Intranasal Oxytocin in Autism: Models, Pain and Oxidative Stress

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There is an increased interest in the current literature in how relevant the administration of oxytocin, mostly by using its intranasal administration, could be in some neuropsychiatric disorders and especially in those manifesting a deficit at their sociability level. These aspects made the possible usage of oxytocin as extremely attractive for some management and treatment solutions in the autistic pathology. Thus, we are describing here some original data and current literature status on how oxytocin could help pathophysiological autistic manifestations in both animal models and human patients, by mainly focusing on some specific behavioural or pain manifestations and related oxidative stress status.

Keywords: oxytocin, autism, model, pain, oxidative stress

Lately there is an increased awareness in how relevant the administration of oxytocin (mostly by using the intranasal route) could be in some neuropsychiatric disorders and especially in those manifested partially by a deficit in their social manifestations [1-3]. In this way, although its social effects are known for quite some time [4], this neuropsychiatric target is actually a new approach for this peptide, classically known for its effects on stimulating lactation and parturition [5].

Thus, the administration of intranasal oxytocin, which will theoretically reach the brain through some various previously described ways [6, 7] seems to exert promising effects in disorders such as schizophrenia [1, 8], anxiety and depression [9, 10], Prader Willi syndrome [3], frontotemporal dementia [11] and of course autism [2, 12, 13].

In fact, there is a variety of studies demonstrating some interesting effects of intranasal oxytocin on the autistic pathology. Probably the most influential study group in this area of research is represented by the one lead by Prof. Adam Guastella in the Autism Clinic for Translational Research/Brain and Mind Centre in Sydney. Thus, firstly this group demonstrated for the first time in the literature in 2010 that the administration of 18 or 24 International Units (IU) of intranasal oxytocin (single dose and a placebo nasal spray one week apart) in a double-blind, randomized, placebo-controlled study on 16 boys (from 12 to 19 years old of age) with autism spectrum disorders resulted in improved performance on the Reading the Mind in the Eyes Task, also with a more prominent effect on the easy items, as compared to the hard items of the test, where no significant effects were reported versus placebo [12].

Still, there were previous publications in this area of research, such as the one of the Hollander group, which showed reduced repetitive behaviours (e.g. need to know, repeating, ordering, need to tell/ask, self-injury and touching) in adults with Asperger's disorder and autism (n=15) [14], as well as some increase in social cognition, manifested by a superior comprehension of affective speech in neutral content sentences [15].

Subsequently, in a publication in 2016 the same Guastella group showed in a five weeks double-blind, randomized, placebo-controlled, crossover study on 31 children with autism, that 24 IU intranasal oxytocin per day (12 in the morning and 12 in the night), with a 4-week washout, results in clear improvements in the primary outcome as given by the caregiver social responsiveness [13].

Of course, all these results are extremely promising in raising the possibility that intranasal oxytocin could be used, in fact, on a larger scale, in the near future as a complimentary treatment option in the autistic pathology, considering also the clear lack of an effective treatment interventions in this disorder right now.

On the other side, there are very well design studies demonstrating that actually things are much more complicated in this area of research and demonstrating no significant effects of intranasal oxytocin administration in autism, such as the one lead by Prof. Dadds [16] also in Australia, which included 38 children with autism (from 7 to 16 years old) receiving 24 or 12 international units (depending on weight) intranasal oxytocin or placebo, administrated only during the specific parent-child interaction training sessions, once daily over 4 days consecutively. In this way, the Dadds report stated that there are actually no differences in the effects of intranasal oxytocin vs. placebo in terms of emotion recognition, social interaction skills and general behaviour [16].

Another worth to mention example in this context, could be represented by the study of Anagnostou et al. [17] which showed in 19 adults (average of 33 years old) with autism spectrum disorders that after 24 IU of intransal oxytocin there were no modifications in parameters such as social function and cognition (the Diagnostic Analysis of Nonverbal Accuracy) or repetitive behaviors (Repetitive Behavior Scale Revised), while still on aspects such as Social Responsiveness Scale, Reading-the-Mind-in-the-Eyes Test and the Yale Brown Obsessive Compulsive Scale there were improvements especially after 6 weeks, in terms of social cognition and quality of life, as measured by World Health Organization Quality of Life Questionnaire.

