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CONTENT | *CONTEÚDO*

Preface

Nutrition, Brain and Behavior

The greatest and last frontier of the world is within ourselves, or between two distinct worlds, our nervous system, brain-mind relationship and mind-body, our origin and end have always been a mystery to humanity. We are curious beings and always alert, interacting strongly with the environment in which we live, and clearly human nutrition directly influencing all those processes that involve growth, integration, maintenance, development and death. Perhaps the most complex machinery to be understood in the whole universe is the evolution of our human consciousness and its fascinating abilities, such as those of dreaming and creating images. All living beings communicate, but only we have the ability to write, speak, express themselves through signs and perceive. Our behavior can be changed at any moment. This brilliant work brings us a reflection of what we are and what the functions of nutrition and its effects on human behavior, gathering basic and related aspects on the most diverse themes of modern neuroscience, in particular of the colleagues and their contributions to and final elaboration of this e-book and central theme of the III Northeastern Symposium on Neuroscience, Nutrition and Human Development (NEURONUTRI UFPE 2019), bringing together friends, colleagues, students and professionals interested in the subject. When the brain studies the brain, and we think about the mysteries that remain to be revealed in the study of its confines, we realize that our knowledge still requires a universe of facts to understand. It is fascinating how the nervous system plasticity can reorganize its neuroanatomical substrate to allow an organism to respond to the demands of its environment. At this meeting we will discuss how maternal nutrition and malnutrition can alter in the long term, the cognitive and physiological development of an individual, predisposing it, even to the development of pathologies or disorders that will affect its performance throughout life and to pass to the offspring some predisposition such as metabolic syndrome or diabetes. As Santiago Ramón y Cajal wrote: "As long as our brain is a mystery, the universe, the reflection of the structure of the brain will also be a mystery". Of particular interest to the participants, is how the brain and behavior is altered when nutrition is inadequate to meet the demands of life and development. Prenatal malnutrition in humans and in animal models of malnutrition leads to reprogramming of the brain which leads to new behavioral strategies to find adequate nutrition. Thank you very much and we hope you like it!

Vasconcelos-Mokler-Duran
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1 - Nutrition, Brain and Behavior: Frontiers of Life

*Carlos Augusto Carvalho de Vasconcelos, PhD**

> "In that dramatic moment when a microscopic male germ cell, after winding and wending its way towards the much larger egg cell and binding to it, the beginning of a new human being takes place. During the following early developmental period which is of vital importance as it is when things can go "right" or "wrong," nutrition may exert a life-long profound influence upon it" Roger J. Williams (1893-1988)**

Starvation and hunger, currently linked to poor socioeconomic conditions, have their roots in the remote past. Humans have long been prone to food shortages arising from climatic disasters, wars, the lack of a nutritious diet etc. Secondary causes of malnutrition include alcoholism, anaemia, malabsorption, celiac and inflammatory bowel disease and more recently nervous anorexy and bulimia. Moreover, a lack of information to ensure that food is prepared containing essential nutrients remains an ongoing global problem even today. An adequate nutritious diet, through its interaction with genetic and biochemical parameters, is also necessary for normal growth, development and survival. It is required for normal brain maturation. Poor nutrition in early life has long-term adverse effects on many conditions and has been implicated in the onset of several behavioral disorders. Both macro- and micronutrients are heavily involved in metabolic processes and are vital for life. Thus there is the need for the consumption of adequate quantities of high quality proteins, lipids, and certain vitamins, especially the water soluble B ones. It is therefore important that nutritional research is regarded as "the science of the future" especially after it has been realised just how essential are vitamins for normal development and the maintenance of a healthy lifestyle. Both the central nervous system (CNS) and peripheral (PNS) are highly dependent on the steady supply of specific nutrients for adequate functioning, especially those essential ones that we are unable to synthesize and rely on nature to provide. Malnutrition during

early human development can lead to medical problems of which the neuropsychiatric and neurological signs and symptoms may only become apparent later in life. However, such conditions are often reversible and can be successfully treated if detected early. It has been recently demonstrated that protein deprivation in the rat fetus later leads to differences in the extracellular concentrations of norepinephrine, dopamine and serotonin in the medial prefrontal cortex of the adult. This would suggest that a long-term rescheduling of the prefrontal cortex in rats exposed prenatally to protein malnutrition might contribute to the attention deficits observed in the adult animals. [1,2,3,4]

Throughout the history of animal experimentation findings have frequently been extrapolated to the situation in the human. These include not only the results from the whole animal, but also those from isolated organs and cells, tissue cultures, subcellular components, modelling and structure-activity relationships. An important discovery was that the lack of a number of essential nutrients in the diet was directly associated with the onset of certain diseases. Currently numerous animal experiments are being undertaken in an attempt to extrapolate the research findings from these to the human situation and hopefully lead to the development of effective and acceptable treatments for patients.[5,6]

It is now apparent that a nutritious diet containing essential minerals and vitamins is required for normal healthy bodily function. In children a low-calorie diet, deficient in protein and lacking vitamins and essential minerals, can lead to irreversible damage to both the PNS and CNS. It is thus paramount that such a situation is diagnosed and addressed as soon as possible. Appropriate treatment can then lead to weight gain and also limit damage to the nervous system thus preventing the onset of behavioral and personality disorders and future mental illness. Moreover, cognitive decline in the elderly appears to develop more rapidly when nutrition is inadequate and vitamins including B12 are deficient. There is thus a direct relationship between nutrition and the development of normal human behavior as referred to above. Recently research into the role of vitamins in human health has gained interest because of their participation in metabolic regulation and their frequent involvement as co-factors regulating the immune system and brain development. A diet deficient in calories and proteins during childhood continues to pose a serious problem in the world today. Such an energy deficit resulting from a lack of macronutrients is usually an indicator that adequate dietary micronutrients are also missing. Weight gain and stature are reduced and although these can be addressed though by commencing to eat healthily, it is vital such measures are undertaken as early as possible to limit further damage, especially to the nervous system. The latter can lead to mental illness and to behavioral disorders such as problems in emotional development. There is a direct relationship between human nutrition and the development of various aspects of human behavior. The importance

of vitamins in the diet is becoming increasingly recognized, as they are involved in so many biochemical and physiological activities within the body particularly those relating to metabolism. An example is that of Vitamin D, considered to be a steroid hormone regulating metabolism. It is involved in the control of lipid, calcium and phosphate levels as well as that of the immune response and brain development. More than a billion people in the world today are affected by a deficiency of vitamin D in their diet. This lack of vitamin D is correlated with neurological problems, including a worsening of mental illnesses, particularly those linked to personality, emotional and other behavioral disorders. Recently the scientific community has realised the importance of vitamins (typified by B12) in not only providing energy, but more importantly in having a major role in regulation of the body's metabolism. More than a billion people around the world are affected by a deficiency of vitamin D in their diet. Although this is associated with impaired cognition, the manner by which this occurs is little understood. The involvement of the extracellular matrix (ECM) has emerged as a major participant in synaptic plasticity. It has now been postulated that vitamin D can interact with the ECM, perineuronal nets (PNNs), to regulate brain plasticity. A deficiency in vitamin D may interfere with this process. An understanding the molecular mechanisms underlying the role of vitamin D on plasticity and brain cognition could help identify ways to successfully treat the symptoms of schizophrenia and other conditions neuropsychiatric disorders. [7, 8, 9, 10]

Increasingly vitamin D deficiency is being recognized as being associated with the incidence of a number of psychiatric disorders. These include those conditions arising from problems during neurodevelopment such as those on the autism spectrum and schizophrenia. Reliable evidence from preclinical data is now accumulating to indicate that vitamin D deficiency early in life affects neuronal differentiation, axonal connectivity and the ontogeny of dopamine release as well as brain structure and function. More recently, epidemiological investigations have suggested a link between low vitamin D intake and psychiatric disorders not typically associated with abnormalities in brain development, such as depression and Alzheimer's disease. Research has revealed that that vitamin D can regulate the levels of catecholamines in the CNS and protect against Alzheimer's type-specific diseases. Vitamin D may also be associated with the pathogenesis of malaria and other parasitic infections. Current research in neuroscience indicates that the development of some neurodegenerative diseases can be related to living conditions and that a nutritious diet containing important vitamins and essential minerals may have a protective effect. [11, 12, 13, 14, 15, 16]

There is now strong evidence to indicate that poor maternal nutrition during pregnancy is associated with mental and behavioral disorders in the offspring. This includes schizophrenia, a term derived from 'schizo' (splitting) and 'phren' (mind), It

was first used in 1908 by the eminent Swiss psychiatrist Paul Eugen Bleuler (1857-1939). Schizophrenia is a psychotic disorder characterized by the presence of delusional beliefs, hallucinations and disturbances of thought, perception and behavior. There are two main types of symptoms, positive ones including hallucinations, delusions and disorders of perception and negative ones such as social and physical anhedonia. Social anhedonia is a disinterest in social contact and a lack of pleasure in social situations, while physical anhedonia is an inability to experience tactile pleasure or that which comes from eating and sex. The symptoms in patients suffering from anhedonia include social withdrawal, negative feelings toward oneself and others, a tendency towards showing fake emotions, a loss of libido and persistent physical problems. However, a diagnosis of schizophrenia can only be arrived at after taking a detailed psychiatric history from the patient and excluding other organic causes of psychosis. Risk factors for the disorder include severe maternal malnutrition including vitamin D deficiency or contracting influenza when pregnant, complications during childbirth and season of birth. The condition has also been linked to childhood trauma, social isolation, being a member of an ethnic minority, city dwelling and cannabis use. Because of the complexity of the possible causes, the pathological mechanisms actually leading to schizophrenia are not fully understood. Although the prevalence is low, the global burden of schizophrenia is immense. More than half of patients suffering from the condition have significant co-morbidities and thus it can be regarded as one of the leading causes of disability worldwide. Those diagnozed with schizophrenia have a 20% reduction in life expectancy with up to 40% of deaths being attributed to suicide. [17,18,19,20,21,22]

The water-soluble vitamin B12 (thiamine) is an essential nutrient that serves as a cofactor in many enzyme reactions, mainly those localized in the mitochondria, and is considered to have antineuritic actions. Some thiamine-dependent enzymes are involved in energy metabolism and in the biosynthesis of nucleic acids, while others are part of the antioxidant machinery. The brain is highly vulnerable to thiamine deficiency due to its strong dependence on mitochondrial ATP production. This is most evident during periods of rapid growth (i.e. in the perinatal period and during early childhood. In these situations thiamine deficiency is commonly associated with malnutrition or genetic defects and contributes to a number of pathological conditions. These range from moderate neurological and psychiatric symptoms (confusion, reduced memory and sleep disturbances) to severe encephalopathy, ataxia, congestive heart failure muscle atrophy and death. There is strong evidence to indicate that thiamine supplementation, especially in childhood, can have beneficial effects in treating those conditions leading to neurological and psychiatric disorders such as autism. [23, 24, 25, 26]

A brain aneurysm is a bulge in the wall of an intracranial artery. It tends to occur on the outer curve of a vessel or on branches of it and is particularly common in the

circle of Willis. Aneurysms can arise through a genetic predisposition or because of vascular disease and if they rupture the consequences can be fatal. Aneurysms may also result from poor nutrition. As mentioned earlier, vitamin D is a crucial component of the diet for ensuring a healthy life. It is involved in mineral homeostasis in the body and plays an important role in regulating skeletal muscle function. A deficiency of vitamin D has been associated with chronic inflammatory conditions, diabetes and obesity which are all risk factors for the development of cardiovascular disease (CVD), CVD is an umbrella term that covers restricted blood flow in the coronary arteries, myocardial infarction, cardiac hypertrophy, cardiomyopathy, cardiac fibrosis, cardiac insufficiency, hypertension, peripheral arterial disease and artherosclerosis. All these conditions may cause morbidity and lead to death. They may also be associated with a deficiency in vitamin D as they improve when it is taken as a supplement thus indicating its therapeutic benefits. Results from the literature would indicate that the extrapolation of research findings from animal experiments can produce clinical benefits. A nutritious diet including essential minerals and vitamins appears to provide a degree of protection from the harmful effects of inflammatory cytokines and endotoxins. Exercise combined with a nutritious diet has a preventative effect on the development of cardiovascular disease. Together they combine to lower glucose levels and thereby increase insulin release leading to an improvement in both appetite and mood. A body mass index greater than 30 is also a risk factor for CVD and is associated with a poor prognosis. In contrast, recent reports have indicated that the survival rate is better in those individuals with only mild to moderate obesity, who are suffering from coronary heart disease and hemorrhagic or ischemic stroke, if undertaking exercise and consuming healthy food. A similar finding has been seen in patients with ruptured intracranial aneurysms, who, although obese, apparently remain metabolically healthy, with protection from further progression of the disease. However, more studies are needed to determine how consistent is this correlation between obesity, body mass index and intracranial aneurysms and the way in which human nutrition directly or indirectly influences the development and pathophysiology of intracranial and other aneurysms in the body. [27.28, 29.30, 31.32]

Finally is necessary to emphasise that a nutritious diet is needed both for normal growth and for the maintenance of a healthy lifestyle through to old age. It is also clear that, especially during the critical periods of development, a lack of essential minerals and vitamins can cause long-lasting damage to many organs and tissues including both the PNS and CNS. There is thus a clear and close relationship between diet and the normal function of brain and behavior; each is dependent on the other.

References

1. Chaves N. A Nutrição o Cérebro e a Mente. Edições o Cruzeiro. MEC/UFPE. Instituto de Nutrição. 1971. 110p. [Portuguese].

2. Morgane PJ, Austin-LaFrance R, Bronzino J, Tonkiss J, Díaz-Cintra S, Cintra L, Kemper T, Galler JR. Prenatal malnutrition and development of the brain. Neurosci Biobehav Rev. 1993 Spring;17(1):91-128.

3. Kumar N. Neurologic presentations of nutritional deficiencies. Neurol Clin. 2010 Feb;28(1):107-70. doi: 10.1016/j.ncl.2009.09.006.

4. Mokler DJ, McGaughy JA, Bass D, Morgane PJ, Rosene DL, Amaral AC, Rushmore RJ, Galler JR. Prenatal Protein Malnutrition Leads to Hemispheric Differences in the Extracellular Concentrations of Norepinephrine, Dopamine and Serotonin in the Medial Prefrontal Cortex of Adult Rats. Front Neurosci. 2019 Mar 5;13:136. doi: 10.3389/fnins.2019.00136.

5. Paton W. Man and mouse. Animals in medical research. 1984. Printed in Great Britain by the Guernsey Press Co. Ltd. Oxford University Press©; 174.

6. Vasconcelos CAC. Animal models of human nutritional diseases: a short overview. Rev Bras Hematol Hemoter. 2012; 34(4): 264. doi: 10.5581/1516-8484.20120068

7. Ashworth A, Bell R, James WP, Waterlow JC. Calorie requirements of children recovering from protein-calorie malnutrition. Lancet 1968 Sep 14;2(7568):600-3. 10.1016/s0140-6736(68)90697-1.

8. Waber DP, Bryce CP, Fitzmaurice GM, Zichlin ML, McGaughy J, Girard JM, Galler JR. Neuropsychological outcomes at midlife following moderate to severe malnutrition in infancy. Neuropsychology 2014 Jul;28(4):530-40. doi: 10.1037/neu0000058.

9. Baroni L, Bonetto C, Rizzo G, Bertola C, Caberlotto L, Bazzerla G. Association Between Cognitive Impairment and Vitamin B12, Folate, and Homocysteine Status in Elderly Adults: A Retrospective Study. J Alzheimers Dis. 2019 Jun 6. doi: 10.3233/JAD-190249.

10. Mayne PE, Burne THJ. Vitamin D in Synaptic Plasticity, Cognitive Function, and Neuropsychiatric Illness. Trends Neurosci. 2019 Apr;42(4):293-306. doi: 10.1016/j.tins.2019.01.003.

11. Eyles DW, Burne TH, McGrath JJ. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Front Neuroendocrinol. 2013 Jan;34(1):47-64. doi: 10.1016/j.yfrne.2012.07.001.

12. Kesby JP, Eyles DW, Burne TH, McGrath JJ. The effects of vitamin D on brain development and adult brain function. Mol Cell Endocrinol. 2011 Dec 5;347(1-2):121-7. doi: 10.1016/j.mce.2011.05.014. Epub 2011 Jun 1.

13. Harms LR, Burne TH, Eyles DW, McGrath JJ. Vitamin D and the brain. Best Pract Res Clin Endocrinol Metab. 2011 Aug;25(4):657-69. doi: 10.1016/j.beem.2011.05.009.

14. Denniss RJ, Barker LA, Day CJ. Improvement in Cognition Following Double-Blind Randomized Micronutrient Interventions in the General Population. Front Behav Neurosci. 2019 May 28;13:115. doi: 10.3389/fnbeh.2019.00115.

15. Bivona G, Agnello L, Lo Sasso B, Scazzone C, Butera D, Gambino CM, Iacolino G, Bellia C, Ciaccio M. Vitamin D in malaria: more hypotheses than clues. Heliyon. 2019 Feb 6;5(2):e01183. doi: 10.1016/j.heliyon.2019.e01183.

Bivona G, Gambino CM, Iacolino G, Ciaccio M. Vitamin D and the nervous system. Neurol Res. 2019 May 30:1-9. doi: 10.1080/01616412.2019.1622872.

16. Omenn GS. Neurochemistry and behavior in man. West J Med. 1976 Dec;125(6):434-51.

17. Hany M, Rehman B, Chapman J. Schizophrenia. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing [Book].; 2019 - 19 Jun 12. Accessed June 17, 2019.

18. Messias EL, Chen CY, Eat on WW. Epidemiology of schizophrenia: review of findings and myths. Psychiatr. Clin. North Am. 2007 Sep;30(3):323-38.

19. Davis J, Eyre H, Jacka FN, Dodd S, Dean O, McEwen S, Debnath M, McGrath J, Maes M, Amminger P, McGorry PD, Pantelis C, Berk M. A review of vulnerability and risks for schizophrenia: Beyond the two hit hypothesis. Neurosci Biobehav Rev. 2016 Jun;65:185-94. 3.

20. Jablensky A. The diagnostic concept of schizophrenia: its history, evolution, and future prospects. Dialogues Clin Neurosci. 2010;12(3):271-87. [PMC free article] [PubMed]

21. Patel KR, Cherian J, Gohil K, Atkinson D. Schizophrenia: overview and treatment options. P T. 2014 Sep;39(9):638-45.

22. Ramel SE, Georgieff MK. Preterm nutrition and the brain. World Rev Nutr Diet. 2014;110:190-200. doi: 10.1159/000358467.

23. Chawla J, Kvarnberg D. Hydrosoluble vitamins. Handb Clin Neurol. 2014;120:891-914. doi: 10.1016/B978-0-7020-4087-0.00059-0.

24. Schwarzenberg SJ, Georgieff MK; COMMITTEE ON NUTRITION. Advocacy for Improving Nutrition in the First 1000 Days to Support Childhood Development and Adult Health. Pediatrics. 2018 Feb;141(2). pii: e20173716. doi: 10.1542/peds.2017-3716.

25. Dhir S, Tarasenko M, Napoli E, Giulivi C. Neurological, Psychiatric, and Biochemical Aspects of Thiamine Deficiency in Children and Adults. Front Psychiatry. 2019 Apr 4;10:207. doi: 10.3389/fpsyt.2019.00207.

26. Ambrosi PB, Vasconcelos CAC, Moret J, Spelle L, Valença MM. Pathogenesis, hemodynamics, and growth of intracranial aneurysms: Future directions. Anat Rec (Hoboken). 2017 Jul;300(7):1175-1179. doi: 10.1002/ar.23530.

27. Rai V, Agrawal DK. Role of Vitamin D in Cardiovascular Diseases. Endocrinol Metab Clin North Am. 2017 Dec;46(4):1039-1059. doi: 10.1016/j.ecl.2017.07.009.

28. Korai M, Kitazato KT, Tada Y, Miyamoto T, Shimada K, Matsushita N, Kanematsu Y, Satomi J, Hashimoto T, Nagahiro S. Hyperhomocysteinemia induced by excessive methionine intake promotes rupture of cerebral aneurysms in ovariectomized rats. J Neuroinflammation. 2016 Jun 27;13(1):165. doi: 10.1186/s12974-016-0634-3.

