysiology of bipolar disorder, Behav. Pharmacol. 18 (2007) 419–430.

[19] B.N. Frey, S.S. Valvassori, G.Z. Réus, M.R. Martins, F.C. Petronilho, K. Bardini, F. Dal-Pizzol, F. Kapczinski, J. Quevedo, Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania, J. Psychiatry Neurosci. 31 (2006) 326–332.

[20] L.K. Jornada, M. Moretti, S.S. Valvassori, C.L. Ferreira, P.T. Padilha, C.O. Arent, G.R. Fries, F. Kapczinski, J. Quevedo, Effects of mood stabilizers on hippocampus and amygdala BDNF levels in an animal model of mania induced by ouabain, J. Psychiatr. Res., in press.

[21] F. Kapczinski, B.N. Frey, A.C. Andreazza, M. Kauer-Sant'Anna, A.B. Cunha, R.M. Post, Increased oxidative stress as a mechanism for decreased BDNF levels in acute manic episodes, Rev. Bras. Psiquiatr. 30 (2008) 243–245.

[22] G. Krishna, M. Hayashi, In vivo rodent micronucleus assay protocol; conduct and data interpretation, Mutat. Res. 455 (2000) 155–166.

[23] M. Kunz, C.S. Gama, A.C. Andreazza, M. Salvador, K.M. Ceresér, F.A. Gomes, P.S. Belmonte-de-Abreu, M. Berk, F. Kapczinski, Elevated serum superoxide dismutase and thiobarbituric acid reactive substances in different phases of bipolar disorder and in schizophrenia, Prog. Neuropsychopharmacol. Biol. Psychiatry 32 (2008) 1677–1681.

[24] R. Li, R.S. el-Mallakh, L. Harrison, D.G. Changaris, R.S. Levy, Lithium prevents ouabain-induced behavioral changes. Toward an animal model for manic depression, Mol. Chem. Neuropathol. 31 (1997) 65–72.

[25] R. Machado-Vieira, A.C. Andreazza, C.I. Viale, V. Zanatto, V. Cereser Jr., R. da Silva Vargas, F. Kapczinski, L.V. Portela, D.O. Souza, M. Salvador, V. Gentil, Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects, Neurosci. Lett. 421 (2007) 33–36.

[26] S.W. Maluf, B. Erdtmann, Follow-up study of the genetic damage in lymphocytes of pharmacists and nurses handling antineoplastic drugs evaluated by cytokinesis-block micronuclei analysis and single cell gel electrophoresis assay, Mutat. Res. 471 (2000) 21–27.

[27] M. McCarren, B.E. Alger, Sodium-potassium pump inhibitors increase neuronal excitability in the rat hippocampal slice: role of a Ca2+-dependant conductance, J. Neurophysiol. 57 (1989) 496–509.

[28] L. Mynett-Johnson, V. Murphy, J. McCormack, D.C. Shields, E. Claffey, P. Manley, P. McKeon, Evidence for an allelic association between bipolar disorder and a Na+, K+ adenosine triphosphatase alpha subunit gene (ATP1A3), Biol. Psychiatry 44 (1998) 47–51.

[29] N.H. Myung, X. Zhu, I.I. Kruman, R.J. Castellani, R.B. Petersen, S.L. Siedlak, G. Perry, M.A. Smith, H.G. Lee, Evidence of DNA damage in Alzheimer disease: phosphorylation of histone H2AX in astrocytes, Age (Dordr). 30 (2008) 209–215.

[30] J.N. Picada, D.G. Flores, C.G. Zettler, N.P. Marroni, R. Roesler, J.A. Henriques, DNA damage in brain cells of mice treated with an oxidized form of apomorphine, Brain Res. Mol. Brain Res. 114 (2003) 80–85.

[31] C.R. Plata-Salamán, Immunoregulators in the nervous system, Neurosci. Biobehav. Rev. 5 (1991) 185–215.

[32] P.K. Ranjekar, A. Hinge, M.V. Hegde, M. Ghate, A. Kale, S. Sitasawad, U.V. Wagh, V.B. Debsikdar, S.P. Mahadik, Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients, Psychiatry Res. 121 (2003) 109–122.

[33] A.M. Rose, B.J. Mellett, R. Valdes Jr., J.E. Kleinman, M.M. Herman, R. Li, R.S. El-Mallakh, Alpha 2 isoform of the Na,K-adenosine triphosphatase is reduced in temporal cortex of bipolar individuals, Biol. Psychiatry 44 (1998) 892–897.

[34] D.J. Ruktanonchai, R.S. El-Mallakh, R. Li, R.S. Levy, Persistent hyperactivity following a single intracerebroventricular dose of ouabain, Physiol. Behav. 63 (1998) 63403–66406.

[35] D. Scibior-Bentkowska, H. Czeczot, Cancer cells and oxidative stress, Postepy Hig. Med. Dosw. (Online) 63 (2009) 58–72.

[36] R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, Y.F. Sasaki, Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen. 35 (2000) 206–221.

[37] S.S. Valvassori, F.C. Petronilho, G.Z. Réus, A.V. Steckert, V.B. Oliveira, C.R. Boeck, F. Kapczinski, F. Dal-Pizzol, J. Quevedo, Effect of N-acetylcysteine and/or deferoxamine on oxidative stress and hyperactivity in an animal model of mania, Prog. Neuropsychopharmacol. Biol. Psychiatry 32 (2008) 1064–1068.

[38] I.V. Villela, I.M. Oliveira, J. Silva, J.A.P. Henriques, DNA damage and repair in haemolymph cells of golden mussel (*Limnoperna fortunei*) exposed to environmental contaminants, Mutat. Res. 697 (2006) 78–86.

[39] D. Wang, D.A. Kreutzer, J.M. Essigmann, Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions, Mutat. Res. 400 (1998) 99–115.

[40] N.T. Watt, M.N. Routledge, C.P. Wild, N.M. Hooper, Cellular prion protein protects against reactive-oxygen-species-induced DNA damage, Free Radical Biol. Med. 43 (2007) 959–967.

[41] Z. Xie, P. Kometiani, J. Liu, J.I. Shapiro, A. Askari, Intracellular reactive oxygen species mediate the linkage of Na+/K+-ATPase to hypertrophy and its marker genes in cardiac myocytes, J. Biol. Chem. 274 (1999) 19323–19328.