The reasons for all these discrepancies and controversial results could be represented by a variety of factors ranging from doses used to the age of patients used, as well as the

individual variability and the small number of patients selected, and were reviewed quite extensively before such as in the paper of Lee in 2015 [18], and with reports also describing and trying to standardize how the intranasal administration should be methodologically performed [19].

Besides this, almost all studies mentioned above reported no serious side effects as a result of the various dosage of intranasal oxytocin in the pathology of autism (e.g. these were mainly represented by thirst, urination and constipation-[13]), which could be quite important for the further use of this peptide as an effective autistic treatment.

Also, another aspect worth mentioning here is represented by the pain perception in autistic pathology, which is known to be quite a problem, especially considering that now the classical theories stating that most of the ASD patients have lowered or even abolished sensitivity to pain [20, 21], are questioned and challenged heavily to this date [22-24], since oxytocin was previously cited as having some interesting analgesic effects [25, 26].

Thus, considering the interests of our group in most of the neuropsychiatric disorders, pain [27, 28] and lately oxytocin, we are planning to further study these aspects in the autistic pathology, by using some rodent models of autism, as we are going to insist in the next section of our mini-report.

Experimental part

Regarding the animal models, and especially when we talk about the animal models mimicking the complex human neuropsychiatric disorders symptomatology, we should state from the beginning that there is impossible to perfectly replicate an animal model for a neuropsychiatric disease [29].

In this way, most of the models for these disorders are based on manipulating some neurotransmitters or affecting brain areas which are fundamental in that pathology (by using for example neurosurgery methods – [30]), or involving various behavioural tests [31] and deleting/ altering some essential genes in those deficiencies.

Of course, the usage of the animal models has some advantages, such as being easier to obtain, maintain and handle, less expensive and displaying increased reproductive series [29]. In this way, various animal models of schizophrenia (based for example on ketamine, methionine or phencyclidine administration), Alzheimer's disease, Parkinson's disease (e.g. – by administrating dopaminergic toxins such as 6-OHDA [32, 33] or MPTP [34] straight into the substantia nigra by neurosurgery), depression (e.g. the usage of forced swim test – [35]), anxiety (the usage of elevated plus maze – [36,37]) and of course autism (as we were going to insist immediately) were design based on genetic, behavioural or specific neurotransmitters manipulations [29].

Regarding the animal models of autism, these are even trickier to develop (of course we cannot replicate feelings in rats!), and they are mainly based on replicating two fundamental behavioural factors of the disorder: the social interaction and the repetitive behaviours [38-40], as well as manipulating the genes known for being implicated in some degree in the autistic pathology [41-43].

In this way, perhaps one of the most important behavioural models of autism is based on the perinatal administration of the antiepileptic valproic acid, considering that it is known for quite some time that the administration of this drug during pregnancy increases very much the risk for autism [44]. Thus, it was firstly showed by Rodier group that the administration of an increased dose (350 mg/kg) of valproic acid around the 12th day of gestation (they firstly tried 11.5, which is actually the day of neural tube closure, day 12 and day 12.5 gestation) are affecting the number of neurons in the cranial nerve motor nuclei [38]. It was later confirmed that this procedure, performed exactly in the 12.5 day of gestation is resulting in the a range of autistic behavioural manifestation, such as reduced social interaction, repetitive and stereotypical locomotor manifestations, lower sensitivity to pain, increased anxiety, depression and some social memory deficits [40,45,46], as well as anatomic manifestation related to cerebellar abnormalities, decreased Purkinje cells in the vermis, deficits in synaptic plasticity, reduced prefrontal dopaminergic activity, alterations in the endocannabinoid system and cranial nerve motor nuclei deficits [47-50], especially when given at the higher doses of 500 or usually 600 [51, 52].

In addition, we have to mention that especially for the social behavioural manifestations in this model, specific behavioural tasks were design with a special focus of the one proposed by the Crawley group, which showed that this could tested in a three-chambered apparatus which gives the rodent a choice between a familiar environment, a novel environment, and a novel environment containing a stranger individual, as well as the usage of T-maze and water-maze tests for repetitive and stereotypical manifestations or specific equipments designed to record the vocalisation degree between these pups with *autism* [53].