29. Ambrosi PB, Valença MM, Vasconcelos CAC. Paradoxical Obesity and Intracranial Aneurysms: A Mini Review. Int J Clin Nutr Diet 2016, 2: 112, 2-6. doi: doi.org/10.15344/ijcnd/2016/112.

30. Lepski G, Lobão CAF, Taylor S, Mesquita Filho PM, Tatagiba M. Bleeding risk of small intracranial aneurysms in a population treated in a reference center. Arq Neuropsiquiatr. 2019 May 1;77(5):300-309. doi: 10.1590/0004-282X20190046.

31. Larsson SC, Bäck M, Rees JMB, Mason AM, Burgess S. Body mass index and body composition in relation to 14 cardiovascular conditions in UK Biobank: a Mendelian randomization study. Eur Heart J. 2019 Jun 13. pii: ehz388. doi: 10.1093/eurheartj/ehz388.

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** Roger John Williams (1893-1988), was an American biochemist who spent his academic career as a Professor at the University of Texas at Austin. He is renowned for isolating folic acid and was recognized as one of the world's foremost authorities on nutrition.

2 - Prenatal Protein Malnutrition Efects on Brain Neurochemistry

David J. Mokler[1], PhD and Jill A. McGaughy [2], PhD*

Prenatal protein malnutrition alters brain neurochemistry throughout the neuroaxis. Changes which have been reported occur in many areas of the brain and in many neurotransmitter systems.

Studies have shown that exposure to PPM increases the risk of developing depression, substance abuse and schizophrenia [1, 2]. However, there is a greater incidence of attentional problems associated with exposure to PPM [3]. Given the reported behavioral changes which occur in adults that were exposed to PPM; and changes that can be causally related to PPM in animal models, it seems likely that the brain has undergone a reprogramming which changes the behavioral repertoire to increase the likelihood of finding adequate nutrition. In this short review, we will focus on observed changes in the monoaminergic neurotransmitter systems.

There are numerous animal models of prenatal protein malnutrition, varying in the length and severity of the malnutrition. The length of the malnutrition may vary from weeks prior to breeding, through gestation into the post-natal period up to until weaning. The level of protein deprivation can vary from the normal level of protein for pregnancy of 25% down to 6%. Diets are mostly isocaloric with the caloric difference made up with casein. In the present review, we will focus on the model that we have used (see [4, 5] for details). In brief, female rats are started on a diet of either 25% (well-nourished) or 6% (malnourished) protein diet five weeks prior to breeding. Breeding males are started on diet one week prior to breeding. At parturition, litters are culled to eight pups (generally six males and two females) and cross fostered to well-nourished dams who have given birth in the past day. At weaning, pups are housed with same sex littermates. Only one animal per litter is used for an individual experiment, although some animals may be used in multiple experiments.

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Microdialysis experiments were performed in adult male rats at 90 to 120 days of age as described in detail in Mokler, McGaughy [6]. Guide cannulae were implanted under general anesthesia. Following three days for recovery, dialysis probes were inserted without anesthesia into conscious rats and perfused with artificial cerebrospinal fluid at a rate of 1 µl/min. Perfusate was collected every 20 min and analyzed for serotonin, dopamine and norepinephrine by high performance liquid chromatography with electrochemical detection. Following the microdialysis experiment, rats were perfused with paraformaldehyde and the site of perfusion determined histologically.

Serotonin

Many studies have focused on the serotonergic system of the brain and how this has been affected by PPM. This focus is due to the links of serotonin to mood and arousal which may be affected by PPM. Serotonin is mostly increased in many brain areas including the hippocampus[7, 8] and the left medial prefrontal cortex [6, 9] (Table 1). It is not, however, increased in the orbital frontal cortex or in the right medial prefrontal cortex (4 and Table 1). The significance of these sub-region and hemispherically specific changes in serotonin will be discussed later.

Studies to determine the mechanisms of these changes have shown that there is no change in the birthdates or number of serotonergic cells in the raphe nuclei [10]. Other studies have shown a decrease in the immunoreactivity for SERT in the dorsal hippocampus [11]. Thus, the increase in extracellular serotonin as determined by in vivo microdialysis may be the result from abnormally low reuptake. If, however, the decrease in expression of SERT also reflects a lower density of nerve terminals, other mechanisms need to be determined for the increase in extracellular serotonin.

Dopamine

Dopamine enervation of the prefrontal cortex (PFC) has also been of interest given the connections between dopamine, attention and the PFC. In attention deficit disorder (ADD), evidence suggests that there is a decrease in dopamine in the PFC. Two studies by our group have shown decreases in basal extracellular concentrations of dopamine in the right ventral medial PFC (vmPFC)[6, 9](Table 1). One study looked at dopamine bilaterally in the vmPFC, reporting a decrease in the right but no change in the left hemisphere [6].

Mechanisms for these decreases in dopamine still need to be elucidated. Both the dopamine transporter (DAT) and the norepinephrine transporter (NET) are important regulators of extracellular DA in the brain. Of significance with discussing the roles of DAT and NET in attention are the current understanding of the roles of these transporters in the brain. It is clear that these transporters are non-selective and have varying affinities for other monoamines. In fact, DA has a higher affinity for NET than for DAT.

In addition, there are great differences in the distribution of these transporters in the brain. The PFC has very low levels of DAT and high levels of NET. In the striatum the reverse is true. Thus, uptake of DA in the PFC is thought to be almost entirely due to NET. Therefore, observed changes in NET would affect both DA and NE extracellular levels.

Norepinephrine

Given the pivotal role of norepinephrine in the frontal cortex in the regulation of cognition [12-14], it is important to evaluate changes in this neurotransmitter system in PPM. In our studies of PPM changes in the frontal cortex using in vivo microdialysis, we have reported a decrease in extracellular NE in the right but not the left vmPFC [6], and an increase in NE in both the left and right hemispheres of the orbital frontal cortex (Table 1). Previous work using this same model of PPM showed no differences in birthdate or number of NE neurons in the locus coeruleus [15]. Newman et al. [5] reported, however, a decrease in NE staining in the frontal cortex of adult rats exposed to PPM. This decrease was accompanied by a decrease in cognitive flexibility in these same rats, in a manner similar to animals with lesions of their noradrenergic system [5].

The lateralization of these neurochemical effects is interesting. There are many examples of hemispheric differences in both human and animal brains. It is clear that the normal, adult rat brain has hemispheric differences [16-18]. Sullivan et al. [19] have shown that stress responses are lateralized in the rat prefrontal cortex. Martinez and Sarter [20] have shown functional lateralization in the cholinergic system of the cortex in a sustained attention task. In regards to PPM, the cortical lateralization of NE and DA in the vmPFC, which includes the infralimbic PFC [6], may be important to the cognitive changes that have been reported [5]. However, the decrease in NE reported by Newman et al. [5] was seen in both hemispheres (McGaughy, personal communication), which raises more questions of mechanisms. If NET moderates extracellular DA and NE in the PFC, then other mechanisms must be involved to give the hemispheric changes which we have seen. Future studies must assess neurochemical changes in animals during cognitive testing as these conditions heavily recruit monoaminergic systems to better link brain and behavioral changes.

The changes which we have observed in these neurotransmitter systems as the result of exposure to PPM, and the cognitive changes which have been observed, suggest a long term repatterning of both neurochemistry and behavior. The cognitive inflexibility that has been observed in both human and rat populations exposed to PPM may be a strategy to increase focus on behaviors to find adequate nutrition. This suggests that a 'program' exists within the brain to produce this rewiring. More work is needed to determine how this occurs and can it be reversed.

References

1. Susser, E., H.W. Hoek, and A. Brown, Neurodevelopmental disorders after prenatal famine: The story of the Dutch Famine Study. Am J Epidemiol, 1998. 147(3): p. 213-6.
2. St Clair, D., et al., Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961. JAMA, 2005. 294(5): p. 557-62.
3. Waber, D.P., et al., Cognitive impairment as a mediator in the developmental pathway from infant malnutrition to adolescent depressive symptoms in Barbadian youth. J Dev Behav Pediatr, 2011. 32(3): p. 225-32.
4. Tonkiss, J. and J.R. Galler, Prenatal protein malnutrition and working memory performance in adult rats. Behav Brain Res, 1990. 40(2): p. 95-107.
5. Newman, L.A., et al., Prenatal protein malnutrition produces resistance to distraction similar to noradrenergic deafferentation of the prelimbic cortex in a sustained attention task. Frontiers in Neuroscience, 2019. 13.
6. Mokler, D.J., et al., Prenatal protein malnutrition leads to hemispheric differences in the extracellular concentrations of norepinephrine, dopamine and serotonin in the medial prefrontal cortex of adult rats. Frontiers in Neuroscience, 2019. 13: p. 136.
7. Mokler, D.J., et al., Effects of median raphé electrical stimulation on serotonin release in the dorsal hippocampal formation of prenatally malnourished rats. Brain Res, 1999. 838(12): p. 95-103.
8. Mokler, D.J., J.R. Galler, and P.J. Morgane, Modulation of 5-HT release in the hippocampus of 30-day-old rats exposed in utero to protein malnutrition. Brain Res Dev Brain Res, 2003. 142(2): p. 203-8.
9. Mokler, D.J., et al., Stress-induced alterations in medial prefrontal cortex and dorsal hippocampus of prenatal protein malnourished rats: A dual-probe microdialysis study. Soc.Neurosci.Abstr., 2003.
10. King, R.S., et al., Birthdates and number of neurons in the serotonergic raphe nuclei in the rat with prenatal protein malnutrition. Nutr.Neurosci., 2002. 5(6): p. 391-397.
11. Blatt, G.J., et al., Prenatal protein malnutrition effects on the serotonergic system in the hippocampal formation: an immunocytochemical, ligand binding, and neurochemical study. Brain Res Bull, 1994. 34(5): p. 507-18.
12. Mokler, D.J., C.E. Miller, and J.A. McGaughy, Evidence for a role of corticopetal, noradrenergic systems in the development of executive function. Neurobiol Learn Mem, 2017. 143(17): p. 94-100.
13. del Campo, N., et al., The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. Biological Psychiatry, 2011. 69(12): p. e145-e157.
14. Robbins, T.W. and A.F.T. Arnsten, The neuropsychopharmacology of fronto-executive function: Monoaminergic modulation. Annual Review of Neuroscience, 2009. 32(1): p. 267-287.
15. King, R.S., et al., Effect of Prenatal Protein Malnutrition on Birthdates and Number of Neurons in the Rat Locus Coeruleus. Nutr Neurosci, 1999. 2(4): p. 267-76.
16. Staiti, A.M., et al., A microdialysis study of the medial prefrontal cortex of adolescent and adult rats. Neuropharmacology, 2011. 61(3): p. 544-9.
17. Sullivan, R.M., M.M. Dufresne, and J. Waldron, Lateralized sex differences in stress-induced dopamine release in the rat. Neuroreport, 2009. 20(3): p. 229-32.

18. Sullivan, R.M. and M.M. Dufresne, Mesocortical dopamine and HPA axis regulation: role of laterality and early environment. Brain Res, 2006. 1076(1): p. 49-59.

19. Sullivan, R.M., Hemispheric asymmetry in stress processing in rat prefrontal cortex and the role of mesocortical dopamine. Stress, 2004. 7(2): p. 131-43.

20. Martinez, V. and M. Sarter, Lateralized Attentional Functions of Cortical Cholinergic Inputs. Behavioral Neuroscience, 2004. 118(5): p. 984-991.

21. Mokler, D.J., et al., Stress-induced changes in extracellular dopamine and serotonin in the medial prefrontal cortex and dorsal hippocampus of prenatal protein malnourished rats. Brain Res, 2007. 1148: p. 226-233.

3 - Prenatal protein malnutrition modi fies amygdalo-hippocampal-cortex dynam ics after restrain stress in young rats

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Summary

It is well known that the brain electric activity reflects the Central Nervous System (CNS) neuronal organization and maturity level. Thus, the changes on this activity reveal functional organization adjustments. Previous studies have shown that prenatal protein malnutrition (PPM) can differentially affect structure and function of several brain areas and generate behavioural disturbances at short or long term. Acute stress has been studied as a noxious event leading to brain dysfunction. This study was designed to analyse in 30 day-old wellnourished and prenatally malnourished male rats the effect of acute stress (movement restrain by 30 min), evaluating Prefrontal Cortex (PFC) Basolateral Amygdala (BLA) and Hippocampal Cornus Ammonis 1 (CA1) electrical oscillation and inter- and intrahemispheric correlation. Using stereotaxic surgery, stainless steel monopolar electrodes were placed into CA1, BLA and PC at both hemispheres.

Spontaneous brain activity was recorded during 4 hr as basal and 4 hr after restrain stress. Spectral inter- and intrahemispheric correlation analyses were carried out. Intrahemispheric correlation showed asymmetrical functionality in well nourished but not in malnourished animals during basal recordings. After stress, brain activity changes were not evident in PPM group. However, in well nourished group (Co) interhemispheric correlation showed dynamical changes after stress.

Spontaneous behaviour was also evaluated and it was found affected in PPM group, mainly grooming behaviour which can be an indicative of anxiousness.

The obtained results suggest that prenatal protein malnutrition alters the dynamics and functional arrangement of those substrates regulating the response to the stress, as well as their circuitry functional integration. Keywords: Stress, malnourished, CA1, Basolateral Amygdala, Prefrontal cortex

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Introduction

Protein malnutrition experienced at a time when the nervous system is developing rapidly affects both the circadian rhythm and the homeostatic processes involved in the sleep-wake cycle (Cintra, et al, 1988, 2002, Durán, et al, 1999).

The electrical neuronal activity in the brain as well as its main manifestation as vigilance states (i.e. sleep-wake states) are function of the neuronal organization, differentiation and level of maturity of the nervous system. Thus, changes in the vigilance states after malnutrition undoubtedly reflects altered functional organization of the Central Nervous System (CNS). One of the most studied rhythms is the hippocampal slow rhythmic activity (or theta rhythm), which is observed during REM sleep and active waking in animals, the latter being related to attentional processes. Moreover, the hippocampus is considered as an important filter of information that comes from external sources (e.g. vision, audition, olfaction, etc.). In addition to the hippocampus, previous studies have illustrated that prenatal protein malnutrition can differentially alter the structure -and function of numerous brain areas, and consequently produce both short- and long-term behavioral disturbances (e.g. cognitive performance, vigilance states, etc. and other biological rhythms as locomotor activity and several behavioral patterns). In a previous study Cintra et al (2002) found homeostatic and circadian alterations on the sleep-wake cycle in prenatally protein malnourished post-weaning rats. Wake and REM (rapid eye movement) sleep phases were delayed, amplitude of slow wave sleep (SWS) rhythm was reduced, suggesting a diminution on the number of cells involved in the cortex synchronization, and theta rhythm during REM sleep was slowest but its amplitude was higher than in normal rats. We found a high correlation in the electrical frequencies between cortex and hippocampus in the left hemisphere mainly during REM sleep. This might indicate a disruption in the inhibitory system and neuronal communication involving these structures (because high correlative values indicate a failure in the modulatory system). In turn, these findings could explain some of the long-term alterations found in behavior and cognitive processes in young and adult malnourished rats. Previous studies in prenatal malnourished young and adult rats have demonstrated histological alterations in several nuclei and brain areas involved in the sleep-wake homeostasis (such as Locus coeruleus, raphé nuclei, hippocampi, cortex, etc.) and the modulation or regulation of some behaviors (e.g. limbic system, prefrontal cortex). (Cintra et al., 1997a,b) Other studies have established that exposing prenatally malnourished animals to additional challenges in postnatal life (e.g., stress or pharmacological agents) affects their behavior differently to that of well-nourished controls (Almeida et al., 1996a,b; Tonkiss et al., 1998a,b, 1999, 2003; Kehoe, et al 2001)

Stress has been studied as a noxious event that produces sleep-wake cycle impairments. In animals, acute or chronic stress produces sleep disturbances characterized

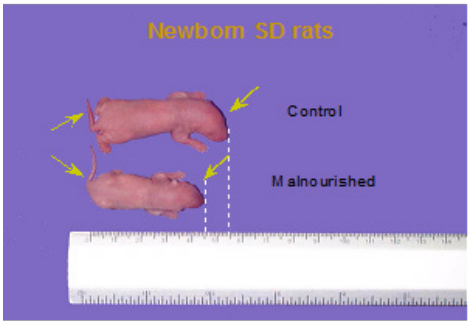
by a decrease in active waking, and an increase on SWS and REM sleep, as well as disruption in the duration, intensity and quality of the sleep-wake cycle. These responses have been associated to mechanisms that may allow the animal to recover and adapt to environmental changes, as part of the homeostatic response for an optimal recovery after stress (Marinesco, et al, 1999). The responses appear to depend on the nature of the stress, since chronic stress is known to decrease REM sleep, whereas, acute stress increase it (Cespuglio, et al, 1995). In addition, some brain areas involved on the homeostatic regulation of sleep, i.e. Locus coeruleus, raphe nuclei, limbic system (particularly the hippocampus), are known to be also involved with stress responses (McEwen, et al 2016). Both, sleep and stress regulatory systems use many of the same neurotransmitters and pathways to modulate their physiological responses. For example, Rampin et al (1991) found that neuropeptide corticotrophin-like-intermediate-lobe-peptide (CLIP) is involved in the regulation of both REM and stress responses. Such results may explain why sleep and stress have a strong reciprocal connection (i.e. not only can stress affect sleep parameters but disturbed sleep patterns can affect stress responsiveness). We already know that prenatal malnutrition alters the neuroanatomical substrates of the sleep-wake cycle; neurophysiological measures associated with it, and may alter the behavioral response to stress. Therefore we would strongly predict and exacerbation of the sleep, wake and behavioral alterations seen in prenatal malnourished animals following stress. This is important because a modification of stress responsiveness could serve a maladaptative function (for cognitive, social and emotional behaviors) in young malnourished individuals.

Methods

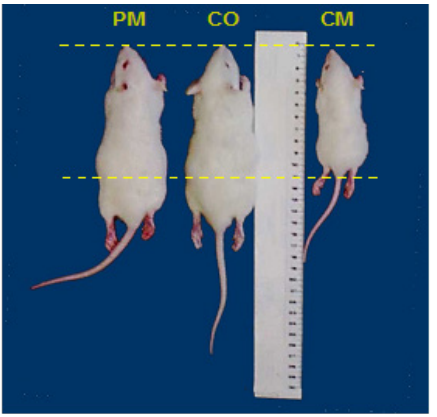
Nutritional Treatment

Five weeks prior to mating, nuliparous female rats (Sprague-Dawley VAF plus; Charles River Laboratories, Kingston, MA) were purchased and allowed ad lib access to one of two isocaloric diets (Teklad, Madison, WI). The diets are formulated to be of adequate protein (25% casein) or low protein (6% casein) content (detailed description given in Galler & Tonkiss, 1991). Males obtained from the same source were acclimated to the experimental diets of the females for one week prior to mating. Each male was then mated with 2 females receiving the same dietary treatment, over a period of 7 days. The presence of sperm in a vaginal smear determined whether mating had occurred. One week prior to the projected delivery date, the females were individually caged in polycarbonate breeding cages (51cm x 41cm x 21cm; Lab Products Inc., Maywood, NJ). Following parturition, litters were culled to 8 pups (6 males and 2 females) and fostered to well-nourished mothers that had given birth no more than 24h previously. Pups born to dams provided with the 6% casein diet and cross-fostered

to lactating dams given the 25% casein diet were assigned the abbreviation "6/25" (prenatally malnourished). Pups born to dams provided with the 25% casein diet and fostered to other lactating dams fed the 25% casein diet were assigned the abbreviation "25/25" (controls). A more detailed description of the nutritional, mating, and fostering procedures is given in a previous paper (Tonkiss and Galler, 1990). All rats were weighed at birth and on the first day of testing, using an electronic balance. After weaning at 21 days all offspring were pair-housed and given ad lib access to Purina rat chow (Formula 5001) and remain so throughout testing at 30-35 days of age.