Thus, when it comes to the relation between oxytocin and this valproic acid-induced rat model of autism, there is only one study available in literature, according to our best of knowledge, by Štefanik et al. [54], which showed that actually valproic acid perinatal administration results in increased sociability and gene expression of oxytocin and its receptor.

In addition, there are a few studies designed to see the effects of intranasal oxytocin administration in some genetic rat models of autism. The Bales group [55] showed for example that in the BTBR T+ Itpr3tf/J inbred strain, which was previously characterized behaviourally by low sociability and increased repetitive behaviours, the administration of 0.8 IU/kg intranasal oxytocin for 30 days results in unclear manifestations over the autistic behavioural manifestations, with no significant effects in tests such as three-chambered social approach, juvenile reciprocal social interactions, open-field, repetitive grooming or some conditioned memory, except for sniffing behaviour in the social interaction test [55].

In addition, the group of Teng [56] previously showed that also the peripheral oxytocin administration for a few weeks can rescue some social deficiencies, as measured one day after the final dosage administration, in another two genetic models of autism (e.g. BALB/cByJ and C58/J) [56].

Even more, the same group later confirmed these results in adults C58/J mice, which after 2 weeks oxytocin treatment displayed increased social behaviour [57]. Even more, in the same study, another genetic model of autism (male Grin1 knockdown mice) showed clear pro-social effects after oxytocin administration, with these effects being superior to classical drugs such as clozapine (66 mg/kg/day) or risperidone (2 mg/kg/day) [57].

Coming back, to VPA model, we should also mention here that oxidative stress metabolism could also represent an important factor in the valproic acid expressed autistic-like effects in this model (as proposed by Mabunga et al. in their review in 2013 [46], where they are stating that

valproic acid could generate embryonic/fetal deficiencies mediated also by the reactive oxygen species).

In fact, it was previously showed in humans, at in least in 2 studies, that valproic acid in children with epilepsy is increasing oxidative stress status, in the urine (through the measuring of isoprostanes) [58] and in the blood (decreased levels of vitamin E and increased malondiadehyde) of overweight children with epilepsy (but not in non-obese epileptic children), after one year of valproic acid treatment [59], while Chang and Abbott showed that oxidative stress may be an important of valproic acid generated hepatotoxicity [60].

Still, this is also a controversial area of research, since some other groups showed for example that small dosages of valproic acid (e.g 1- 10 mg/kg) could attenuate some memory deficits induced by neurotoxin trimethyltin in Morris water maze and passive avoidance task, by a possible antioxidant effect, as opposite to the pro-oxidant effects of the trimethyltin (manifested as increased levels of MDA and decreased thiol concentrations in the brain) [61].

In fact, there are previous studies demonstrating that even the perinatal exposure to valproic acid and the generation of the rat model of autism, with its novel object recognition deficits and hippocampal dendritic loss, can be rescued by 5 weeks of VPA treatment (30 mg/kg), starting at the age of 4 weeks [62], while also the perinatal model generated by valproic acid administration does increase synaptic plasticity in prefrontal cortex, suggesting some mechanisms for this pathology [63].

While of course, the influence of the oxidative stress status in most of the neuropsychiatric disorders is lately gaining a lot of attention with the reactive oxygen species being cited as having important implications in most of the neurological [64, 65] or psychiatric disorders [66, 67], things are not different in autism, where it was previously demonstrated through systematic reviews and meta-analysis that oxidative stress could be implicated in autistic pathology, with special focus on glutathione metabolism [68, 69] or in relation to mitochondrial dysfunction [70] and inflammation [71].

In fact, previous groups showed also in the animal model of autism based on the valproic acid perinatal administration that the administration of some products or drugs which could exert some antioxidant actions are reducing the behavioural deficits associated with the respective model. Thus, Al-Amin group [72] demonstrated that astaxanthin, described by the authors as being 500 times more potent antioxidant then á-tocopherol, is reducing behavioural deficits and increased oxidative stress status in valproic acid induced model of autism, while other groups showed similar effects, including histopathological changes, for a combined Extract of Purple Rice and Silkworm Pupae [73]. In addition, related or not to oxidative stress status, several other products were demonstrated to rescue also the anatomic, behavioural (Korean Red Ginseng – [74,75]) and cognitive deficits (bumetanide – [76]) in the aforementioned perinatal valproic rat model of autism.

As stated above, for example the Morakotsriwan group [73], which worked on the Purple Rice and Silkworm Pupae extracts, clearly showed these increase oxidative stress status in the VPA induced perinatal model of autism, demonstrated by a significant decrease in the specific activity of the all the three antioxidant enzymes: superoxide dismutase, glutathione peroxidase and catalase, as well as an increase of the malondialdehyde concentration, as a main marker of the lipid peroxidation processes [73].

Results and discussions

Our research group previously demonstrated that the perinatal administration of the valproic acid in rats could result in increased oxidative stress status in the temporal lobe of weaning pups born from rat-female which received valproic acid, as demonstrated by a significant ($p < 0.05$) decrease of glutathione peroxidase specific activity (fig. 1) and an increased ($p < 0.001$) in the temporal lobe concentration of malondiadehyde (fig. 2).

Moreover, our research group also recently demonstrated that probably by its antioxidant effects [77], the administration of intranasal oxytocin for 10 consecutive days (20 IU) will result in reduced memory, anxiety and depression-related deficits in a valproic acid-induced rat model of autism [78].

Also, regarding pain, while there is of course a correlation between pain and oxidative stress status [79, 80], the study of pain in the autism models showed some interesting results, as for example the Schneider group demonstrated similar results to those in humans, mainly expressed of course as a reduced general pain sensitivity [40], tested in tail flick test (e.g. for spinal pain) and paw withdrawal task (use for supraspinal pain), plus an increased sensitivity to non painful stimuli [40]. In addition, similar aspects regarding decreased reactivity to thermal nociceptice stimuli were previously described in a BTBR T+tf/J genetic mouse model of autism [43].

Moreover, some antioxidants applied in the VPA model, which were described above, seems to improve this pain-

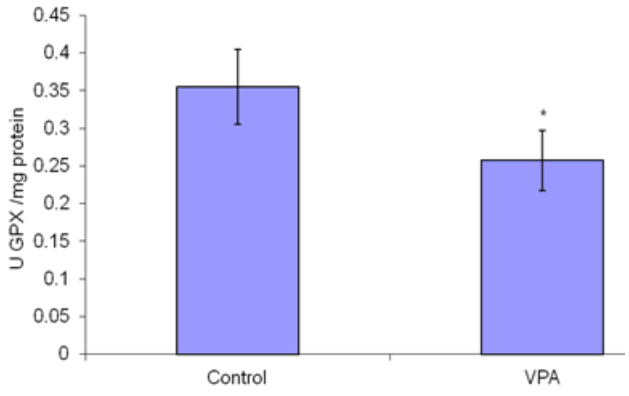

Fig. 1. The effects of perinatal administration of the valproic acid (VPA) in weaning pups born from rat-female which received valproic acid on glutathione peroxidase specific activity from the temporal lobe. The values are mean± S.E.M. (n = 13 per control group and n = 7 for VPA group). *p > 0.05 vs. control

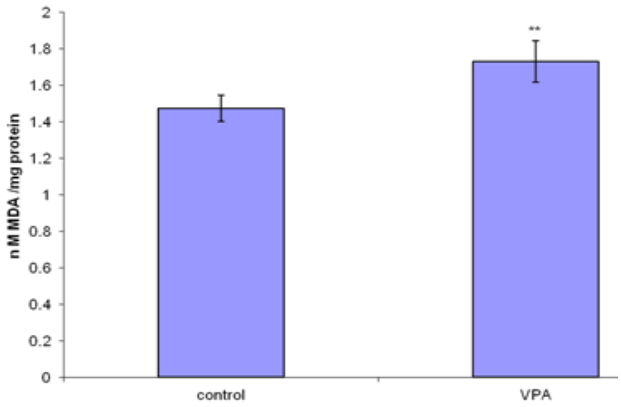

Fig. 2. The effects of perinatal administration of the valproic acid (VPA) in weaning pups born from rat-female which received valproic acid on malondiadehyde concentration from the temporal lobe. The values are mean± S.E.M. (n = 13 per control group and n = 7 for VPA group). **p > 0.01 vs. control

related behavioural manifestations of these animal models, as for example astaxanthin reduced the latency to foot withdrawal in the hot-plate task [72], while the Morakotsriwan [73] specific extract of purple rice + pupae of silkworm did not significantly affected pain manifestations in this VPA model, by using the same hot-plate test.