0-day old rats



30-day old subjects

Electrode Implantation

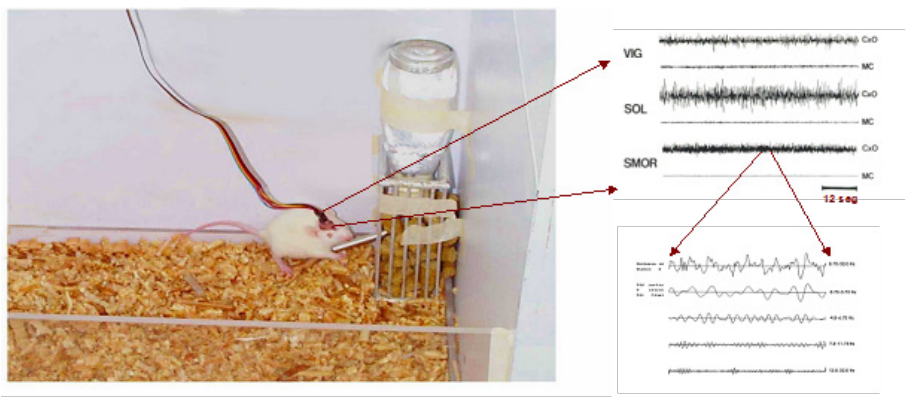
All surgical procedures were performed stereotaxically under aseptic conditions seven days before recording and behavioral manipulation. Animals were anesthetized (Pentobarbital, 50 mg/kg, i.p.) and placed in a stereotaxic apparatus (Kopf, model 1730) using blunt rodent ear bars. The scalp will be cleaned and painted with providone iodine (Betadine). A scalp incision was made, the skin retracted, skull surface cleaned for the implantation of electrodes. To record the behavioral states of vigilance, electrocorticogram (ECoG), electromiogram (EMG), and hippocampal activity (HA) electrodes were implanted. To record ECoG, stainless steel screw (jewelers') electrodes connected to Teflon-coated stainless steel wires were screwed bilaterally in the skull plates over the prefrontal cortex (2.2 mm anterior and 1.0 mm lateral to the bregma). An additional screw electrode was screwed in the bone located above the cerebelar region to act as a reference electrode. To record hippocampal activity Teflon-coated stainless steel macroelectrodes were bilaterally placed in the CA1 hippocampal field (A: 4.8, L: 2.5, H: 4.0 and 3.0). A pair of Teflon-coated stainless steel

wire electrodes was implanted bilaterally in dorsal neck muscles for recording EMG. All electrodes were connected to an amphenol cap that was secured to the skull with craneoplastic (Plastics One, Inc. VA) acrylic.

Recordings Procedure

Three vigilance states, waking (WAK), slow wave sleep (SWS) and Rapid Eye Movements Sleep (REMS), were distinguished from polygraph tracings using a neurodata GRASS acquisition system. Vigilance states were scored visually at the beginning of the active phase for a period of 4 hours during the baseline day and after the stress session and processed for further statistical analyses.

As aforementioned, all recordings started 7 days after surgery at 30 days of age. Twenty four hours before the scheduled baseline day, experimental subjects were placed for habituation with water and food ad libitum, in a electrically shielded, sound--attenuated chamber (38x30x114 cm) illuminated with a reversed 12:12 Light/dark cycle with lights on from 20:00 to 8:00 automatically controlled by a timer. Twenty-four hr on-line recording as baseline was obtained. Stress session was carried out from 9:00 am to 9:30 am followed by 4 or 24 hr-digitalized recordings. Spontaneous behavior (grooming, rearing, sniffing, drinking, eating, lying and walking) after stress was monitored (via video tape recordings) for further analysis. Electrical signals from bilateral prefrontal cortex and CA1 hippocampal fields were collected for further analysis.



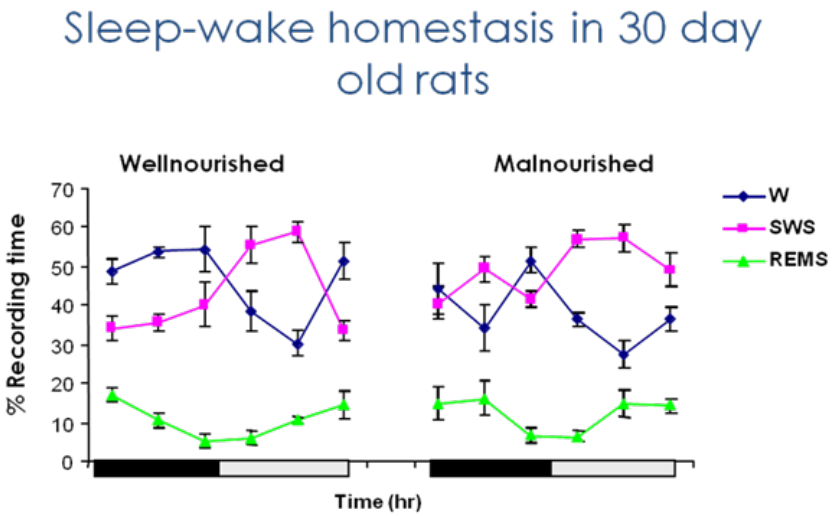
EEG recording and Spectral analysis in a 30-day old rat.

Restraint Stress Procedure

Rats were immobilized for 20 min in a Plexiglas adjustable restraining device. Following immobilization rats were attached to the recording chambers and EEG and spontaneous traits were recorded continuously for the following 4 hr.

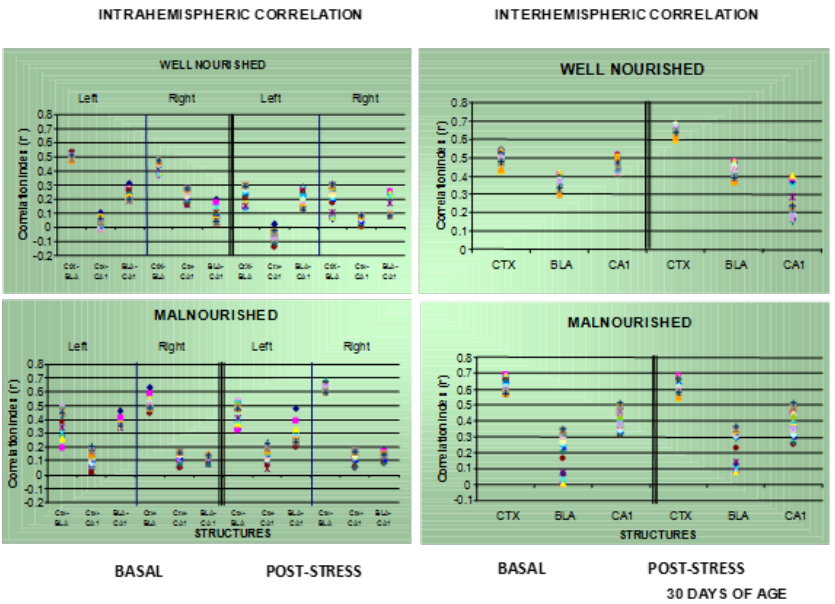
Results

Temporal distribution of the individual components of the sleep-wake cycle (sleep architecture). Three vigilance states were distinguished waking (W), slow wave sleep (S) and REM sleep (R). Baseline and after restraint stress polygraphic recordings were visually scored during the first 4 hours after lights were turned off, showed in prenatally malnourished rats an increase on the SWS and REM sleep, as a consequence of the nutritional condition.



Control rats showed an increase on waking once their circadian activity showed up. It seems prenatally malnourished rats have difficulty to synchronize their circadian and homeostatic processes.

The correlation analysis between prefrontal cortex and hippocampus in 25/25 and 6/25 rats in baseline and post-stress condition. In control animals, base line cortical frequency values are more dispersed (0.1-0.5 Hz), however hippocampal frequencies are distributed between 0.3 and 0.5 Hz. 6/25 rats in the baseline showed a dispersion of cortical frequencies from 0.2 to 0.7 Hz, on the other hand hippocampal frequencies were distributed from-0.2 to 0.3 Hz, indicating a lost of harmony between this structures probably due to malnutrition. Post-stress frequency cortical dispersion in 25/25 animals showed negative values of −0.1 until 0.8 and hippocampal frequencies showed values from 0.0 to 0.7. However 6/25 animals showed the dispersion of cortical frequencies from-0.2 to 0.6 and hippocampal frequencies presented values from -0.2 to 0.2, dispersion of frequencies very different from control animals.



Freely behaving videotaped display for each rat throughout four-hour period after restraint movement stress session (grooming, rearing and rest and activity periods) was analyzed afterwards showed not differences between gropus.

Discussion

Sleep-wake cycle is affected by prenatal protein malnutrition on several ways, Prenatal protein malnutrition has a homeostatic and circadian deleterious effect over sleep-wake cycle, a homeostatic increase on SWS amount, a decrease on the delta waves power density are usually reported in the prenatally malnourished rat. Also, phase shifts and advances are found in the vigilance states, mainly on the REM sleep state, altering their temporal distribution (circadian rhythm) (Cintra, et al. 1988, 2002, Durán, et al, 1999).

The present study investigated the effects of acute stress by movement restriction during 30 min. in control and malnourished adult rats. Malnourished animals showed different architecture pattern in the first and second 2h blocks in comparison with controls. Sleep-waking cycle has been reported previously that is different for 25/25 and 6/25 rats (Durán et al 1999, Cintra et al 2002), this results are in agreement with these reported. On the other hand, it is well documented that stress alters the vigilance states in normal animals, depending on the stress technique and the duration of it. Protein malnutrition produces sleep-wake adjustments, where REM sleep rebound, often reported as a consequence to stress exposure, is not present, at least on the first 24

hours after the stress experience. It is important to note that adjustments over the sleep-wake cycle are depending on the applied technique to produce stress, its duration and its intensity. In the baseline 25/25 rats increased significantly REMS in the second 2h block. However post-stress in 25/25 rats altered vigilance states, increasing waking and REMS and decreasing SWS in the second 2h block in relation to baseline. This increase in REMS has been reported previously as a consequence of movement restrained-acute stress and have been associated to mechanisms that may allow the animal to recover and adapt to environmental changes, as part of the homeostatic response for an optimal recovery after stress (Marinesco, et al, 1999).The post-stress pattern of sleep wake cycle in 6/25 animals was different, reducing SWS and no REMS was found in the second 2h block indicating that malnutrition disrupts the normal response to this acute stress.

Correlation and brain activity.

The obtained results suggest that are different neuro-anatomo-physiological substrates regulating brain activity, as well as they should be harmonized between each other. And may be using the same neural pathways during their functional integration.

Prenatal protein malnutrition affects the harmony and functional arrangement.

Relative power density was found increased on the PM group.

Considering 0.75 as a significant eigenvalue we found in the prenatally malnourished group intra hemispheric and interhemispheric correlation highest in the theta band frequencies rather than in the others.

Dynamics.

The modulation of the neuronal transmission is altered by malnutrition. That modulation implies the action of several, diverse neurotransmitters into the hippocampal GABAergic circuitry, or some kind of alteration on the receptor level, by example, reduction of receptor of some neurotransmitters like serotonin in to the hippocampal formation preventing the hippocampal function to be adequate and complicating communication between structures and hemispheres. As the hippocampal theta activity and electrical oscillations are dynamic processes altered by malnutrition we could expect alterations at a more integrative level (vigilance states, behavior, cognitive processes, etc). However, this is not exact, at least on the light of the present and preceding results. Integrative levels seem to be less affected by malnutrition than lower levels. On the present study dynamic between hippocampal structures was found altered as we measured the frequency correlation, because of that we could expect learning and memory processes to be affect, but reported studies have revealed not to much at this regard. Behaviorally malnourished rats had been found to be less sensitive

of minimally affected by prenatal protein malnutrition (Tonkiss, et al 2002). Regarding stress, previous studies had found contradictory results; this could be related to the stress procedure and experimental conditions as well as to the severity and duration of the malnutrition.

Acute stress induces neuronal activation on several brain regions including hypothalamic paraventricular nucleus (PVN), Locus coeruleus (LC), cerebral cortex and hippocampus. The adaptation of these structures to the chronic or acute stress depends, however, on the type, intensity and duration of the estressor. Thus, a moderate stress, like movement restraint, is sufficient to induces c-Fos expression into the amygdala, hippocampus and cingular and somatosensorial cortex, meanwhile, a more severe stress, i.e. total immobilization, increases c-Fos expression into the PVN, LC, amygdala, hippocampal CA2. Those results suggest PVN, LC and amygdala are involved in the perception of the stress severity. (Chowdhury, et al 2000). On the other hand, duration and quality of sleep states can be modified by occurred experiences during previous waking. Stress is one of those situations known to alter this cycle. Opposite effects into the sleep-wake cycle has been reported as consequence of stress, these apparent controversial reports may be explained depending on the severity and type of stressor. Thus, a low intensity moderate stress is a sleep promoter (Cespuglio, et al, 1995), meanwhile, chronic stress alters the sleep architecture and has more dramatic effects on the sleep (Kant et al 1995, Cheeta, et al 1997). In animals, after 1 or 2 hr of stress by immobilization (restraining movements into acrylic tubes) an increase on slow wake and paradoxical sleep had been reported, suggesting that duration, intensity and rebound of sleep could permit a recovery and adaptation to the environment. However, a movement restriction by expanded periods (4h), produces a total sleep disruption, where sleep rebound disappears, affecting normal recovery and adaptation to novel situations (Marinesco, et al 1999). It has been hypothesized sleep rebound induced by stress is part of those mechanisms denominated as reactive homeostasis, and it is necessary for an optimal recuperation after stress. During long stress duration situations, this process could be altered, conducing deleterious effects in the organism. Also, it is well known the induction and adaptation in the c-Fos expression as response of acute stressor vary from region to region in the brain, again depending on the intensity and duration of stress treatments. By example, the hippocampal dentate gyrus and cingulate cortex present a reduced c-Fos expression when animals had been exposed to acute stress, indicating neuronal inhibitory circuits are involved on this response. Meanwhile, PVN and LC are more resistant to acute stress, suggesting that brainstem structures and hypothalamic nuclei influence the stress induced activity into the hypothalamic-pituitary-adrenal axis. Severe stress induce c-Fos expression in PVn, Lc and amigdaloid nuclei, implying a role in the perception of the stress severity of

these structures, where neuronal activation could be attributed to higher emotional responses.

Meanwhile PVN and LC are more resistant to acute stress, suggesting certain pons structures and hypothalamic nuclei influence the stress induced activity in the hypothalamic-pituitary-adrenal axis. Severe stress produces C-Fos expression in the PVN, LC and amygdaloid nuclei, implying a possible role of those structures on the perception of the stress severity, where neuronal activation can be attributed to major emotional responses. On the other way, interest has increase on the study of interactions between the animal's emotional state and its ability to learn or remember. Particularly, studies are focused to the subjacent neural mechanisms of these interactions and alterations on the synaptic plasticity induced by stress in the hippocampus. It is well known stress exposition produces hippocampal neurons dead and has deleterious effects on the cognitive and memory processes. Thus, has been documented the effect produced by stress on several hippocampus-dependent cognitive task (McEwen y Sapolsky, 1995, Bodnoff et al 1995, de Quervain et al 1998, Baker y Kim, 2002).

Also, stress has been found to suppress the ability to induce long term potentiation (LTP) in the hippocampus (Pavlides et al 1996, Akirav y Richter-Levin, 1999, Wang et al, 2000). However, other brain areas, including amygdala and prefrontal cortex have been suggested as mediators of some aspects of stress response, particularly, among emotional interactions and memories formation. Thus, by example, basolateral amygdala stimulation or lesion module hippocampal LTP (Ikegaya et al, 1996, Akirav y Richter-Levin, 1999). It has been suggested hippocampal- amygdala interaction is part of the emotional memories modulatory mechanism (Akirav y Richter-Levin, 1999). Medial prefrontal cortex is an important component of the neuronal circuitry mediating responses to stressful situation, in addition to be related to working memory and attentive functions (Williams y Goldman–Rakic, 1995, Maroun y Richter-Levin, 2003).

Stress has been studied as a noxious event altering the sleep-wake cycle, additionally affecting, the hypothalamus-pituitary-adrenal axis circadian activity in adult rats(Koehl, et al 1999). Acute or chronic stress produces alterations characterized by a decrease in the active waking, increase of slow wave sleep and paradoxical sleep, also modifying intensity, duration and quality of the vigilance states. Those responses are been related to the mechanisms that permit the animal's recovery and adaptation to the environmental changes as part of the homeostatic response for an optimal recovery after stress (Bouyer et al 1975, 1998; Marinesco, et al, 1999). Responses seems to depend on the stress nature, since chronic stress diminish paradoxical sleep, meanwhile, acute stress increases it (Cespuglio, et al, 1995). Besides, some brain areas involved on the sleep regulation, i.e. LC, raphé nuclei and limbic system (hippocampus particularly) are suggested to be involve in the stress responses.

Both sleep and stress regulatory systems employ similar neurotrasmitters and pathways to modulate their physiological responses. As an example, Rampin et al (1991) found neuropeptide CLIP (corticotrophin-like-intermediate-lobe-peptide) involvement on the paradoxical sleep regulation and stress responses. Such results can explain why sleep and stress have a strong reciprocal connection (means stress affects sleep but sleep affects the stress response). Finally, the rhythmic oscillations coincidence degree in two or more structures or source (simultaneously measured) can be obtained by coherence or correlation analysis. These structures (or sources) can have a direct or indirect connectivity, and the eigenvalues (correlation units) obtained of these analysis can be used to infer the nature of the relationship.

These type of analyses are frequently employed to determine the connectivity level between hemispheres, as well as to determine the occurrence of communication interruptions among brain structures after trauma or neurodegenerative diseases (Corsi--Cabrera et al 2006).. Particularly, correlation analyses has been an invaluable technique to study functional hemispheric interactions during vigilance states (Corsi-Cabrera et al, 1996), they are also useful to reveal cortical organization sexual dimorphism, as well as intracerebral differences among structures in the juvenile malnourished rat (Durán et al., 1999, Cintra, et al 2002).

In this study, prenatal malnutrition revealed an hippocampal activity dissociation after a stressful experience in the adult rat (20 min of restraint movement) These results indicate protein prenatal malnutrition produces alteration on the normal hippocampus-cortical communication, it is possible this bioelectrical activity frequencies are involve in the memory and learning processes modulation and their emotive charge. At the moment we know malnutrition alters the sleep-wake cycle neuroanatomical substrates, associated neurophysiological measures and probably the behavioral response to stress. This is important because a modification on the stress response could play a maladaptive function in malnourished individuals.

References

1. Akirav I, Richter-Levin G. Biphasic modulation of hippocampal plasticity by behavioral stress and Basolateral amygdala stimulation in the rat. J. Neurosci 19:10530-10535, 1999

2. Almeida SS, Tonkiss J, Galler JR Prenatal protein malnutrition affects exploratory behavior of female rats in the elevated plus-maze test. Physiol Behav. 60(2):675-801996a

3. Almeida, SS, Tonkiss, J, Galler, JR Prenatal protein malnutrition affects avoidance but not escape behavior in the elevated T-maze test. Physiol. Behav. 60:191-195,1996b.

4. Baker KB, Kim JJ. Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. Learn Mem 9:58-65, 2002

5. Bodnoff SR, Humphreys AG, Lehman JC, Diamond DM, Rose GM, Meaney MJ. Enduring effects of chronic corticosterone treatment on spatial learning synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. J Neurosci 15:61-69, 1995

6. Bouyer JJ, Deminière JM, Mayo W, Le Moal M. Inter-individual differences in the effects of acute stress on the sleep-wakefulness cycle in the rat. Neurosci Lett 225:193-196, 1975

7. Bouyer JJ. Vallée M, Deminière JM, Le Moal M, Mayo, W. Reaction of sleep-wakefulness cycle to stress is related to differences in hypothalamo-pituitary-adrenal axis reactivity in rat. Brain Res 804:114-124, 1998

8. Cespuglio R, Marinesco S, Baubet V, Bonnet C, El Kafi, B. Evidence for a sleep-promoting influence of stress. Adv Neuroimmunol. 5:145-154, 1995

9. Cintra L, Diaz-Cintra S, Galvan A, Morgane PJ. Circadian rhythm of sleep in normal and undernourished rats. Bol Estud Med Biol. 36(1-4):3-17. 1988

10. Cintra L, Aguilar A, Granados L, Galván A, Kemper T, DeBassio W, Galler J, Morgane P, Durán P, Díaz-Cintra S. Effects of prenatal protein malnutrition on hippocampal CA1 pyramidal cells in rats of four age groups. Hippocampus 7:192-203, 1997a

11. Cintra L, Granados L, Aguilar A, Kemper T, DeBassio W., Galler J, Morgane P, Duran P, Diaz-Cintra S. Effects of prenatal protein malnutrition on mossy fibers of the hippocampal formation in rats of four age groups. Hippocampus. 1997;7(2):184-91b

12. Cintra L, Durán P, Guevara MA, Aguilar A Castañón-Cervantes O. Pre- and Post-natal protein malnutrition alters the effect of Rapid eye movements sleep-deprivation by platform-technique upon the electrocorticogram of the circadian sleep-wake cycle and its frequency bands in the rat. Nutr. Neurosci. 5(2):91-101, 2002.

13. Cheeta S, Ruigt G, Vanproosdij J, Willner P. Changes in sleep architecture following chronic mild stress. Biol Psychiat 41:419-427, 1997

14. Chowdhury GMI, Fujioka T, Nakamura S. Induction and adaptation of Fos expression in the rat brain by two types of acute restraint stress. Brain Res Bull 52(3):171-182, 2000

15. Corsi-Cabrera M, Arce C, Ramos J, Lorenzo I, Guevara MA. Time course of reaction time and EEG while performing a vigilance task during total sleep deprivation. Sleep.19(7):563-569, 1996

16. de Quervain DJ, Roozendaal B, McGaugh JL. Stress and glucocorticoids impair retrieval of long term spatial memory. Nature 394:787-790, 1998

17. Corsi-Cabrera M, Muñoz-Torres Z, del Río-Portilla Y, Guevara MA Power and coherent oscillations distinguish REM sleep, stage 1 and wakefulness.International Journal of Psychophysiology,60 (1): 59-66, 2006

18. Durán P, Galván A, Granados L, Aguilar-Roblero R, Cintra L. Effects of protein malnutrition on the vigilance states and their circadian rhythms in 30-day-old rats submitted total sleep-deprivation. Nutritional Neuroscience, 2:127-138, 1999

19. Herman P, Cullinan WE, 1997 Neurocircuitry of stress: central control of the hypothalamo–pituitary–adrenocortical axis.Trends in Neurosciences, 20(2): 78-84

20. Ikegaya Y, Saito H, Abe K. The basomedial and Basolateral amigdaloid nuclei contribute to the induction of long-term potentiation in the dentate gyrus in vivo. Eur J Neurosci 8:1833-1839, 1996

21. Kant GJ, Pastel RH, Bauman RA, Meininger GR, Maughan KR, Robinson TN, Wright WL, Covington

22. PS. Effects of chronic stress on sleep in rats. Physiol Behav 57:359-365, 1995

23. Kehoe P, Mallinson K, Bronzino J, McCormick CM. Effects of prenatal protein malnutrition and neonatal stress on CNS responsiveness. Dev Brain Res. 2001 Dec 14;132(1):23-31Koehl M, Darnaudéry M, Dulluc J, Van Reeth O, Le Moal M, Maccari S. Prenatal stress alters circadian activity of hypothalamo-pituitary-adrenal axis and hippocampal corticosteroid receptors in adult rats of both gender. J. Neurobiol 40:302-215, 1999

24. McEwen BS, Nasca C, Gray JD. Stress Effects on Neuronal Structure:Hippocampus, Amygdala, and Prefrontal Cortex. Neuropsychopharmacology. 41(1):3-23, 2016

25. Marinesco S, Bonnet C, Cespuglio R. Influence of stress duration on the sleep rebound induced by immobilization in the rat: a possible role for corticosterone. Neurosci 92(3):921-933, 1999

26. Maroun M, Richter-Levin G. Exposure to acute stress blocks the induction of long-term potentiation of the amygdala-prefrontal cortex pathway in vivo. J Neurosci 23(11):4406-4409, 2003

27. McEwen BS, Sapolsky RM. Stress and cognitive function. Curr Opin Neurobiol 5:205-216, 1995

28. Pavlides C, Ogawa S, Kimura A, McEwen BS. Role of adrenal steroid mineralocorticoid and glucocorticoid receptors in long-term potentiation in the CA1 field of hippocampal slices. Brain Res 738:229-235, 1996

29. Rampin C, Cespuglio R, Chastrette N, Jouvet M. Immobilisation stress induces a paradoxical sleep rebound in rat. Neurosci Lett 126:113-118, 1991

30. Tonkiss J, Almeida SS, Galler JR. Prenatally malnourished female but not male rats show increased sensitivity to MK-801 in a DRL task. Behav. Pharmacol. 9:49-60, 1998a

31. Tonkiss J, Trzcinska M, Galler, JR, Ruiz-Opazo, N, Herrera VL Prenatal malnutrition-induced changes in blood pressure: dissociation of stress and nonstress responses using radiotelemetry. Hypertension. 1998 Jul;32(1):108-14 b

32. Tonkiss J, Bonnie KE, Hudson JL, Shultz PL, Duran P, Galler JR. Ultrasonic call characteristics of rat pups are altered following prenatal malnutrition. Dev Psychobiol. 2003 Sep;43(2):90-101.

33. Tonkiss J, Galler J.R. Prenatal protein malnutrition and working memory performance in adult rats. Behav. Brain Res. 40: 95-107, 1990

34. Tonkiss J, Galler JR, Formica RN, Shukitt-Hale B, y Timm RR. Fetal protein malnutrition impairs acquisition of a DRL task in adult rats. Physiol. Behav. 48: 73-77,1990

35. Tonkiss J, Galler JR, Shukitt-Hale B, Rocco FJ. Prenatal protein malnutrition impairs visual discrimination learning in adult rats. Psychobiology 19:247-250, 1991

36. Wang J, Akirav I, Richter-Levin G. Short-term behavioral and electrophysiological conse-

quences of underwater trauma. Physiol Behav 70:327-332, 2000

37. Wiliams GV, Goldman-Rakic PS. Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376:572-575, 1995

4- Autonomic innervation of visceral and peri-vascular adipose tissue

Craig J Daly[1]

Abstract

Obesity, hypertension, and diabetes are together recognized as a metabolic syndrome which is more prevalent with ageing. An element common to both hypertension and obesity is hyperactivity of the sympathetic nervous system. With ageing we experience broader alterations in general autonomic function, including hyperactivity. Therefore, with globally increasing incidence of obesity (and associated disorders) it is important to more fully understand the complex interplay that exists between the autonomic nerves and adipocytes. This short review charts the development of our appreciation of the importance of innervated fat and considers the physiological interactions between both systems.

Keywords: Adipose Tissue, sympathetic, autonomic, adrenergic, vascular, lipolysis.

Introduction

It is becoming common to think of human body fat as an endocrine organ [1]. Previously regarded as merely the storeroom for triglycerides, we now know that fat provides a large variety of paracrine activities that are essential for normal physiological function. It has been estimated that adipocytes secrete around 900 adipokines [2] which demonstrates the complexity and importance of this system. However, autonomic (nervous) control of the adipocytes is still not clearly understood even though research stretches back 40 years or more. This review draws on key observations from the literature over the past four decades that have shaped our understanding of the autonomic and adrenergic control of fat. In particular, the innervation of peri-vascular adipose tissue (PVAT) will be considered.

There are three main types of fat cell. The most abundant is the white adipose tissue (WAT). This constitutes around 20-25% of body mass and emphasises the important

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physiological role that such a large endocrine system must play. White adipocytes contain unilocular lipid droplets and have relatively few mitochondria. The main function of WAT is the storage of triglycerides and subsequent release of free fatty acids following lipolysis. Brown adipose tissue (BAT) plays a mainly thermogenic role, comprises multilocular lipid droplets; it contains large quantities of mitochondria. Beige (or brite) adipocytes are a hybrid that develops in WAT but shares many of the BAT characteristics (i.e. dense mitochondria and multilocular lipid droplets). BAT receives a rich innervation and vascular supply whilst WAT tends to have less, except where populated by brite adipocytes [3].

Early studies

In the mid-late 1960's a picture was emerging suggesting that the sympathetic nervous system modulated metabolism under certain conditions (i.e. cold). It was recognised that adrenaline and noradrenaline played a role, but attributing a distinct α- or β-adrenoceptor role was difficult. Adenyl cyclase was known to be involved, but the overall picture was unclear. The review by Jean Himms-Hagen in 1967 serves as an excellent overview of the thinking around that time [4]. As experimental techniques improved, so did the understanding of the adrenergic control of metabolism. Using dihydroergotamine and propranolol, in an anaesthetized dog, it was shown that stimulation of the nerve supply to subcutaneous inguinal fat caused a rise in the plasma levels of both glycerol and free fatty acid (FFA), thus demonstrating that nerve activity promoted lipolysis. The β-adrenoceptor antagonist propranolol inhibited the nerve induced lipolysis suggesting that the sympathetic neurotransmitter, noradrenaline, acted at β-adrenoceptors to stimulate lipolysis [5]. Further experiments in humans found that adrenaline, in the presence of propranolol, caused a profound inhibition of lipolysis. This inhibition was blocked by phentolamine and could therefore be attributed to the action of α-adrenoceptors [6]. Further work in isolated human adipocytes showed that clonidine (α_2-adrenoceptor agonist) was far more effective in inhibiting lipolysis than either methoxamine or phenylephrine (both α1-adrenoceptor agonists) [7].

With regards to the now known existence of nine adrenoceptor subtypes (all G-protein coupled receptors (GPCRs)), it has been proposed that β_3-adrenoceptors stimulate lipolysis and inhibit lipogenesis. α_2-adrenoceptors inhibit lipolysis and α_1-adrenoceptors inhibit lipogenesis [8]. α_1-adrenoceptors may also stimulate lipolysis. The presence of at least three adrenoceptor subtypes on fat cells suggests that either adrenaline or noradrenaline (or both) play an important role in the regulation of fat and metabolism. It is well established that cAMP drives lipolysis and of the 163 GPCRs that have been detected in adipose tissue, 48 either stimulate or inhibit lipolysis [9]. Following stimulation by the catecholamines adrenaline and noradrenaline, β-adrenoceptors will enhance cAMP

production whilst α_2-adrenoceptors inhibit it. Receptors for ATP and neuropeptide-Y have also been detected in adipose tissue which therefore provides targets for all three of the main sympathetic neurotransmitters.

Innervation of fat

The presence of nerves within BAT has been established through the use of histological methods and neurotoxic drugs. Using 6-hydroxydopamine, it was demonstrated that destruction of the nerves via chemical sympathectomy reduced histological detection of noradrenaline and NPY staining in rat interscapular brown adipose tissue (ISBAT) [10]. The same study showed that reserpine only abolished the noradrenergic staining, confirming that NPY has a different localisation within ISBAT. Both chemicals demonstrate a strong adrenergic presence within BAT, and therefore a likely physiological role for nerve released noradrenaline.

The presence of a functional innervation of WAT has been more difficult to establish. Fishman and Dark wrote, in 1987, 'mammalian white adipose tissue is considered neuronally quiescent' and also that sensory innervation either does not exist or has not been studied [11]. Using small crystals of 'true blue' implanted into inguinal or dorsal subcutaneous fat, it was shown that dye transferred back along sensory nerves and stained the cell bodies within the dorsal root ganglia (DRG) [11]. Thus, these fat deposits probably contain sensory nerves that can detect, or 'taste', the local environment in order to signal higher brain centers of the CNS. The study found that inguinal fat sensory neurons have their cell bodies in the DRG at T13-L2 whilst the dorsal fat sensory fibres have their cell bodies at T1-T3.

More recent work on sensory innervation of fat has concentrated on the peri-vascular adipose tissue (PVAT) surrounding blood vessels. Sensory nerves, containing calcitonin gene related peptide (CGRP), which promotes vasodilation in response to electrical field stimulation in vitro can only do so when the surrounding PVAT is present [12]. It is therefore hypothesized that sensory nerves in PVAT release CGRP which can cause direct relaxation of the vascular smooth muscle. In addition, CGRP-stimulated leptin release from PVAT can activate leptin receptors in adventitial sensory nerves to release additional CGRP. It has also been hypothesized that substance P (SP) released from sensory nerves in PVAT can stimulate release of leptin and can also promote lipolysis [8]. The presence of pre-junctional P2x-receptors on the sensory nerves in PVAT also facilitates an interaction between the sympathetic nerves (via ATP release) and the sensory system.

The circumstantial evidence provided above suggests a possible local interaction between sympathetic and sensory nerves within a given adipose tissue bed. These interactions may serve to regulate adipocytes that are local to the individual sensory

and sympathetic fibers. Other local interactions exist between the adipose tissue and the vascular and neuronal supply. Brown and beige adipocytes can, upon activation by sympathetic nerves, release nerve growth factors which can influence the degree of innervation. The brown fat can also release vascular endothelial growth factors to influence the degree of vascularisation [3]. Therefore, adipose tissue can potentially control its own blood and nerve supply. This all suggests an extremely complex system of local regulation within adipose tissue.

A more systemic interaction is described as the adipose afferent reflex [13]. It is hypothesized that sensory afferent fibers in WAT through detection of local environmental conditions or direct stimulation (neurotransmitters or adipokines) can signal the paraventricular nucleus of the CNS. This in turn communicates with the rostral ventrolateral medulla which ultimately leads to an increase in sympathetic nerve activity [13]. Therefore, local factors in the body's adipose tissues can have a direct effect on blood pressure, the heart and kidney function.

Peri-vascular adipose tissue

Most blood vessels are covered in adipose tissue. This is particularly evident in the thoracic aorta and mesenteric arteries (figure 1). Closer examination of the type of PVAT reveals that the thoracic aorta has a more beige appearance and thus comprises a mix of white, brite and possibly brown adipocytes. In contrast, the mesenteric arteries appear to be covered in mainly white adipocytes. PVAT was originally reported to have mainly anti-contractile properties. However, as stated above, the number of releasable compounds (around 900) makes it highly likely that adipose tissue (and PVAT) can have both anti- and pro-contractile effects. A detailed assessment of the balance of these effects is beyond the scope of this short article.

The mouse aorta has few (if any) autonomic nerves within its wall and is presented in figure 1 only as an example of how extensive PVAT can be; extending from the largest conduit artery to the smallest resistance arteries of the mesenteric bed. Interestingly, despite a lack of significant sympathetic innervation, the aortic PVAT has been shown to release noradrenaline in response to applied tyramine [14]. This suggests that PVAT is capable of catecholamine uptake and release of noradrenaline as a potentially pro-contractile factor.

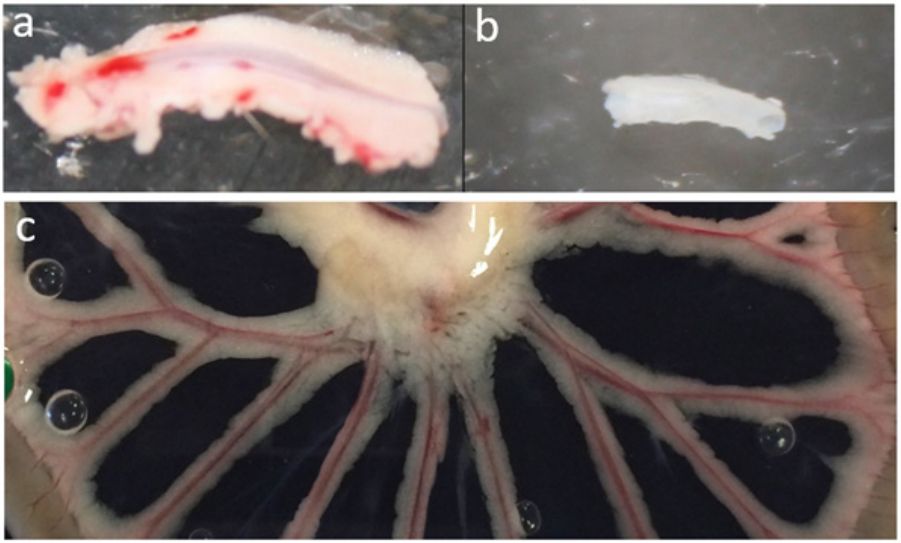

Figure 1. Perivascular adipose tissue (PVAT). a) mouse thoracic aorta with PVAT intact. b) a short segment of the aorta shown in (a) after removal of PVAT. c) rat mesenteric arteries with PVAT intact (mouse mesentery is very similar).

The mesenteric resistance arteries have been well characterized as vessels that remodel significantly in hypertension where they exhibit an increased media-lumen ratio [15] They also have a convenient size and optical properties that are well suited for phase contrast microscopy [16], widefield fluorescence microscopy [17] and laser scanning confocal microscopy [18]. Therefore, their detailed 3D structure has been well examined and described. However, it is only relatively recently that the innervation of the PVAT around mesenteric arteries has been studied [8, 12]. It appears from 3D laser scanning microscopy work that the same nerves that innervate the vascular wall also supply the PVAT. Figure 2 shows different views of a mouse mesenteric artery that has been stained with an antibody to the nerves (PGP 9.5). The PVAT has also been stained using a fluorescent α1-adrenoceptor antagonist (QAPB aka BODIPY FL-prazosin) which is highly lipophilic. The main concentration of nerves, within the tunica adventitia, run along the axis of the vessel (figure 2a & 2b). Nerves can also be seen branching out to the PVAT and individual fibers can easily be traced from the fat to the vessel's nerve supply. It should be stated that the type(s) of nerves shown in figure 2 cannot be specifically identified. Whilst the vast majority will be sympathetic, some will be sensory, and the presence of parasympathetic fibres cannot be ruled out. Much more detailed work is now required to fully understand the interplay between the vascular sympathetic nerves, the PVAT and the adventitia.

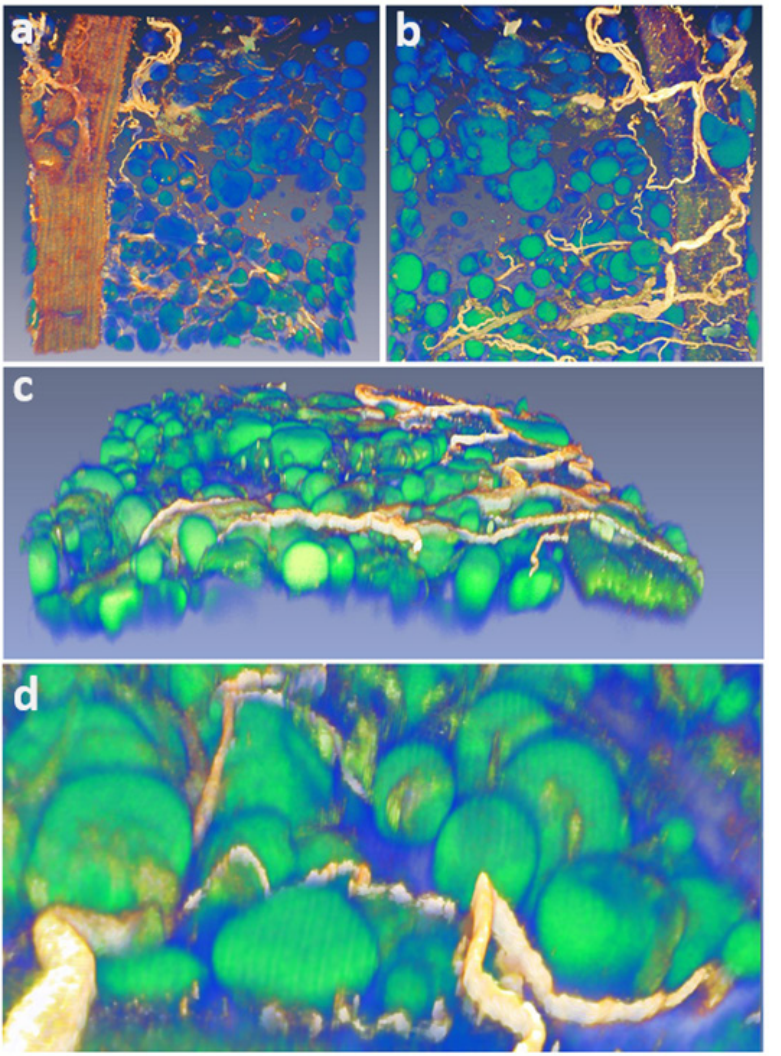

Figure 2. Confocal Laser Scanning Microscopy (CLSM) of a mouse mesenteric artery with attached perivascular adipose tissue (PVAT) PVAT from on top and to one side of the artery has been removed to facilitate viewing. The nerves are stained with the specific antibody PGP 9.5 (yellow). The PVAT is stained with a fluorescent from of prazosin (lipophilic α1-adrenoceptor antagonist, blue/green). a) The mesenteric artery (vertical structure to the left) is viewed from the inner endothelial surface with nerves, on the upper arterial surface, branching into the PVAT (Green/blue). b) Top surface of the sample with the vessel viewed adventitial side up. The dense vascular nerve network can be seen running the length of the vessel and branching into the PVAT. c) & d) Alternative views of the 3D volume shown in a & b. These alternative views have been selected to highlight the close apposition of the nerves and PVAT. Size bars are not shown due to the rotation and zoom introduced by viewing at different angles.