Thus, considering these discrepancies, but also the aforementioned effects of oxytocin, our group is also working right now in trying to understand the effects of intranasal oxytocin on pain manifestation in the perinatal valproic acid-induced rat model of autism, an area of research with warranted future relevance, especially considering the effects of oxytocin in pain perception [25, 26].

In fact, it is worth mention that right now there are clinical studies undergoing the usage of intranasal oxytocin as a possible treatment in patients in persistent pain (e.g. pelvic pain- phase 3), such as the one lead by Campbell et al. group from the University of Calgary [81].

Conclusions

There is an increased interest lately in understand the possible relevance of oxytocin as a possible treatment solution in the autistic pathology. In this way, we described here some original data and current literature on how oxytocin could help pathophysiological autistic manifestations in both animal models and human patients, by mainly focusing on some specific behavioural or pain manifestations and related oxidative stress status. Future directions in this area of research could also include, including the efforts of our group, a better understanding on pain and oxidative stress manifestations, as a result of intranasal oxytocin administration in the autistic pathology.

Acknowledgements: This work was supported by a PN-II-RU-TE-2014-4-1886 grant called "A complex study regarding the relevance of oxytocin administration in some animal models of neuropsychiatric disorders", number 120 from 01/10/2015. In addition, Radu Lefter was supported by an internal UAIC grant GI-13-2014.