Discussion

Our understanding of the physiological role of adipose tissue is progressing rapidly. It is very clear that body fat acts as an extremely important endocrine organ. Furthermore, adipose tissue appears to contain sensory afferent fibers that can signal the central nervous system to modulate the autonomic nervous system in an adipose afferent reflex. Metabolic syndrome links obesity, hypertension and diabetes. The work cited here, and other published literature, draws clear links between adipose tissue and sympathetic activity. Although not discussed here, it is worth mentioning that adiponectin release from adipocytes can interfere with the ability of insulin to function as a vasodilator [8]. Therefore, it is possible that adipose tissue (and thus obesity) may have a much greater role in controlling blood pressure and glucose levels than we are currently aware of. Furthermore, the vascular wall, by virtue of its autonomic nerve supply and abundant PVAT may be the perfect model for studying and understanding the intricate interplay between neurotransmitters and adipokines.

Future directions

Textbook diagrams of the autonomic innervation of the vascular wall will always show the neurotransmitters travelling towards the smooth muscle of the tunica media. Very few, if any, will show the neurotransmission path being equally capable of traveling away from the smooth muscle and towards the outer adventitia and PVAT [8]. The presence of sympathetic, sensory and parasympathetic nerves in the vascular wall and PVAT provides the possibility of eight or more neurotransmitters within the local environment. Adding the likelihood of hundreds of adipokines serving as vasoconstrictors, vasodilators and growth mediators presents an unimaginable complexity. The same argument can be made for any organ that is covered in fat and has an autonomic and sensory innervation. Research is now required that looks specifically at the way in which local fat deposits are neuronally connected to their adjacent organs. A clearer understanding of the 'adipocrine'- tissue coupling could provide important new therapeutic targets for metabolic syndrome disorders.

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References

1. Coelho M, Oliveira T & Fernandes R (2013) Biochemistry of adipose tissue: an endocrine organ. Arch Med. Sci. 9(2): 191-200

2. Lehr S, Hartwig S & Sell H (2012) Adipokines: A treasure trove for the discovery of biomarkers for metabolic disorders. Proteomics Clin Appl. 6(1-2):91-101

3. Wang W & Seale P (2016) Control of brown and beige fat development. Nat Rev, Mol Cell Biol, 17(11) 691-702.

4. Himms-Hagen J (1967) Sympathetic regulation of metabolism. Pharmacological Reviews. 19 No. 367-461

5. Fredholm BF & Karlsson J (1970). Metabolic effects of prolonged sympathetic nerve stimulation in canine subcutaneous adipose tissue. Act Physiol., Scand. 80. 567-576

6. Lafontan M, Dang-Tran L & Berlan M (1979) Alpha-adrenergic antilipolytic effect of adrenaline in human fat cells of the thigh: comparison with adrenaline responsiveness of different fat deposits. Eu. J. Cin Pharm. 9, 261-266.

7. Lafontan M, Berlan M. (1980)Evidence for the alpha 2 nature of the alpha-adrenergic receptor inhibiting lipolysis in human fat cells. Eur J Pharmacol. 22;66(1):87-93.

8. Bulloch JM & Daly CJ (2014). Autonomic nerves and perivascular fat: interactive mechanisms Pharmacology & Therapeutic;143(1):61-73

9. Amisten S, Neville M, Hawkes R, Persaud SJ, Karpe F, Salehi A. (2015) An atlas of G-protein coupled receptor expression and function in human subcutaneous adipose tissue. Pharmacol Ther. Feb; 146:61-93

10. Mukherjee S, Lever JD, Norman D, Symons D, Spriggs TL & Jung RT. (1989) A comparison of the effects of 6-hydroxydopamine and reserpine on noradrenergic and peptidergic nerves in rat brown adipose tissue. J Anat. 167:189-93.

11. Fishman RB and Dark J (1987) Sensory innervation of white adipose tissue. Am J Physiol. 253: R942-R944.

12. Bakar HA, Dunn WR, Daly CJ & Ralevic V (2017). Sensory innervation of perivascular adipose tissue: a crucial role in artery vasodilatation and leptin release. Cardiovascular Research, Vol 113, Issue 8, 962-972.

13. Xiong XQ, Chen WW & Zhu GQ (2014) Adipose afferent reflex: sympathetic activation and obesity hypertension. Acta Physiol 210, 468-478

14. Nadia Ayala-Lopez, Marisa Martini, William F Jackson, Emma Darios, Robert Burnett, Bridget Seitz, Gregory D Fink, & Stephanie W Watts (2014) Perivascular adipose tissue contains functional catecholamines. Pharmacol Res Perspect. 2014; 2(3): e00041.

15. Mulvany MJ (2002) Small artery remodeling in hypertension. Current Hypertension Reports 4:49-55

16. Mulvany MJ & Halpern W (1976) Mechanical properties of vascular smooth muscle cells in situ. Nature 260, 617-619

17. Daly, C.J., Gordon, J.F. & McGrath, J.C. (1992) The use of fluorescent nuclear dyes for the study of blood vessel structure and function: novel applications of existing techniques. J. Vascular Research; 29 41-48.

18. Daly CJ, McGee A, Vila E, Briones A, Arribas SM, Pagakis S, Adler J, Merle A, Maddison J, Pedersen J, McGrath JC. (2002) Analysing the 3D structure of blood vessels using confocal microscopy. Microscopy & Analysis November, 92, 5-8.

5 - An update on the causes and subsequent effects of gender differences in the human brain

Dr Des Gilmore[1]

Summary

Recently controversy has arisen regarding the presence of sexual dimorphism in the central nervous system (CNS) despite numerous well-documented studies confirming its existence. These include anatomical examination of specific regions in the brain, experimental manipulations of steroid hormones during development and observed differences in both non-sexual and sexual behavior in both animals and humans. It is now very well established that androgens, acting early in fetal life, bring about masculinization of the genitalia and thereafter act upon the CNS. The latter takes place only during limited critical periods when the neural tissue is sufficiently plastic to respond permanently and irreversibly to their action. In addition to androgen-induced structural changes in several regions of the brain there are also effects on future behavior. However, it is vitally important to recognize that because clear sex differences do exist in the CNS and these cannot be ignored, but in no way do these make one gender superior or inferior to the other.

Key Words: Brain; Dimorphism; Masculinization; Gender Differences; Behavior

Introduction

Currently some controversy has arisen as to whether there are actually distinct differences between the sexes with regard to brain structure and function. The recent use of neuroanatomical imaging has contributed to the debate. Two stalwart opponents of hardwired sexual dimorphism in the brain are Gina Rippon (1) and Cordelia Fine (2), who describe themselves as staunch feminists. Fine alleges that the evidence for the existence of innate biological gender differences in the minds and brains is faulty and exaggerated and is a form of "neurosexism". She believes that both social and envi-

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ronmental factors strongly influence behavior and claims that weaknesses in research techniques have affected the results obtained. Rippon, working at Aston University in Birmingham in the field of cognitive neuroimaging, describes how reported sex differences in the brain can be traced back as far as the 18th and 19th centuries. She claims that many male researchers from this time onward sought to prove men were superior to women in all aspects of life. Rippon insists that the notion there are males and female brains is bunkum and "neurotrash" and any observed differences can be attributed to social and environmental influences. Thus she argues that it is life experiences that mould our highly plastic brains well into adult life. Rippon therefore believes that the reason boys and girls prefer gendered toys and show other behavioral differences from a very early age is largely due to their carers' expectations. Her arguments fail, however, to explain observations as regards sexually divergent behavior in animals, both natural and experimentally induced, nor the effects of hormonal disruption that may occur during limited critical stages in human development. Moreover, her claims are also at odds with much research including that by Ingalhalikar et al. (3), and that which showed that sex-typed toy preferences are also present in vervet monkeys (4). The latter findings were later confirmed.in rhesus monkeys (5).

Hormonal effects in early development

Like the undifferentiated genitalia, specific regions of the brain are primary targets for gonadal steroids when they are sufficiently responsive to become irreversibly differentiated along the male pathway; this determines future patterns of both sexual and non-sexual behavior (see Gilmore 6 for a review and comprehensive list of references). In humans, fetal testes are active by the eighth week of gestation, stimulated by the action of human chorionic gonadotropin from the placenta. Male differentiation of the external genitalia is brought about at this time by the conversion of testosterone, produced in the Leydig cells, to 5α-dihydrotestosterone (5α-DHT). However, masculinization of the CNS takes place later. Numerous studies upon rodents have shown that in those regions of the brain where steroid hormone receptors are present, testosterone crosses the blood brain barrier and as a result of intraneuronal aromatization to estradiol-17β (E2) masculinization of the brain takes place. This alters the basic processes of neural development and also plays an important role in influencing later reproductive behavior. It would appear that the developing female brain in rodents is largely prevented from masculinization by E2 being irreversibly bound to α-fetoprotein and thereby unable to pass into neuronal tissue. Interestingly, findings using a knock out mouse model lacking α-fetoprotein, indicated that parental as well as sexual behavior is also sexually differentiated perinatally (7). In primates it has been suggested that it is sex hormone binding globulin (SHBG) to which E2 is attached and so unable

to enter neurons (see Hong et al, 8). However, an alternative view regarding brain masculinization in humans has recently been put forward (9, 10). It was argued after reviewing evidence, that a functional androgen and not an estrogen receptor in the CNS is required for the developing brain to be programmed for future male sexual orientation and behavior. It was also pointed out (9) that men lacking the aromatase enzyme still show masculine behavior despite their inability to aromatize testosterone to E2.

Sexual differentiation of the CNS

Irrespective of claims that little if any sexual dimorphism exists in neural tissue in humans and animals, these have indeed been shown to be present in the structure and function at certain locations in the brain and spinal cord (see 11). With the advent of neuroimaging methodology, multiple studies have found sex differences in the brain that may underlie the behavioral differences seen between the genders. It was recently re-emphasized that males have larger crania proportionate to their greater body size, and a higher percentage of white matter containing myelinated axonal fibers, and cerebrospinal fluid, whereas women demonstrate a higher percentage of gray matter after correcting for sex differences in intracranial volume (3). It has also been shown how testosterone, acting during fetal life in humans, influences specific areas in the brain that later develop in a sexually dimorphic fashion (11). Moreover, very recent findings support the hypothesis of sex differences in cognitive strategies used to solve mental rotation tasks (12).

Sex differences in the relative size and shape of specific brain structures have also frequently been reported. These include the hypothalamus, hippocampus, amygdala. corpus callosum and prefrontal cortex. Furthermore, developmental differences in tissue growth suggest that there is an anatomical sex difference during maturation, although links to observed behavioral divergence have not yet been established. Sexual differentiation of the CNS is more subtle than that of the reproductive system, but of equal importance. Sex hormones exert a dual influence on it. In the male, as already mentioned, during fetal or early neonatal life, androgens act in an inductive capacity on the undifferentiated brain to bring about its masculinization. Later, following puberty, the gonadal steroids act on the CNS in an excitatory or inhibitory manner to influence gonadotropin secretion and the expression of sexual behavior. Both the sex and stress hormones play a critical role in mammalian brain development regulating the number, location and connectivity of neurons and by exerting potent influences on neurogenesis, programmed cell death and survival, neuronal migration and synaptogenesis. Exposure to testosterone, E2 and the glucocorticoids needs to be critically timed. The effects on hormone release, homeostasis, learning, memory and emotions have to be co-ordinated to ensure the correct orchestration of

development in the CNS through the control of key developmental processes.

The period of maximum susceptibility of the brain to gonadal steroids depends on the maturity of the CNS, and is independent of parturition. Testicular androgens exert an organizational influence on the CNS only during limited critical periods. They act as signal transduction molecules, neurotropic factors and/or neuromodulators by binding to specific intracellular receptors and so altering gene expression and/or transcriptional factors within the target cell to affect protein synthesis in neuronal tissue (13). In a similar manner estrogens have been shown to attach to specific intracellular receptors within the brain to modify them in such a way that they can bind DNA and so alter the expression of estrogen-sensitive genes (14).

It has been pointed out by Goldstein et al (15) that not unsurprisingly, in those brain areas involved in the control of sexual behavior, there is co-localization of gonadal steroid receptors with those of neurotransmitters including the monoamines, γ-amino butyric acid (GABA) and growth factors. Years ago we were able to demonstrate that, when brain serotonin (5-HT) levels are lowered in male hamsters around the time of sexual differentiation, there is an increase in the level of female sexual behavior these animals later exhibit as adults (16). This would suggest that 5-HT may affect a neural substrate already differentiated by androgens during the perinatal period. Exogenously-administered opiates can also interfere with neural development, and consequently affect adult male sexual behavior, as we were able to show (17, 18).

It must be emphasized that it is only during limited times the neural tissue is sufficiently plastic to respond permanently and irreversibly to the hormones. These periods have been delineated in a number of placental mammals including the mouse, rat, hamster, guinea pig, rabbit, sheep, and rhesus monkey by the examination of the effects of timed hormone treatments on sexually-dependent neuroendocrine and behavioral patterns. In humans, however, it is only by observing the effects of hormonal anomalies that occur during embryonic and fetal development that information can be gathered regarding their involvement in differentiation of the CNS. It is important to note that the critical period is an empirical concept, and does not represent a clearly-defined stage of development. Moreover it cannot be assumed that all sexually differentiated CNS structures are maximally sensitive to gonadal steroids at the same time. Critical sensitive periods vary temporally for different sexually dimorphic traits.

As previously alluded to, the effects of the sex steroids on neural structures take place quite some time later than differentiation of the genitalia. Thus in certain instances, due to a failure of the brain to become masculinized, there may be a mismatch between one's external genitalia and their sexual identity. This could explain the belief of some males that they are trapped in the wrong body and identify as male to female transsexuals. Furthermore the inappropriate masculinization of the brain of a genetic

female would result in the opposite situation. It is important to recognize that transsexualism is quite independent of sexual orientation and that transgender individuals may identify as heterosexual, homosexual, bisexual or asexual. It has been estimated by Amnesty International that 0.3% of people in the European Union regard themselves as transsexual. Recently transgender rights have become widely discussed, in not least with regard to the participation of transexual women in female sporting events. Debate relating to this contentious issue has involved the potential use of antiandrogens to reduce testosterone levels, gender reassignment surgery and separate categories for non-binary athletes.

Sex differences in behavior

There are many instances of sexual dimorphism in the human as regards behavior. Research undertaken over decades has indicated that women are more sensitive to touch, and have better fine motor co-ordination than do men. Men are reported to perform well in tasks that require visual spatial skills, map reading, negotiating mazes, at mathematics and at perceiving and manipulating objects in space. Women are appreciably better at tasks that require a language ability. Obviously these are general and not absolute attributes. However, even in the infant, sex differences with regard to behavior are apparent. Baby boys will respond more to what is visually catching in the environment, e.g. lights, patterns and three dimensional objects. Baby girls will respond preferentially to faces of people rather than to inanimate objects. However, it is now becoming clear that postnatal socialization is also an important factor contributing to differences in gender-related behavior.

The organization of the brain itself also seems less laterally delineated in females. In right-handed males language is rigidly segregated in the left hemisphere, while visual spatial skills are contained in the right hemisphere. In right-handed females, the hemispheres are less functionally distinct and more diffusely organized. It is evident that testosterone, acting during fetal life, has a major influence on the development of visual spatial skills as e.g. in the testicular feminization (androgen insensitivity) syndrome, these skills are less than in other XY men. Such XY individuals are born with female external genitalia and generally raised as girls. Their testes are intra-abdominal and although these produce androgens their tissues, lacking androgen receptors, are unresponsive. Consequently neither their external genitalia nor brain are masculinized. They tend to be very beautiful (e.g. the 1930s actress Jean Harlow was believed to be such an individual) and regard themselves as women both physically and mentally. A recent example is the Belgian model Hanne Gaby Odiele. On the other hand XYY men have been found to have even greater visual spatial skills than might be expected, whereas in XXY (Klinefelter's syndrome) males these are normal.

XO (Turner's syndrome) females tend to be exaggeratedly female with regard to their behavior. Moreover, many years ago it was reported how testosterone administration to pregnant rhesus monkeys increases the incidence of male behavior in their female offspring (19).

Manipulation of the hormonal environment in rodents during critical periods of early development affects many aspects of sex specific behavior including play, scent marking, feeding and aggression as well as that relating to reproduction (see 20). These behavioral changes, due to early hormonal administration, are accompanied by very noticeable alterations in brain structures. It is thus now obvious that also in humans prenatal exposure to raised levels of androgens results in psychosocial development moving towards a masculine direction with regard to both childhood play behavior and sexual orientation. Girls born with congenital adrenal hyperplasia, and thereby exposed to high levels of androgens in utero, show varying degrees of genital virilization. Moreover, these individuals also exhibit increased masculinized play behavior as children and a minority are more likely than other women to be homosexual or bisexual in adulthood. In those rare situations where infant boys have been reassigned as girls following damage to the penis, there have frequently been major problems in them accepting their newly assigned gender because their brains have already been masculinized. Observations by Imperato-McGinley (21, 22) shed considerable light on this situation. She discovered groups of related individuals in the Dominican Republic and later in Papua New Guinea with a genetically-determined 5α-reductase deficiency. This resulted in an inability to convert testosterone to the more metabolically active 5α-DHT. Due to this there was a marked hypoplasia of the external genitalia present at birth; consequently most of the children, although genetically male, were raised as girls. Nevertheless, because of testosterone having masculinized their brains in utero, the boys had less difficulty than might have been expected in adopting a masculine gender reassignment at puberty as their sexual orientation was male. However, it has to be recognized that humans are surprisingly flexible as regards their core sexual identity and may thus exhibit a wide spectrum of gender and non-gender specific behavior in later life. It is thus not really possible to categorize humans and also other animals as exclusively hetero- or homosexual. However, evidence has accumulated to indicate that in some individuals there is a predisposition to homosexuality, i.e. that a gene (or genes) influence sexual orientation without necessarily determining it. Results from research on a genome-wide linkage scan carried out on 409 pairs of homosexual brothers revealed some interesting results (23). Two regions of linkage were identified on the X_{q28} region of the X chromosome and the pericentromeric region of chromosome 8. How the genes might operate to predispose individuals to a homosexual orientation is as yet unclear. They

may act directly or indirectly on a sex specific region of the brain e.g. the $INAH_3$ nucleus, or indirectly through affecting a personality trait.

The response of the hypothalamic-pituitary-adrenal axis to stress is also sexually differentiated and has been characterized as fright, fight or flight in males, but as tend, defend or befriend in females. Sex differences during development in the exposure to the glucocorticoids are also very important. In a comprehensive review Gillies et al.(24) it was pointed out that in males, but not females, there may be competing epigenetic influences, as the glucocorticoids briefly overlap with testosterone or its estrogenic metabolites on organizational processes in the CNS, thus contributing to its sexual dimorphism. If for some reason the glucocorticoids are inappropriately elevated during pregnancy, either by the mother being severely stressed or by the administration of several doses of exogenous glucocorticoids when premature birth is threatened, brain structure and function may be affected leading to some feminization of reproductive behavior in later life. Moreover, it can also alter the hypothalamic-pituitary-adrenal axis reactivity to stress and consequently physiological stress-coping mechanisms. An impairment of the latter during development, associated with a faulty programming of the hypothalamic-pituitary adrenal axis, is a risk factor for the appearance of psychiatric and other brain disorders in later life, many of which show clear sex differences. Near the end of pregnancy both sexes in utero experience a critically timed rise in the bioavailability of glucocorticoids released from the fetal/maternal adrenal axis. This is vital for the maturation of many organs and tissues, in particular the lungs (production of surfactant) and the brain.