References

1. GUASTELLA, A., WARD, P., HICKIE, I., SHAHRESTANI, S., HODGE, M., SCOTT, E., LANGDON, R., Schizophr Res., **168**, no. 3, 2015, p. 628.
2. GUASTELLA, A., GRAY, K., RINEHART, N., ALVARES, G., TONGE, B., HICKIE, I., et al. J Child Psychol Psychiatry., **56**, no. 4, 2015, p. 444.
3. EINFELD, S., SMITH, E., MCGREGOR, I., STEINBECK, K., TAFFE, J., RICE, L., et al., Am J Med Genet A., **164**, no. 9, 2014, p. 2232.
4. PEDERSEN, C., PRANGE, A., Pharmacol Ther., **28**, no. 3, 1985, p. 287.
5. FISCH, L., SALA, N., SCHWARCZ, R., Am J Obstet Gynecol., **90**, 1964, p. 108.
6. STRIEPENS, N., KENDRICK, K., HANKING, V., LANDGRAF, R., WÜLLNER, U., MAIER, W. et al., Scientific Reports, **3**, 2013.
7. NEUMANN, I., MALOUMBY, R., BEIDERBECK, D., LUKAS, M., LANDGRAF, R., Psychoneuroendocrinol., **38**, no. 10, 2013, p. 1985.
8. PADURARIU, M., LEFTER, R., CIOBICA, A., PAULET, M., DOBRIN, R., European Neuropsychopharmacol., **26**, no. 2, 2016, p. 289.
9. PADURARIU M., PREPELITA R., CIOBICA A., DOBRIN R., TIMOFTE D., STEFANESCU C., et al. Neurophysiology., **48**, no. 4, 2016, p. 312.
10. CIOBICA, A., BALMUS, I., PADURARIU, M., Acta Endocrinol., **12**, no 1, 2016, p. 65.
11. FINGER, E., MACKINLEY, J., BLAIR, M., Neurology., **84**, no. 2, 2015, 174.
12. GUASTELLA, A., EINFELD, S., GRAY, K., RINEHART, N., TONGE, B., LAMBERT, T., HICKIE, I., Biol Psychiatry., **67**, no. 7, 2010, 692.
13. YATAWARA, C., EINFELD, S., HICKIE, I., DAVENPORT. T., GUASTELLA, A., Mol Psych., **21**, no. 9, 2016, p. 1225.
14. HOLLANDER, E., NOVOTNY, S., HANRATTY, M., YAFFE, R., DECARIA, C., ARONOWITZ, B., Neuropsychopharmacol. **28**, no. 1, 2003, p. 193.
15. HOLLANDER, E., BARTZ, J., CHAPLIN, W., PHILLIPS, A., SUMNER, J., SOORYA, L., ANAGNOSTOU, E., WASSERMAN, S. Biol Psychiat., **61**, no. 4, 2007, p. 498.
16. DADDS, M., MACDONALD, E., CAUCHI, A., WILLIAMS, K., LEVY, F., BRENNAN, J., J Autism Dev Disord., **44**, no. 3, 2014, p. 521.
17. ANAGNOSTOU, E., SOORYA, L., CHAPLIN, W., BARTZ, J., HALPERN, D., WASSERMAN, S., Mol Autism., **3**, no 1, 2012, p. 16.
18. LEE, S., LEE, A., HWANGBO, R., HAN, J., HONG, M., BAHN, G.H., Experimental Neurobiol., **24**, no. 4, 2015, p. 312.
19. GUASTELLA, A., HICKIE, I., MCGUINNESS, M., OTIS, M., WOODS, E., DISINGER, H., CHAN, H., Psychoneuroendocrinol. **38**, no. 5, 2013, p. 612.
20. TORDJMAN, S., ANDERSON, G., BOTBOL, M., PLoS ONE., **4**, no. 8, 2009, p. 5289.
21. MILITERNI, R., BRAVACCIO, C., FALCO, C., J Headache Pain. **1**, 2000, p. 53.
22. NADER, R., OBERLANDER, T., CHAMBERS, C., CRAIG, K., Clin J Pain. **20**, no. 2, 2004, p. 88.