Gender differences in numerous pathological conditions

Critics of Fine, including Baron-Cohen and his colleagues (25), insist she has fused science with politics in her strident extreme denial that structural gender differences exist in the brain. Moreover, it has been emphasised by McCarthy et al, (26) how there are clear differences between the sexes with regard to cognitive and emotional responses related to learning, memory and language, fear, anxiety and nociception. There are also such sex differences in the outcome following traumatic brain injury, stroke, and neurodegenerative diseases including Parkinson's, Alzheimer's and Huntington's. Neurological conditions such as dyslexia and stuttering are also three to four times more frequent in boys than in girls, and attention deficit hyperactivity disorder is 10 times more common in boys (see 20). However, this may be partly due to the condition being underdiagnozed in girls. A lack of brain sexual dimorphism would also not explain why gender is such an important risk factor for the development of both autism and early onset schizophrenia. Males are much more vulnerable to autism spectrum conditions as incidentally so are transsexual individuals and the reason for this bias is at present unclear, However, it has been suggested by Baron-Cohen et al. (25) that a possible biological mechanism that may account

for it is the effect of fetal testosterone on the developing brain.

In summary it would appear that the denial of evidence for any sex differences in the brain, or at least those attributed to the action of androgens in early development, is largely due to a somewhat biased view of political correctness. Moreover, as has been pointed out by Gillies et al. (24) different preventive, diagnostic and treatment approaches may be required for men and women with certain pathological conditions. It has been emphasized that this highlights the urgent need for a greater understanding of the specific pathways that exhibit biological sexual dimorphism in the brain, along with mechanisms which generate these differences (24). Finally one should celebrate rather than ignore all the evidence that has accumulated to indicate that the CNS is altered early in life by the action of androgens and accept that in no way does this make the brain of one gender of greater or lesser value than that of the other.

References

1. Rippon G. The Gendered Brain 2019 Bodley Head 448pp. ISBN-13.9781847924766
2. Fine C. Delusions of Gender (2010) WW Norton & Company 338pp. ISBN 0393968382
3. Ingalhalikar M, Smith A, Parker D, Satterthwaite TD, Elliott MA, Ruparel K, et al. Sex differences in the structural connectome of the human brain. Proc Nat Acad. Sci USA 2014; 111:823–828.
4. Aexander GM, Hines M. Sex differences in response to children's toys in nonhuman primates (Cercopithecus aethiops sabaeus) Evol Hum Behav 2002; 23:467–479.
5. Hassett JM, Siebert ER, Wallen K. Sex differences in rhesus monkey toy preferences parallel those of children. Horm Behav 2008; 54:359–364.
6. Gilmore DP. Sexual Dimorphism in the Central Nervous System 2015 Chapter 1 pp.1-30 in Mammalian Endocrinology and Male Reproductive Biology Edited by Shio Kumar Singh CRC Press 331pp. ISBN-13:978-1-4987-2735-8.
7. Keller M, Pawluski JL, Brock O, Douhard Q, Bakker J. The alpha-fetoprotein knock-out mouse model suggests that parental behavior is sexually differentiated under the influence of prenatal estradiol. Horm Behav 2010; 57:434-440
8. Hong H, Branham WS, Ng HW, Moland CL, Dial SL, Fang H, et al. Human sex hormone binding globulin binding affinities of 125 structurally diverse chemicals and comparison with their binding to androgen receptor, estrogen receptor and alpha-fetoprotein. Toxicol Sci 2015; 143:333-348.
9. Motta-Mena NV, Puts D. Endocrinology of human sexuality, mating and reproductive behavior. Horm Behav 2018; 91:19-35.
10. Puts D, Motta-Mena NV. Is human brain masculinization estrogen receptor-mediated? Reply to Luoto and Rantala. Horm Behav 2018; 97:2-4.
11. Lombardo MV, Ashwin E, Auyeung B, Chakrabarti B, Taylor K, Hackett G, et al. Development/Plasticity/Repair Fetal Testosterone Influences Sexually Dimorphic Gray Matter in the Human

Brain. J Neurosci 2012; 32:674-680.

12. Kurth F, Spencer D, Hines M, Luders E. Sex differences in associations between spatial ability and corpus callosum morphology J Neurosci Res 2018; 96:1380-1387.

13. McEwen BS. Non-genomic and genomic effects of steroids on neural activity. TIPS 1991; 12:141-147.

14. Parker MG. Mechanisms of action of steroid receptors in the regulation of gene transcription. J Reprod Fert 1990; 88:717-720.

15. Goldstein JM, Seidman LKJ, Horton NJ, Makris N, Kennedy DN, Caviness VS, et al, Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. Cereb Cortex 2001; 11:490-497.

16. 16. Johnston H, Payne AP, Gilmore DP. Effect of exposure to morphine throughout gestation on feminine and masculine adult sexual behaviour in golden hamsters. J Reprod Fert 1994; 100:173-176.

17. Johnston HM, Payne AP, Gilmore DP. Perinatal exposure to morphine affects adult sexual behaviour of the male golden hamster. Pharmacol Biochem Behav 1992; 42:41-44.

18. Johnston HM, Payne AP, Gilmore DP, Wilson CA. Neonatal serotonin manipulation alters the adult sexual behaviour of golden hamsters. Pharmacol Biochem Behav; 1990; 35:571-575.

19. Goy RW, McEwen BS. Sexual Differentiation of the Brain. 1980 MIT Press London 236pp. ISBN-9780 262070775.

20. Gilmore DP. Human brain sexual dimorphism: causes and consequences. Neurobiologia 2010; 73:19-33.

21. Imperato-McGinley J, Miller M, Wilson JD, Peterson RE, Shackleton C, Gajdusek DC. A cluster of male pseudohermaphrodites with 5α-reductase deficiency in Papua New Guinea. Clin Endocrinol 1991; 34:293-298.

22. Imperato-McGinley J, Peterson RE, Gautier T, Sturla E. Androgens and the evolution of male gender identity among male pseudohermaphrodites with 5α-reductase deficiency. New Engl J Med 1979; 300:1233-1237.

23. Sanders AR, Martin ER, Beecham GW, Guo S. Genome wide scan demonstrates signjficant linkage for male sexual orientation. Psychol Med 2015; 45:1379=1388.

24. Gillies GE, Virdee K, McArthur S, Dalley JW, Sex-dependent diversity in ventral tegmental dopaminergic neurons and developmental programming: A molecular, cellular and behavioural analysis. Neuroscience 2014; 282:69-85

25. Baron-Cohen S, Lombardo MV, Auyeung B, Ashwin E, Chakrabarti B, Knickmeyer R. Why are autism spectrum conditions more prevalent in males? PLoS Biol 2011; 9:e10001081.

26. McCarthy MM, Arnold AP, Ball GF, Blaustein JD, De Vries GJ. Sex Differences in the Brain: The Not So Inconvenient Truth J Neurosci 2012; 32:2241-2247.

6 - The Involvement of the Autonomic Nervous system in the Sexual Response

Des Gilmore[1]

Summary

The parasympathetic and sympathetic branches of the autonomic nervous system along with somatic innervation are all involved in the sexual response. Afferent stimuli from the genitalia can be processed supraspinally in the brain or just be dealt with spinally. Unusually, it is parasympathetic activity leading to the release of nitric oxide (NO) that causes relaxation of the arteries and arterioles in the erectile tissue resulting in their engorgement with blood. Sympathetic input, however, brings about vasoconstriction and loss of any erection, but by causing the release of principally norepinephrine and neuropeptide Y is responsible for ejaculation. NO raises the intracellular concentration of cyclic guanosine monophosphate (cGMP) in the vascular smooth muscle cells of the penis and thereby maintains the erection. cGMP is normally broken down by the enzyme phosphodiesterase type 5 (PDE5) therefore limiting the extent of the erection. Recently PDE5 inhibitors, including sildenafil (Viagra®), have been employed as effective treatments for erectile dysfunction by preventing the degradation of cGMP and thus enabling normal reproductive function in men.

Key Words: ANS; NO; cGMP; Erection; Ejaculation

Introduction

The sexual response in both adult males and females is the result of a complex neurovascular process involving the co-ordinated action of sympathetic, parasympathetic and somatic innervation leading to numerous behavioral changes and physiological alterations in the body including vascular dilation of the erectile tissue in the penis and clitoris. This is accompanied by secretions from the accessory sex glands, smooth muscle contraction of the vas deferens during ejaculation, rhythmic vaginal contrac-

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tions during orgasm in females and contractions of the somatic pelvic muscles that accompany orgasm in both sexes. Although a number of circulating hormones are also vitally important for normal reproductive functioning, this review will concentrate on the involvement of the autonomic nervous system (ANS) in the process, in particular on sexual arousal. However, it is nevertheless necessary to be aware that androgens, in particular, influence the sex drive and arousal and consequently the frequency of sexual behavior.

Centrally certain parts of the brain, in particular the limbic system and the anterior-medial and medial-tuberal regions of the hypothalamus, play a pivotal role in regulating sexual behavior by linking the nervous and endocrine systems and also through being important integration centers for promoting libido. Furthermore, within the brain there are several regions containing receptors for androgens and estrogens which, in the human as in many animals, are irreversibly altered during early development to determine male and female gender identification and future reproductive behavior (see Gilmore 1, 2 for reviews). If lesions occur there prior to puberty sexual development may be affected; if these happen in the adult the result can be impotence in men and amenorrhea in women. Amongst the large number of central neurotransmitters which play either a facilitatory or an inhibitory role in the control of reproduction are norepinephrine (NE), acetylcholine (ACh), dopamine (DA) and serotonin (5-HT) along with the endogenous opioids. Over many decades much of our research has been devoted to this topic (see 3-11).

A detailed account of the central neurotransmitters involved in erectile control has been provided by Andersson (12). He relates how, during sexual arousal in men, cell bodies of neurons found in the hypothalamic paraventricular nucleus and surrounding regions secrete oxytocin, contributing to erections by activating excitatory neural pathways from the spinal cord to the penis. The importance of oxytocin and of vasopressin (ADH), largely produced in the hypothalamic supraoptic nucleus, in the control of genital reflexes and associated sociosexual behavior is only now becoming apparent (see 13). Interestingly, one consequence of taking 5-HT reuptake inhibitors as a treatment for depression is a high incidence of erectile dysfunction in men as reported by Modell et al. (14). 5-HT inhibits erections by opposing the effects of proerectile neurotransmitters and oxytocin.

Neural control of erection

The testes, epididymides, accessory sex glands and erectile tissue all receive innervation from the parasympathetic and sympathetic branches of the ANS through the pelvic and hypogastric nerves respectively and somatic input primarily via the pudendal nerve. Erection is basically a spinal reflex that can be initiated through the recruit-

ment of afferent neurons in the genital region by touch, but also be induced by visual, olfactory and imaginary input to the brain. The primary tactile input comes from highly sensitive mechanoreceptors in the genital area, especially in the glans penis. Enhanced sensitivity to touch during erection generates a positive feedback loop. Afferent signals from genital stimulation travel in nerve fibers which form bundles and become the dorsal nerve of the penis. This eventually, along with other contributions, becomes the pudendal nerve that enters the spinal cord by way of the dorsal roots at level S2-S4. The pudendal nerve terminates on spinal and interspinal neurons in the region from where messages can be conveyed to the somatic sensory cortex. An excellent account of the physiology of human erection and the various pathological conditions affecting it has been provided by Dean & Lue (15). Afferent stimuli can be processed and integrated supraspinally in the brain, arriving there via spinothalamic and spinoreticular pathways. On the other hand, the stimuli may just be dealt with spinally. In the latter situation afferent fibers carrying the impulses synapse in the lower spinal cord on interneurons and so trigger an efferent outflow without any central involvement, to produce an erection. This is commonly observed in paraplegics who suffer from lesions of the spinal cord above the sacral segments, but exhibit reflexive erections. Other sensory information is conveyed centrally by the pelvic and hypogastric nerves, primarily from the internal pelvic organs. Studies on the rat have demonstrated that all of the afferent nerves are sensitive to circulating sex steroids, as well as to noxious stimuli arising from the internal genitalia, vagina and skin (16). Moreover, their distribution is such that they are able to carry a great deal of sensory information to the brain that will initiate sexual arousal.

Erection of the penis and clitoris is a rare example of parasympathetic activity leading to relaxation of the smooth muscle in the penile arteries and arterioles and their consequent dilation. In contrast sympathetic input causes vasoconstriction and loss of any erection. Preganglionic sympathetic neurons at synapses within the ganglia release Ach which activates nicotinic ACh receptors on postganglionic neurons. In response, postganglionic neurons, in most instances, release NE, which activates adrenergic receptors on the peripheral target tissues that bring about the effects associated with the sympathetic innervation. Interestingly, the vascular sympathetic neurotransmitters, in addition to causing vasoconstriction, can also inhibit parasympathetic transmission via prejunctional alpha2-adrenoceptors. The reverse is also true as parasympathetic-derived ACh can inhibit sympathetic transmission via prejunctional muscarinic receptors.

Parasympathetic activity leading to an erection originates in neurons located in the intermediolateral region of the sacral spinal cord at level S2-S4. Axons from these neurons emerge from the ventral roots and reach the genital area via the pelvic nerve

after passing through the hypogastric plexus. They terminate within smooth muscle cells lining the arteries and arterioles in the erectile tissue. The parasympathetic preganglionic neurons and their pathway to the penis and clitoris are, unsurprisingly, substantially similar in both sexes. There is preganglionic sympathetic innervation of the genital region via the hypogastric nerve and somatic input via the pudendal nerve from α-motoneurons located in the ventral horn of the lower thoracic and upper lumbar spinal cord. As regards the sympathetic innervation, preganglionic fibers emerge through white rami at the level of T12, L1 and L2 in the spinal cord. These fibers synapse with postganglionic ones in the inferior mesenteric paravertebral ganglion and it is the postganglionic fibers from these neurons that pass through the hypogastric plexus to innervate the penis and other parts of the genitalia including the accessory sex glands.

Autonomic Input to the Genital Region

Innervation	Principal Nerve Involved	Major Neurotransmitter(s)
Parasympathetic	Pelvic	NO
Sympathetic	Hypogastric	NE + NPY

Vascular supply to the genital region

Blood is supplied to the penis by three branches of the internal pudendal artery. The greatest amount of the blood supplying the corpora cavernosa reaches them through the dorsal arteries of the penis which run alongside the deep dorsal vein. Most of the blood to the corpus spongiosum also arrives there by the same arteries and by those supplying the bulb of the penis (see 17). In the flaccid state the arterial branches supplying the penis are constricted and there is very little blood in the erectile tissue there. Activity in the postganglionic neurons in the relevant parasympathetic ganglia causes dilation of penile and clitoral arteries, and a corresponding relaxation of the smooth muscles in the venous sinusoidal spaces in the erectile tissues. Consequently, there is a great increase in blood flow causing the cavernous spaces in the penis to expand, the corpora cavernosa more than the corpus spongiosum. Concurrent with this occurring, a decrease in sympathetic tone allows relaxation of the smooth muscle in the corpora cavernosa, further contributing to the tumescence of the penis, while the ischiocavernosus and bulbospongiosus muscles mechanically compress the veins of the corpora cavernosa thereby obstructing the outflow of blood from the penis and causing the erectile tissue to become even more rigid. The pattern of blood flow bringing about penile erection can change very quickly and in some instances the latter can occur within as little as five to 10 seconds! Blood pooling in the glans penis also causes

it to enlarge and become darker in colour. During erection the corpus spongiosum expands, but not nearly as much as do the corpora cavernosa; to do so would close the urethra and so interfere with ejaculation. The increase in pressure in the corpus spongiosum and glans is reported to be only one third to half of that in the corpora cavernosa (15). The angle of the erect penis is dependent on its size and on its attachment by ligaments to the puboischial rami and the anterior surface of the pubic bone. In men with a long heavy penis, or a loose suspensory ligament, the angle when erect may not be more than 90 degrees (see 15), but much more in most individuals when it becomes fully rigid.

Role of nitric oxide in erection

Although, whereas in most postganglionic parasympathetic actions the mediator is ACh, the innervation of the penile and clitoral arteries instead involves neurons that release nitric oxide (NO). Neurogenic NO is synthesized enzymatically from the amino acid L-arginine by three isoforms of NO synthase (NOS), including endothelial NOS (eNOS). NO is now known to be the most important influence in bringing about the relaxation of smooth muscle in the arterial walls leading to dilation of the vessels and consequently large amounts of blood entering the erectile tissue. Erection subsides when parasympathetic activity returns to baseline. Another parasympathetic effect associated with sexual arousal is an excitatory input to the vaginal (Bartholin's) glands and to the vas deferens, seminal vesicles and prostate as well as to the bulbourethral (Cowper's) glands; the secretions of the latter lubricating the glans penis and helping remove toxic substances from the urethra.

ACh and vasoactive intestinal peptide (VIP) have also been implicated as vasodilating neurotransmitters involved in erection, but their role is minor in comparison to that of NO. Many cases of impotence are caused by insufficient release of NO, the function of which is to raise the intracellular concentration of cyclic guanosine monophosphate (cGMP) in vascular smooth muscle cells. It is the activation of the enzyme guanylate cyclase by NO which converts guanosine-5'-triphosphate (GTP) to cGMP and thereby lowers intracellular calcium levels. The presence of cGMP in high concentrations in the vascular smooth muscle cells lining the blood vessels of the erectile tissue triggers relaxation of arterial and trabecular smooth muscle bringing about the arterial dilation and venous constriction thereby leading to erection (see 18). Breakdown of cGMP by the enzyme phosphodiesterase type 5 (PDE5) limits the extent of vasodilation and thereby the intensity of the erection. PDE5 is the predominant phosphodiesterase in the corpora cavernosa and is made up of two identical subunits each with a catalytic and a regulatory domain. Francis et al. (19) reported how the catalytic domain contains a single binding site for cGMP and is thus the target for PDE5 inhibitors. These block the

degrading action of PDE5 on cGMP in the vascular smooth muscle cells supplying the penis by preventing its breakdown at the catalytic site. Moreover, it has been stated (18) that, since the PDE5 inhibitors raise the level of cGMP, they potentiate their own actions because cGMP binding to the allosteric site stimulates further PDE5 inhibitor binding to the catalytic site. These drugs are now employed in the treatment of erectile dysfunction, and were the first effective oral treatment available for the condition. They include sildenafil (Viagra®), vardenafil (Levitral®) and tadalafil (Cialis®). It is reported (20) that the former two have a half-life of around four hours, whereas that of (Cialis®) is over 17 hours.

Development and action of pde5 inhibitors

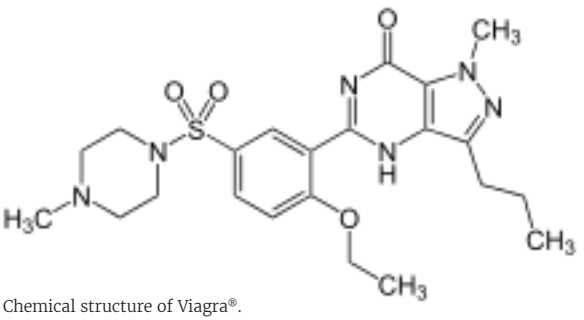
An interesting account of the research leading to the discovery of Viagra® was provided in a lecture given in early 2015 at the University of Glasgow by Professor Sir Simon Campbell, a synthetic organic chemist, who received his PhD from the University of Birmingham in 1965. Interestingly, he was Visiting Professor at the University of São Paulo from 1969 to 1972 and later became the Senior Vice President of Medicinal Discovery at Pfizer where he oversaw the development of Viagra®. The drug was isolated from a research program attempting to block the action of PDE5 and increase tissue levels of cGMP, even although the endogenous ligand that increased the activity of guanylate cyclase was unknown at that time. Starting from zaprinast, a weak and non-selective PDE5 inhibitor, computer modelling guided rational medicinal chemistry to bring about significant increases in potency and selectivity within a novel series of pyrazolopyrimidines. Optimization of selective adrenergic receptor drugs and pharmacokinetics led to the development of Viagra®.

Unfortunately Viagra® was found to be devoid of any appreciable cardiovascular activity and thus ineffective for the treatment of angina and hypertension. However, the growing realization that NO and cGMP had a role in controlling blood flow to the penis suggested that Viagra® might be effective for the treatment of male erectile dysfunction. This hypothesis was verified from extensive clinical trials in nearly 5000 patients and Viagra® was approved by the American FDA in March 1998 as a treatment for this condition. Viagra® is now one of the world's most widely prescribed medicines and has been, and continues to be, used by hundreds of millions of patients. Within three months of its launch it had earned $US400 million for Pfizer and thereafter it has generated sales of $US1.8 billion annually. . However, it is extremely important to understand that drugs such as Viagra® are potentially dangerous when taken along with nitrates and α-adrenergic receptor blockers because these all lower blood pressure and their combined use may lead to severe hypotension.

The molecular structure of Viagra® is similar to that of cGMP. It functions as a

competitive binding agent of PDE5 in the corpora cavernosa, resulting in the presence of more cGMP and better erections. In the absence of sexual stimulation, and therefore lack of activation of the NO/cGMP pathway, Viagra® will not bring about an erection. Moreover, because during sexual arousal this pathway is stimulated specifically in the penis, PDE5 inhibitors including Viagra® only exert a relatively small effect on smooth muscle in other tissues. However, despite this being the case, some recent findings from studies at the University of Manchester, have indicated that Viagra® may indeed have a cardioprotective role in certain conditions. Research by Professor Andrew Trafford and his colleagues from observations of 6,000 diabetic patients revealed that PDE5 inhibitors have a pronounced effect in slowing the progression of heart failure and reducing the risk of fatal arrhythmias (21).

Viagra® is metabolised in the liver by cytochrome p450 enzymes, mainly CYP4503A4 (major route), but also by CYP2C9 (minor route) hepatic isoenzymes. The major metabolic product produced by the action of these enzymes is N-desmethylated sildenafil, which is then further metabolised. This compound itself has an affinity for the PDE binding site, but only around 40% of that of Viagra®. The metabolites are excreted predominantly in the feces and to a much lesser extent in the urine. If taken with a high-fat meal absorption is reduced; the time taken to reach the maximum plasma concentration increases by around one hour, and the maximum concentration itself is decreased by nearly one-third. Certain drugs, including alcohol block NO release, thus interfering with erection, as do many diseases including diabetes.



Chemical structure of Viagra®.

Interestingly, in New Zealand red deer farming is a big business. Early antler growth (velvet) is a soft cartilaginous tissue, well supplied with blood vessels and nerves, and which can grow more than two centimetres daily. This velvet is harvested and exported to Asia for medicinal use. It was once extensively taken as an aphrodisiac, but the discovery of Viagra® with its proven effect led to an appreciable drop in velvet exports and consequent reduction in an important source of revenue!

Control of ejaculation

NE and neuropeptide Y (NPY) are released in the penis by the postganglionic sympathetic nerve terminals present there (see 22). NE has a major contractile effect on the smooth muscles of the penis and blood vessels by acting through stimulation of α1-adrenoceptors and its action is increased in the presence of NPY. When the impulses provoking erection reach a critical level, a spinal reflex is initiated and a massive discharge of impulses occurs over the sympathetic nerves supplying the genital organs - largely at the level of L1 and L2. This leads to ejaculation. Therefore, in contrast to erection, ejaculation is a sympathetic reflex that causes closure of the smooth muscle sphincter at the base of the urinary bladder. As a result, urine is not discharged at this time and semen does not enter the bladder through retrograde ejaculation. The somatic component of ejaculation arises from α-motoneurons in the lumbar and sacral regions of the spinal cord that stimulate the bulbocavernosus and ischiocavernosus muscles. The somatic input also contributes to the contractions in the striated muscles of the perineal floor that are associated with orgasm in both sexes. Even before ejaculation occurs, rhythmic peristaltic contractions occur in the ampulla of the vas deferens, the seminal vesicles and prostate, propelling semen into the penile section of the urethra. Typically this leads to the discharge of a small amount of semen before ejaculation proper, which can result in pregnancy when the withdrawal method is relied upon as a form of contraception. Ejaculation itself results from peristaltic contractions in the smooth muscle of the accessory sex glands leading to pulses of semen spurting out from the penile urethra. Associated with orgasm are rapid rhythmic contractions of the anal sphincter. Following ejaculation the arterioles supplying the erectile tissue constrict and the smooth muscle within it contracts, causing a reduction in size of the blood sinuses. This relieves pressure on the veins supplying the penis and consequently blood drains away allowing the penis to return to its more usual flaccid state. Priapism is a persistent and generally painful erection of the corpora cavernosa unrelated to sexual desire or stimulation. It may last several hours and results from abnormalities in the blood vessels and nerves, frequently in response to medication taken to induce erections.

As mentioned earlier, in the female the ANS also has a major role to play in the sexual response. During the arousal phase in the female (similar to the initiation of erection in the male) many of the responses reflect activity in the parasympathetic division of the autonomic nervous system. Dilation of the blood vessels in the erectile tissue causes their engorgement with blood and erection of the clitoris is accompanied by distension of the perineal tissues and subsequent lowering of the posterior third of the vagina. Parasympathetic fibers arising from the sacral plexus innervate the erectile tissues just as they do in the male. Orgasm in the female also appears to be related to the sympathetic division of the ANS, as is ejaculation in the male. Psychic stimuli involved in the female orgasm are co-ordinated through the cerebrum, which also

modulates the autonomic response. Orgasm is itself co-ordinated via a spinal reflex involving the pudendal nerve arising in the sacral region, which causes rhythmic contractions of the perineal muscles. The spinal reflexes that take place during orgasm in the female also appear to increase uterine and cervical activity facilitating sperm transport to the upper regions of the reproductive tract.

Both mechanical damage to, and disease-related effects, on the spinal cord and brain can result in impairment of sexual arousal and orgasm in both sexes. Sedatives, narcotics and tranquillizers in high doses can also have this effect because of a lowering of nerve conductance in the central nervous system. Some drugs such as β-blockers, used to treat hypertension, can alter neural control of blood flow to the genitalia resulting in difficulty in achieving an erection in the male and decreased vaginal swelling and lubrication in the female. In older men fertility is often maintained into the 80s or even 90s, as testosterone is still being secreted, though in lower amounts than in younger individuals. However, erection takes longer and the penis is likely to be less firm when fully erect. The length of the refractory period (i.e. the time following orgasm, during which another erection and ejaculation cannot occur), is also prolonged. Moreover, the volume of semen at ejaculation is less and testicular size and elevation during arousal are reduced. In older (post-menopausal) women there is a lack of estrogen, resulting in decreased blood flow to the vagina and consequently reduced vaginal secretions for lubrication. This can result in painful intercourse, damage to the vaginal epithelium and consequently increased risk of infection. Many responses to sexual arousal are also reduced.

Thus, in summary mechanical stimulation of the penis, or descending input from the brain act on interneurons in the spinal cord. These bring about changes in the activity of the sympathetic and parasympathetic neurons that project to small arteries supplying the erectile tissue. Activity in the sympathetic neurons supplying these arteries decreases, while that in the parasympathetic neurons increases. Therefore when erection commences, the vessels in the penis dilate, blood flow increases and the vascular channels become engorged with blood. At the same time expansion of the blood sinuses compresses the veins that drain the penis; resulting in the slowing of blood leaving the organ and so helping to maintain the erection. Sexual arousal also brings about increased blood flow to the testes, causing them to increase in size and be lifted closer to the body. In many, but not all men (as in most women) there is also erection of the nipples during arousal. This is caused by muscle contractions in the breast accompanied by increased blood flow to the nipples. Eventually a spinal reflex leads to a massive discharge in the sympathetic nerves serving the genital region leading to ejaculation. The somatic contribution to ejaculation contributes to the contractions of the perineal floor that are associated with orgasm in both sexes.

References

1. Gilmore DP. Sexual Dimorphism in the Central Nervous System 2015 Chapter 1 pp.1-30 in Mammalian Endocrinology and Male Reproductive Biology Edited by Shio Kumar Singh CRC Press 331pp. ISBN-13:978-1-4987-2735-8.
2. Gilmore DP. Human brain sexual dimorphism: causes and consequences. Neurobiologia 2010; 73:19-33.
3. McDonald PG, Gilmore DP. The effect of ovarian steroids on hypothalamic thresholds for ovulation in the female rat. J Endocr; 1971; 49:421-429.
4. Al-Hamood MH, Gilmore DP, Wilson CA. Evidence for a stimulatory beta-adrenergic component in the release of the ovulatory LH surge in pro-oestrous rats. J Endocr 1985; 106:143-151.
5. Siddiqui A, Gilmore DP. Regional differences in the catecholamine content of the rat brain: effects of neonatal castration and androgenization. Acta Endocrinologica (Copenh) 1988; 118:483-494.
6. Johnston HM, Payne AP, Gilmore DP, Wilson CA. Neonatal serotonin manipulation alters the adult sexual behavior of golden hamsters. Pharmacol Biochem Behav 1990; 35:571-575.
7. Johnston HM, Payne AP, Gilmore DP. Perinatal exposure to morphine affects adult sexual behaviour of the male golden hamster. Pharmacol Biochem Behav 1992; 42:41-44.
8. Brown CH, Gilmore DP, Kalia V, Leigh AJ, Wilson CA. Dose-dependent opioid modulation of the preovulatory luteinizing hormone surge in the rat. Mediation by catecholamines. Biogenic Amines 1994; 10:119-128.
9. Gilmore DP. The involvement of the endogenous opioids in the regulation of reproduction. Biogenic Amines 1998; 14:331-354.
10. Yilmaz B, Gilmore DP. Opioid modulation of catecholaminergic neurotransmission and the pre-ovulatory LH surge in the rat. Neuroendocrinol Lett 1999; 20:115-121.
11. Gilmore DP. Reflections of a reproductive physiologist living and working in two hemispheres. pp 225-275 in Neurociência e Desenvolimento Humano o infinito não acaba jamais, Edited by Carlos Augusto Carvalho de Vasconcelos, & Modesto Leite Rolim Neto. ISBN 978-85-8013-317-2. 2014.
12. Andersson KE. Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. Pharmacol Rev 2011; 63:811-859.
13. Normandin LL, Murphy AZ. Somatic genital reflexes in rats with a nod to humans: anatomy, physiology, and the role of the social neuropeptides. Horm Behav 2011; 59:656-665.
14. Modell JG, Katholi CR, Modell JD, DePalma R. Comparative sexual side effects of buproprion, fluoxetine, paroxetine and sertraline. Clin Pharmacol Ther 1997; 61:476-487.
15. Dean RC, Lue TF. Physiology of penile erection and pathophysiology of erectile dysfunction. Urol Clin North Am 2005; 32:379-395.
16. Peters LC, Kristal, MB, Komisaruk BR. Sensory information of the external and internal genitalia of the female rat. Brain Res 1987; 408:199-204.
17. Jung J, Jo HW, Kwon H, Jeong NY. Clinical neuroanatomy and neurotransmitter-mediated regulation of penile erection. Int Neurourol J 2014; 18:58-62.
18. Corbin JD. Mechanisms of action of PDE5 inhibition in erectile dysfunction. Int J Impot Res 2004; 16 Suppl 1:S4–S7.

19. Francis SH, Busch JL, Corbin JD, Sibley D. cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action. Pharmacol Rev 2010; 62:525–563.

20. Wright PJ. Comparison of Phosphodiesterase Type 5 (PDE5 inhibitors) Int J Clin Pract 2006; 60:967-975.

21. Anderson SG, Hutchings DC, Woodward M, Rahimi K, Rutter MK, Kirby M, et al. Phosphodiesterase type-5 inhibitor use in type 2 diabetes is associated with a reduction in all-cause mortality. Heart 2016; 101:1750-1756.

22. Giuliano F, Rampin O. Neural control of erection. Physiol Behav 2004; 83:189-201.

7- Estimulação elétrica nervosa transcutânea sobre a autoavaliação do sono na doença de parkinson

Transcutaneous electric nerve stimulation in sleep of patients with parkinson's disease

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Resumo

Os distúrbios do sono estão entre os sintomas não motores mais frequentes na doença de Parkinson (DP). Esta série de casos avaliou os efeitos da Estimulação Elétrica Nervosa Transcutânea (TENS) em acupontos sobre a autoavaliação do sono em 14 pacientes com DP classificados entre os estágios 1 e 3 da Escala de Hoehn e Yahr original (HY). Pacientes foram avaliados através da Escala de Sono para Doença de Parkinson (PDSS) e submetidos ao tratamento com oito sessões de TENS sobre acupontos, sendo a corrente do tipo Burst. Utilizou-se o teste t para amostras pareadas, considerando-se $p \leq 0,05$. Houve melhora estatisticamente significativa sobre o escore total da PDSS e seu domínio psicose noturna após o período de intervenção. A terapia parece promissora sobre os distúrbios do sono associados a DP, porém são necessárias mais pesquisas.
Palavras-chave: Analgesia por acupuntura, Estimulação Elétrica Nervosa Transcutânea, Doença de Parkinson.

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Abstract

Sleep disorders are among the non-motor signs more common in Parkinson's disease (PD). This case series evaluated the effects of Transcutaneous Electrical Nerve Stimulation (TENS) on acupoints in the self-assessment of sleep in 14 PD patients classified between stages 1 and 3 of Hoehn and Yahr original (HY). Patients were assessed using the Sleep Scale for Parkinson's Disease (PDSS) and subjected to treatment with eight TENS sessions on acupoints, with the current Burst. We used the paired sample t-test, considering $p \leq 0.05$. There was a statistically significant improvement in the total score of the PDSS and its domain night psychosis after the intervention period. The therapy seems promising about sleep disorders associated with PD, but more research is required.
Keywords: Acupuncture analgesia, Transcutaneous Electric Nerve Stimulation, Parkinson's Disease.

Introdução

A doença de Parkinson (DP) é um distúrbio neurodegenerativo progressivo comum em idosos[1,2]. Seus sintomas motores cardinais são a bradicinesia, o tremor de repouso, a rigidez muscular e a instabilidade postural[3,4].

Embora os sintomas motores cardinais sejam aqueles utilizados para o diagnóstico clínico da DP[3,4], os sintomas não motores (SNM) também assumem importância pela possibilidade de ocorrerem durante todo o curso da doença, por diminuir a qualidade de vida do indivíduo e antecipar a hospitalização[5].

Os distúrbios do sono estão entre os SNM mais comuns da DP, com uma prevalência de 60 a 90%[6,7]. Sua fisiopatologia não está totalmente elucidada, sendo provável a etiologia multifatorial. Acredita-se que sejam fatores predisponentes a presença de sintomas motores, distúrbios do sono primário (apneia do sono e distúrbio comportamental do sono REM), efeitos colaterais de medicamentos e a neurodegeneração de sistemas reguladores centrais de sono-vigília[8].

Os distúrbios do sono podem ser subjetivos ou objetivos. Os subjetivos abrangem: insônia, sono fragmentado, redução do tempo de sono e nictúria. Já os objetivos incluem a redução da latência do sono, a perda do fuso do sono e o distúrbio do movimento noturno excessivo[9]. Para avaliar as manifestações da doença relacionadas ao sono, o uso da Escala de Sono para a Doença de Parkinson (PDSS) se destaca por ser um instrumento específico, confiável, válido e de fácil aplicação[10].

Para o tratamento destes distúrbios, aproximadamente 40% dos pacientes com DP usam terapia complementar como a acupuntura[11]. O acesso aos acupontos e ao sistema de meridianos não se limita as agulhas, mas também pode ser obtido por estimulação elétrica nervosa transcutânea (TENS)[12].

A aplicação TENS sobre acupontos é uma modalidade não-invasiva que combina as vantagens da acupuntura e TENS[13,14]. Os impulsos elétricos atuam sobre os nervos sensoriais, que através de estímulos aferentes podem desencadear a produção de endorfinas e ajuste da fisiologia corporal[15].

Apesar da TENS ser um método bastante utilizado em nosso meio não foram identificados estudos da sua aplicação para tratamento dos problemas do sono em pacientes com DP. Dessa forma, este estudo tem como objetivo avaliar os efeitos da TENS em acupontos sobre a autoavaliação do sono em pacientes com DP.

Métodos

Esta série de casos foi desenvolvida no Programa Pró-Parkinson do Hospital das Clínicas da Universidade Federal de Pernambuco e aprovado pelo Comitê de Ética em Pesquisa com Seres Humanos do Centro de Ciências da Saúde desta Universidade (CAAE: 14877213.8.0000.5208).

Os pacientes do estudo foram recrutados de forma aleatória no momento de sua consulta de rotina no serviço de acordo com a agenda do dia, sendo incluídos aqueles com diagnóstico clínico de DP idiopática entre os estágios 1 e 3 de acordo com a versão original da escala de Hoehn e Yahr (HY)[16]. Foram excluídos os pacientes com Mini Exame do Estado Mental (MEEM) menor do que 18 pontos[17], com outras doenças neurológicas associadas e em uso de medicação para depressão, ansiedade, psicose e indutores do sono.

O instrumento de avaliação utilizado foi a Escala de Sono para Doença de Parkinson (PDSS). A PDSS é uma escala analógica visual específica e autoaplicável que avalia os distúrbios do sono em pacientes com DP. Compreende 15 itens com sintomas associados aos distúrbios do sono, sendo analisados em oito domínios: qualidade geral do sono à noite (item 1); início e manutenção do sono (itens 2 e 3); agitação noturna (itens 4 e 5); psicose noturna (itens 6 e 7); nictúria (itens 8 e 9); sintomas motores noturnos (itens 10-13); sono relaxante (item 14) e adormecer durante o dia (item 15). Pacientes foram convidados a marcar um traço ao longo de uma linha 10 centímetros, quantificando-se a distância. O escore máximo da escala é 150, sendo correspondente ao paciente livre de qualquer sintoma[10].

Foram realizadas oito sessões, uma vez por semana, com duração de vinte minutos. Para tanto foram fixados eletrodos de superfície nos acupontos Taichong (LR-3), Hé gǔ (Ll-4), Yanglingquan (GB-34), Neiguan (PC-6)[18] ligados ao equipamento de TENS (EL 608, marca NKL). A corrente de escolha foi a do tipo BURST, com pulsos intermitentes, isolados em frequência de 2 Hz, variando de 1 a 10 mA. Os dados obtidos foram analisados através de teste t pareado utilizando o software BioEstat 5.3 e considerando um $p \leq 0,05$.

Resultados

A amostra foi composta por 14 pacientes que finalizaram as 8 sessões, sendo 7 classificados como HY1, 6 como HY2 e 1 como HY 3. A tabela 1 apresenta as características gerais da amostra quanto ao estadiamento da doença e a idade, bem como o escore total da PDSS antes e após o período de intervenção. Observa-se que o escore total da escala aumentou de forma significativa após o período de estudo (Tabela 1).

Também, na análise dos domínios da PDSS antes e após a intervenção, sete dos oito domínios apresentaram um aumento dos seus subescores (Tabela 2). Entretanto, a melhora foi estatisticamente significativa apenas para o domínio psicose noturna. O subescore do domínio adormecer durante o dia foi o único a apresentar uma redução, indicando uma piora significativa.

Ao analisar o escore total da PDSS antes e após a intervenção em relação a grupos com diferentes estágios da doença, observa-se que a diferença foi significativa apenas para o estágio mais leve da DP (HY1) (Tabela 3). Já ao analisar em relação ao sexo, observa-se uma diferença significativa para o sexo feminino (Tabela 4).

Discussão

Os distúrbios do sono são um dos SNM mais comuns na DP, comprometendo a qualidade de vida dos pacientes[6]. Neste estudo foi utilizada a TENS em acupontos específicos como tratamento complementar nos distúrbios do sono em pacientes com DP.

Analisando os domínios da escala PDSS, nossos resultados revelaram que houve melhora em alguns aspectos dos distúrbios do sono utilizando TENS em acupontos específicos. Apesar da TENS ser um método bastante utilizado em nosso meio, não foram identificados outros estudos sobre a sua aplicação para o tratamento dos problemas do sono em pacientes com DP.

Estudos de revisão mostram que muitos pacientes com DP optam pelo uso da acupuntura como tratamento alternativo em algum momento da sua vida, melhorando alguns aspectos da doença. Porém, estes estudos apresentaram metodologia ainda ofuscante[19].

Estudo de Shulman et al.[20] com 20 pacientes com DP mostrou que 85% dos pacientes tratados com acupuntura relataram uma melhora subjetiva nos problemas do sono e estes achados foram confirmados pela melhora nos escores do Sickness Impact Profile. Em outro estudo randomizado controlado duplo-cego, pacientes com DP em uso de eletroacupuntura mostraram melhora significativa nos distúrbios do sono[21].