23. CLARKE, C., Case Reports in Psychiat., **930874**, 2015, pp. 1-25.
24. ALLELY, C., The Scientific World Journ., **916178**, 2013, pp. 1-20.
25. RASH, J., CAMPBELL, T., Psychosom Med., **76**, no. 6, 2014, p. 422.
26. WANG, Y., YUAN, Y., YANG, J., WANG, C., PAN, Y., LU, L., Neuropeptid., **47**, no. 2, 2013, p. 93.
27. ANTIOCH, I, CIOBICA, A., PAULET, M., BILD, V., LEFTER, R., TIMOFTE, D., Psychiatr Danub, **27**, No. 2, 2015, p. 142.
28. ANTIOCH I, CIOBICA A, BILD V, ANTON E, TIMOFTE D. Analele ªtiintifice ale Universitatii Alexandru Ioan Cuza, Sectiunea Geneticã ºi Biologie Moleculara., **16**, no. 1, 2015, 27.
29. LEFTER, R., COJOCARU, D,. CIOBICA, A., PAULET, I., SERBAN, I., ANTON, E.,. Archives of Biological Sciences Belgr. **66**, no. 3, 2014, p. 105.
30. HRITCU, L., CIOBICA, A., ARTENIE, V., Cent. Eur. J. Biol**., 3**, no. 3, 2008, p. 250-257.
31. BILD, W., CIOBICA, A, Journal of Affective Disord., **145**, no. 2, 2013, p. 165.
32. CIOBICA, A., OLTEANU, Z., PADURARIU, M., HRITCU, L., Journal of Physiology and Biochem. **68**, no 1, 2011, 59.
33. CIOBICA A, PADURARIU M, HRITCU L, Psychiatr Danub., **24**, no. 2, 2012, p. 194.
34. ABABEI, D., LEFTER, R., BILD, V., ANTIOCH, I., BALMUS, I., CIOBICA, A., TIMOFTE, D., Veterinary Drug., **9**, no. 1, 2015, p. 59.
35. FOYET, H., HRITCU, L., CIOBICA, A., STEFAN, M., KAMTCHOUING, P., COJOCARU, D., Journal of Ethnopharmacol., **133**, no. 2, 2011, p. 773.
36. CIOBICA, A., HRITCU, L., NASTASA, V., PADURARIU, M., BILD, W., J Med Biochem, **30**, no. 2, 2011, p. 109.
37. CIOBICA, A., BILD, V., HRITCU, L., PADURARIU, M., BILD, W., Central European Journal of Med., **6**, no. 3, 2011, p. 331.
38. RODIER, P., INGRAM, J., TISDALE, B., NELSON, S., ROMANO. J., J Comp Neurol., **370**, no. 2, 1996, p. 247.
39. INGRAM, J., PECKHAM, S., TISDALE, B., RODIER, P., Neurotoxicol Teratol. **22**, no. 3, 2000, p. 319.
40. SCHNEIDER, T., PRZEWOCKI, R., Neuropsychopharmacol., **30**, no. 1, 2005, p. 80.
41. MOY, S., NADLER, J., YOUNG, N., PEREZ, A., HOLLOWAY, L., BARBARO, R., BARBARO, J., WILSON, L., THREADGILL, D., LAUDER, J., MAGNUSON, T., CRAWLEY, J., Behav Brain Res., **176,** no. 1, 2007, p. 4.
42. CHADMAN, K., Pharmacol Biochem Behav., **97**, no. 3, 2011, p. 586.
43. SILVERMAN, J., YANG, M., TURNER, S., KATZ, A., BELL, D., KOENIG, J., CRAWLEY, J., Neurosc., **171**, no. 4, 2010, p. 1197.
44. BROMLEY, R., MAWER, G., CLAYTON-SMITH, J., BAKER, G., Neurol., **71**, no. 23, 2008, p. 1923.
45. OLEXOVA, L., SENKO, T., ŠTEFANIK, P., TALAROVICOVA, A., KRSKOVA, L., Interdisc Toxicol., **6**, no. 4, 2013, p. 222.

46. MABUNGA, D., GONZALES, E., KIM, J., KIM, K., SHIN, C., Exp Neurobiol., **24,** no. 4, 2015, p. 285.
47. INGRAM, J., PECKHAM, S., TISDALE, B., RODIER, P., Neurotoxicol Teratol., **22**, no. 3, 2000, p. 319.
48. MARTIN, H., MANZONI, O., Front Cell Neurosci., **31**, 2014, p. 8.
49. HARA, Y., TAKUMA, K., TAKANO, E., KATASHIBA, K., TARUTA, A., HIGASHINO, K., HASHIMOTO, H., AGO, Y., MATSUDA, T., Behav Brain Res., **289**, 2015, p. 39.
50. KERR, D., DOWNEY, L., CONBOY, M., FINN, D., ROCHE, M., Behav Brain Res., **15**, no. 249, 2013, p. 124.
51. FAVRE, M., BARKAT, T., LaMENDOLA, D., KHAZEN, G., MARKRAM, H., MARKRAM, K., Front Behav Neurosc., **7**, no. 88., 2013, pp. 1-10.
52. BANERJEE, A., ENGINEER, C., SAULS, B., MORALES, A., KILGARD, M., PLOSKI, J., Front Behav Neurosci., **12**, no. 8, 2014, p. 387.
53. CRAWLEY, J., Ment Retard Dev Disabil Res Rev.,**10**, no. 4, 2004, p. 248.
54. ŠTEFANIK, P., OLEXOVA, L., KRSKOVA, L., Pharmacol Biochem Behav., **4**, no. 131, 2015, p. 42.
55. BALES, K., SOLOMON, M., JACOB, S., CRAWLEY, J., SIVERMAN, J., LARKE, R. et al., Transl Psychiatry., **11**, no. 4, 2014, p. 480.
56. TENG, B., NONNEMAN, R., AGSTER, K., NIKOLOVA, V., DAVIS, T., RIDDICK, N., BAKER, L. et al., Neuropharmacol., **9**, no. 72, 2013, p. 187.
57. TENG, B., NIKOLOVA, V., RIDDICK, N., AGSTER, K., CROWLEY, J., BAKER, L., KOLLER, B., PEDERSEN, C., JARSTFER, M., MOY, S., Neuropharmacol., **6**, no. 105, 2016, p. 61.
58. MICHOULAS, A., TONG, V., TENG, X., CHANG, T., ABBOTT, F., FARRELL, K., J Pediatr., **149**, no. 5, 2006, p. 692.
59. VERROTTI, A., SCARDAPANE, A., FRANZONI, E., MANCO, R., CHIARELLI, F., Epilepsy Res., **78**, no. 2-3, 2008, p. 171.
60. CHANG, T., ABBOTT, F., Drug Metab Rev., **38**, no. 4, 2006, p. 627.
61. EDALATMANESH, M., HOSSEINI, M., GHASEMI, S., GOLESTANI, S., SADEGHNIA, H., MOUSAVI, S., VAFAEE, F., Ir J Med Sci., **185**, no. 1, 2016, p. 75.
62. TAKUMA, K., HARA, Y., KATAOKA, S., KAWANAI, T., MAEDA, Y., WATANABE, R., TAKANO, E., HAYATA-TAKANO, A., HASHIMOTO, H., AGO, Y., MATSUDA, T., Pharmacol Biochem Behav., **126**, no. 11, 2014, p. 43.
63. SUI, L., CHEN, M., Brain Res Bull., **87**, no. 6, 2012, p. 556.
64. PADURARIU, M., CIOBICA, A., HRITCU, L., STOICA, B., BILD, W., STEFANESCU, C., Neuroscience Lett., **469**, no. 1, 2010, p. 6.
65. PADURARIU, M., CIOBICA, A, LEFTER, R., SERBAN, I., STEFANESCU, C., CHIRITA, R., Psychiatr Danub., **25**, no 4, 2013, p. 401.
66. PADURARIU, M., CIOBICA, A., DOBRIN, I., STEFANESCU, C., Neurosci Lett. **479**, no. 3, 2013, p. 317.
67. CIOBICA, A., PADURARIU, M., DOBRIN, I., STEFANESCU, C, DOBRIN, R., Psychiatr Danub. **23**, no. 3, 2011, p. 237.
68. FRUSTACI, A., NERI, M., CESARIO, A., ADAMS, J., DOMENICI, E., DALLA, BERARDINA, B., BONASSI, S., Free Radic Biol Med., **52**, no. 10, 2012, p. 2128.
69. CHAUHAN, A., CHAUHAN, V., Pathophysiol., **13,** no. 3, 2006, p. 171.
70. ROSSIGNOL, D., FRYE, R., Front Physiol., **5**, no. 150, 2014, pp. 1-10.
71. HENDREN, R., BERTOGLIO, K., ASHWOOD, P., SHARP, F., Med Hypotheses., **73**, no. 6, 2009, p. 950.
72. AL-AMIN, M., RAHMAN, M., KHAN, F., ZAMAN, F., MAHMUD REZA, H., Behav Brain Res., **1**, no. 286, 2015, p. 112.
73. MORAKOTSRIWAN, N., WATTANATHORN, J., KIRISATTAYAKUL, W., CHAISIWAMONGKOL, K., Oxid Med Cell Longev., **3206561**, 2016.
74. KIM, P., PARK, J., KWON, K., KIM, K., KIM, H., LEE, J., KIM, H., HAN, S., SHIN, C., Food Chem Toxicol., **51**, no. 1, 2013, p. 288.
75. GONZALES, E., JANG, J., MABUNGA, D., Food & Nutrition Res., **60**, no. 10, 2016.
76. LIU, S., FEIYONG, J., TIANLIANG, X., NAIJUN, X., YING, Z., HUIYI, J., Int J Clin Exp Med., **9**, no. 12, 2016, p. 23363.
77. HONCERIU, C., CIOBICA, A., STOICA, B., CHIRAZI, M., PADURARIU, M., Rev. Chi. (Bucharest), **67**, no. 11, 2016, p. 2246.
78. LEFTER, R., CIOBICA, A., ANTIOCH, I., BALMUS, I., PADURARIU, M., DOBRIN, R., 5th International Congress on Neurobiology, Psychopharmacology and Treatment Guidance, **27**, 2017, p. 108.
79. ARCAN, O., CIOBICA, A., BILD, W., HRITCU, L., COJOCARU, D., J Med Biochem, **32**, no. 1, 2013, p. 52.
80. ARCAN, O., BILD, W., CIOBICA, A., SERBAN, D., ANTON, E., PETRARIU, F., TIMOFTE, D., NASTASA, V., Rom Biotech Let, **19**, no. 5, 2014, 9763.
81. TAVIS, C., Clinicaltrials.gov, 3, 2017, pp. 1-3.

Manuscript received: 18.01.2017