A psicose noturna foi o domínio com melhora expressiva após a intervenção. Tem sido sugerido que os transtornos comportamentais do sono REM (movimentos rápidos dos olhos) aumentam a probabilidade da presença de alucinações, particularmente, em pacientes com idade avançada e que têm maior alteração motora[22]. Há relatos de que as pessoas com DP mais avançada são mais propensas a apresentar

alucinações e outros sintomas psicóticos relacionados à própria doença ou à associação com medicações[23].

A característica mais importante da sonolência diurna excessiva é a tendência para adormecer durante o dia, geralmente acompanhada de uma sensação de aumento de sonolência. Os achados sugerem que a intervenção aumentou a sonolência diurna nesses sujeitos. Um grande estudo no Canadá, incluindo 638 pacientes com DP, mostrou uma prevalência de 3,8% de episódios de adormecimentos diurnos durante a condução de veículos[24].

A sonolência excessiva diurna é multifatorial[25], estando seu aumento neste estudo, provavelmente associado a fatores como as desordens cognitivas e os transtornos do humor. Entretanto, os acupontos utilizados não tinham espectro de ação sobre estes sintomas.

Nossos resultados sugerem que os indivíduos classificados no menor nível da doença (HY 1) apresentaram melhora da autoavaliação dos distúrbios do sono. Apesar disso a nossa série de casos está composta por sujeitos nos estágios leves da doença (HY 1 e HY 2) tendo apenas 1 caso no estágio moderado (HY 3).

Em relação ao sexo, podemos notar que no presente estudo houve uma diferença significativa no escore total do PDSS para o sexo feminino. Isso pode ser explicado dada a variedade de fatores que influenciam na qualidade do sono em mulheres: oscilações hormonais, envelhecimento, obesidade, doenças crônicas, depressão e ansiedade, conforme descrito por Costa et al.[26] em seu artigo de revisão integrativa. Apesar da multifatoriedade relacionada a qualidade do sono nas mulheres, Stavitsky et al.[27] investigando o sono de 35 pacientes com DP observou que os homens apresentaram pior qualidade do sono e sonolência excessiva diurna em relação as mulheres.

Em estudo de revisão da literatura, Kim e Jeon[28] descreveram críticas as diversas metodologias dos estudos localizados, afirmando que em nenhum houve avaliação dos efeitos da acupuntura ao longo do tempo após a cessação do tratamento. A evidência da eficácia da acupuntura, segundo estes autores, ainda não é convincente, necessitando de estudos com melhor qualidade metodológica e seguimento em longo prazo. Ademais, relataram heterogeneidade na escolha dos acupontos, resultando em interpretações diferentes e falta de protocolo de acupuntura validado para DP. A maioria dos estudos desta revisão[28] utilizaram os acupontos GB34 e ST36, seguido por LR 3, LI4, LI11 e SI3.

Apesar dos resultados positivos é importante citar que as limitações relacionadas a amostra não controlada contituem um fator a ser considerado. Também, apesar do método despontar como promissor, não encontramos na literatura estudos que comprovem sua eficácia no tratamento dos problemas do sono em sujeitos com DP, constituindo um campo ainda não explorado.

Conclusão

A utilização da TENS em acupontos teve efeitos positivos sobre sintomas relacionados ao sono de pacientes com DP deste estudo, sendo observados maiores benefícios para o domínio psicose noturna e pacientes com estágio HY1 e do sexo feminino.

Porém houve limitações metodológicas atreladas ao desenho do estudo. Sugerem-se estudos como ensaios clínicos randomizados com análise por intenção de tratar e outros que proponham protocolos de acupontos específicos para DP conforme recomendações dos guias internacionais CONSORT e STRICTA.

Sugerimos ainda a elaboração de uma avaliação mais abrangente do paciente com DP, dos sintomas motores e não motores, utilização de instrumentos e/ou escalas estabelecidas universalmente, como a Escala Unificada de Avaliação da Doença de Parkinson e avaliação do benefício desta terapia complementar na redução da terapêutica com L-dopa e de seus efeitos adversos.

Referências

1. Wirdefeldt K, Adami H-O, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson ' s disease : a review of the evidence. 2011;

2. Souza CFM, Almeida HCP, Batista J, Costa PH, Silveira YSS, Bezerra JCL. A Doença de Parkinson e o Processo de Envelhecimento Motor : Uma Revisão de Literatura. 2011;19(4):718–23.

3. Brasil. Portaria nº 228, de 10 de maio de 2010: Protocolo Clínico e Diretrizes Terapêuticas - Doença De Parkinson [Internet]. 2010. Available from: http://bvsms.saude.gov.br/bvs/saudelegis/sas/2010/prt0228_10_05_2010.html

4. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson ' s disease : a clinico-pathological study of 100 cases. 1992;181–4.

5. Munhoz RP, Moro A, Silveira-Moriyama L, Teive HA. Non-motor signs in Parkinson's disease: a review. Arq Neuropsiquiatr [Internet]. 2015;73(5):454–62. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0004=282-2015000500015X&lng=en&nrm=iso&tlng-en

6. Suzuki K, Miyamoto M, Miyamoto T, Iwanami M, Hirata K. Sleep disturbances associated with Parkinson's disease. Parkinsons Dis. 2011;2011:219056.

7. Alatriste-Booth V, Rodríguez-Violante M, Camacho-Ordoñez A, Cervantes-Arriaga A. Prevalence and correlates of sleep disorders in Parkinson's disease: a polysomnographic study. Arq Neuropsiquiatr [Internet]. 2015;73(3):241–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25807131

8. Videnovic A, Noble C, Reid KJ, Turek FW, Marconi A, Rademaker AW, et al. Circadian melatonin rhythm and excessive daytime sleepiness in Parkinson's disease. 2014;71(4):463–9.

9. Trotti L, Ray D. Neurobiology of sleep: the role of dopamine in Parkinson's disease. Chaudhuri KR, Tolosa E, Schapira AH V., Poewe W, editors. Non-motor symptoms of Parkinson's disease. Oxford: Oxford University Press; 2009. 165-176 p.

10. Chaudhuri KR, Pal S, DiMarco a, Whately-Smith C, Bridgman K, Mathew R, et al. The Parkinson's disease sleep scale: a new instrument for assessing sleep and nocturnal disability in Parkinson's disease. J Neurol Neurosurg Psychiatry [Internet]. 2002;73(6):629–35. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1757333&tool=pmcentrez&rendertype=abstract

11. Rajendran PR, Thompson RE, Reich SG, P.R. R, R.E. T, S.G. R. The use of alternative therapies by patients with Parkinson's disease. Neurology [Internet]. 2001;57(5):790–4. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L32845496\nhttp://sfx.bibl.ulaval.ca:9003/sfx_local?sid=EMBASE&issn=00283878&id=doi:&atitle=The+use+of+alternative+therapies+by+patients+with+Parkinson's+disease&stitle=Neurology&ti

12. Davis C. Fisioterapia e reabilitação: terapias complementares. 2ª ed. Rio de Janeiro: Guanabara Koogan; 2006. 364-365 p.

13. Chen L, Tang J, White PF, Sloninsky A, Wender RH, Naruse R, et al. The effect of location of transcutaneous electrical nerve stimulation on postoperative opioid analgesic requirement: acupoint versus nonacupoint stimulation. Anesth Analg. 1998;87(5):1129–34.

14. Chao A-S, Chao A, Wang T-Z, Chang Y-C, Peng H-H, Chang S-D, et al. Pain relief by applying transcutaneous electrical nerve stimulation (TENS) on acupuncture points during the first stage of labor: A randomized double-blind placebo-controlled trial. Pain [Internet]. 2007;127(3):214–20. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0304395906004210?showall=true

15. Mann F. Acupuntura: A antiga arte chinesa de curar. 1ª ed. Hemus, editor. São Paulo; 1994.

16. Hoehn, M.M., Yahr MD. Parkinsonism : onset , progression , and mortality. Neurology. 1967;

17. FOLSTEIN MF, FOLSTEIN SE MP. Mini-mental state: a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189–98.

18. Focks C. Atlas de Acupuntura. São Paulo: Manole; 2005. 192 p.

19. Zeng B-Y, Zhao K. Effect of Acupuncture on the Motor and Nonmotor Symptoms in Parkinson's Disease-A Review of Clinical Studies. CNS Neurosci Ther [Internet]. 2016;n/a – n/a. Available from: http://doi.wiley.com/10.1111/cns.12507

20. Shulman LM, Wen X, Weiner WJ, Bateman D, Minagar A, Duncan R, et al. Acupuncture therapy for the symptoms of Parkinson's disease. Mov Disord. 2002;17(4):799–802.

21. Cristian A, Katz M, Cutrone E, Walker RH. Evaluation of acupuncture in the treatment of Parkinson's disease: A double-blind pilot study. Mov Disord. 2005;20(9):1185–8.

22. Sinforiani E, Pacchetti C, Zangaglia R, Pasotti C, Manni R, Nappi G. REM behavior disorder, hallucinations and cognitive impairment in Parkinson's disease: A two-year follow up. Mov Disord. 2008;23(10):1441–5.

23. Forsaa EB, Larsen JP, Wentzel-Larsen T, Goetz CG, Stebbins GT, Aarsland D, et al. A 12-year pouplation-based study of psychosis in Parkinson Disease. Arch Neurol. 2010;67(8):996–1001.

24. Hobson DE, Lang AE, Martin WRW, Razmy A, Rivest J, Fleming J. Excessive daytime sleepiness and sudden-onset sleep in Parkinson disease: a survey by the Canadian Movement Disorders Group. JAMA. 2002;287(4):455–63.

25. Adler CH, Thorpy MJ. Sleep issues in Parkinson ' s disease. 2005;

26. Costa RHS, Silva RAR da, Morais MF de AB de, Costa MM do N, Costa DAR da S. Integrative Review Article Triggering Aspects of the Sleep Disorders in Women : Literature Review Aspectos Desencadeantes Dos Transtornos Do Sono Em Mulheres : Revisão De. J Nurs UFPE line. 2013;7(4):1234–40.

27. Stavitsky K, Cronin-Golomb A. Sleep Quality in Parkinson's Disease: An Examination of Clinical Variables. Cogn Behav Neurol. 2011;24(2):43–9.

28. Kim HJ, Jeon BS. Is acupuncture efficacious therapy in Parkinson's disease? J Neurol Sci. 2014;341(1-2):1–7.

8 - Aspartame and the nervous system: a systematic review

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Abstract

The consumption of food products containing the non-caloric sweetener aspartame has increased since the 80's, when these products were introduced in the market. Since then, several pieces of evidence suggest that aspartame may be potentially harmful to the nervous system, although some controversy on this subject still persists. Some research suggests an association between aspartame intake and metabolic damage to the central nervous system (CNS), such as changes in enzyme and neurotransmitter activities. Considering the possible adverse neuronal actions of aspartame, we consider it important to study the biological effects of this sweetener on the functional aspects of the CNS. Objective: To analyze the pertinent literature on important aspects of the possible neurophysiological alterations associated with aspartame consumption. Methods: We conducted a search for studies, whose strategy was developed for Embase, Medline, Lilacs and Pubmed databases, according to the descriptors: Aspartame, brain function and nervous system. We present here a narrative review of the literature over the period from 1992 to 2012. Conclusion: experimental and clinical studies indicate the risk of neural adverse reactions, which are associated with the use of aspartame, even in relatively low doses. This risk might be higher in developing organisms. Because of such evidence we recommend that the sweetener consumption, if any, should be performed with moderation and caution, under the guidance of a nutritionist or a medical doctor, in order to avoid aspartame-associated deleterious effects on brain function, mainly in children.
Key Words: Aspartame; nervous system; brain function; brain development; brain excitability

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Introduction

Artificial sweeteners are substances with a high sweetening power when compared to sucrose. They are used as sugar substitute in foods and dietary beverages in order to reduce their caloric value [1]. In Brazil, several sweeteners are permitted for use in foods and beverages, among which the two most sold are the mixture of saccharin plus cyclamate and aspartame [1]. This last sweetener (aspartame) is the subject of the present systematic review.

The non-caloric sweetener Aspartame (L-aspartyl-L-phenylalanine methyl ester) was randomly discovered in 1965 in the United States by James Schlatter, a researcher from the pharmaceutical company G. D. Searle Laboratories. Schlatter was attempting to find a new drug for treating gastric ulcer when he licked his fingers to pick up a piece of paper; the licked fingers contained a small amount of a newly synthetized compound and Schlatter immediately noticed the intense sweet taste of that compound, which was identified as being aspartame. The consumption of Aspartame as sweetener was authorized in the United States of North America in 1981 by the Food and Drug Administration (FDA), after various toxicological studies. In Brazil, the free trade of low-calorie products and sweeteners, including those containing aspartame, was authorized in 1988 [2]. Currently, the consumption of Aspartame-containing diet- and light-products has become a great success due to their taste characteristics that are similar to sucrose, and help to reduce the energy value of food [1,3,4] .The calorific value of Aspartame is equal to sucrose (4 kcal / g). However, the quantity of aspartame needed to produce a sweet taste is much lower than sucrose, as Aspartame is about 200 times sweeter. Therefore, the caloric contribution of Aspartame is negligible, in comparison with that of sucrose. Aspartame can be consumed by diabetic patients, but it should be avoided by patients suffering from phenylketonuria, the genetic disease that alters the metabolism of phenylalanine [2, 5].

Currently, the sweetener can be found in more than six thousand products including soft drinks, powdered drinks, chewing gums, gelatins, dessert mixes, puddings and fillings, frozen desserts, yogurt, tabletop sweeteners, and some pharmaceutical products like vitamins and cough tablets[1]. Its acceptable daily intake is 50 mg/kg body weight, which is estimated to be the equivalent of 18-19 cans (1 can = 355 ml) of diet cola [8,9]. For the health organization and Canada's welfare (Health and Welfare Canada), the acceptable daily intake is 40 mg/kg body weight [9]. Many products containing Aspartame as an additive are indicated for consumption by diabetic patients or persons on caloric restriction. This sweetener has been used on a large scale, not only by those groups, but also by the healthy population [4]. On the other hand, data on the amount of non-nutritive artificial sweeteners that are added to industrialized foods and beverages are not easily accessible [8, 9].

The consumption of Aspartame may be potentially damaging to the nervous system. This consumption has increased since the 80's, when these products were introduced in the market. In recent years, consumption of diet- and light-foods has increased neatly, promoting an increment of the research investment aimed at the development of new Aspartame-based products. These products are primarily directed to people who have a disorder in the metabolism of sugars (diabetes); also, in the last two decades healthy consumers are looking for foods with low-calorie[10].

Nowadays, researchers still differ about their ideas on the possible harmful effects of aspartame on neuronal function. Some studies suggest association of aspartame intake with metabolic damage to the central nervous system (CNS), such as changes in enzyme and neurotransmitter activities.[11,12]. Considering the possible neuronal adverse effects of aspartame, it is important to gain a better understanding of the biological effects of this sweetener on the functions of the CNS. The knowledge that dietary changes can affect the mammalian nervous system is very well established. However, in relation to the involvement of Aspartame consumption on neurological disorders, the data in the literature are conflicting. In view of that, and considering that Aspartame consumption may be causally related to adverse neural effects, we considered it important to clarify the possible biological effects of this sweetener on the functions of the CNS. Thus, the objective of this work is to review the studies about the possible action of aspartame on brain function.

Methods

We conducted a systematic review according to a protocol that was constructed to ensure the research design. Therefore, the protocol was organized according to the following components: a) the main question of the review; b) inclusion and exclusion criteria; c) strategies for the search of the scientific research universe d) collection and synthesis of data. The searches were conducted on Embase, Medline, Lilacs and Pubmed databases, according to the descriptors: Aspartame, brain function and nervous system. The narrative review of the literature was performed for the period from 1992 to 2012. Data were systematized based on their strength of evidence. The process of data synthesis was carried out through a descriptive analysis of selected studies, and the final product presented in a narrative form analysis.

Results

An initial observation seemed very important to us: the fact that many pieces of evidence suggest that Aspartame may be involved in the onset of headache and epileptic symptoms in humans [11–19]. Regarding the use of sweeteners in food for children and adolescents, evidence does exist, for example, which indicates the association between

the use of aspartame and the appearance of seizures or neural irritability in children, as well as the risk of exceeding the maximum daily dose accepted[20]. The maximum daily amount for a 3 year old child with 14kg (at a dose of 40mg/kg/day) would be easily exceeded by consuming one can of diet soda, 1 serving of dietary sweet, 1 serving of dietary yogurt and 3 envelopes sweetener to sweeten liquid, total 517mg [8,20]. However, there is lack of conclusive studies on the effects of these additives on long-term growth and development.

The use of aspartame has been debated since its approval by the FDA and currently there are still controversies about their safety. Some evidence suggests possible neurological and behavioral adverse effects due to the components of aspartame metabolization in the organism (phenylalanine, aspartic acid, diketopiperazine and methanol). Other pieces of evidence suggest that aspartame would not be cytotoxic [4]. Magnuson et al. (2007), in an extensive review concluded that the available data did not support an association between aspartame as a component of the human diet, and neurotoxic effects. On the other hand, in a systematic review Humphries; Pretorius; Naude [4] (2008) observed in several studies that aspartame disrupts the neuronal function and modifies the brain concentrations of catecholamines. It is also reported that aspartame and its degradation products indirectly increase the neuronal depolarization rate. The authors concluded that, given the doubts and polemics about the adverse effects of this sweetener, more research is needed to clarify the controversies about it.

The use of aspartame has been associated with headaches, panic attacks and convulsions [12, 11, 13, 18, 21, 19] . Also, memory impairment has been reported in rats after chronic exposure to aspartame [22] . Its consumption has also been linked to seizures in humans [17, 16,15, 14], neurotoxicity [23] and genotoxic risk [24, 25].

Regarding induction of malignancy, Lim et al.[26] (2006), in a cohort study, have concluded that their findings did not support the hypothesis that aspartame increases the risk of hematopoietic or brain cancer. In contrast, Soffritti et al. [27] (2008) found that aspartame significantly increases the risk of tumors in rodents, particularly lymphomas and mammary tumors, with a doubled risk in those cases in which the exposition to Aspartame begins in the prenatal period. These authors concluded that the FDA should review the approval of the sweetener.

Changes in metabolism in the central nervous system (CNS), as a result of intake of aspartame, have also been described. Data obtained by Vences-Mejia et al. [28] (2006) demonstrated that a daily intake of aspartame below the amount recommended by the FDA in 30 days, causes a substantial increase in cytochrome P450 (CYP). This enzyme complex is responsible for endogenous and exogenous molecular metabolism in the CNS, such as the metabolism of xenobiotics. The authors state that biological consequences of this phenomenon should be investigated with a view to increasing

the number of individuals exposed to the additive. The quantities used in the research referred to above, 75 and 125mg / kg in mice, respectively represent an amount of 15 to 25 mg / kg for humans, after using a correction factor (CF = 5), ie, below the amount recommended by the FDA.

Simintzi et al.[27] (2007) observed that aspartame components may be directly and / or indirectly acting on acetylcholinesterase (the enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine) activity in the frontal cortex. This enzymatic reaction is necessary for the cholinergic neuron to return to its resting state after activation, avoiding then excessive neurotransmitter action, which would produce a neuronal overstimulation and as a consequence weakness and fatigue of the organism. Elevated (toxic) doses of the sweetener significantly decreased the activity of the enzyme. If we can compare the results of this in vitro study to the human reality, it may be suggested that cholinergic symptoms such as rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis, are related to the consumption of aspartame in high doses (150 or 200 mg / kg; 30 or 40mg / kg for human).

The extracellular dopamine levels can be reduced by the intake of aspartame when administered systemically in a single dose of 500 mg/kg for rats and 100 mg/kg for humans [29]. It is important to note that, according to these authors, the dopaminergic scarcity is a condition found in diseases such as Parkinson's disease and schizophrenia.

Conclusion

Given the above, studies indicate adverse reactions to the use of Aspartame on the nervous system. Thus, the sweetener should be consumed moderately and cautiously, under guidance of a nutritionist or a medical doctor, because the consumption of this sweetener doses as low as 75 mg/kg/d can lead to deleterious effects brain function [28]. It was observed that there are very few studies on aspartame intake during pregnancy and lactation, thus suggesting that the competent controlling organizations (e.g. FDA) influence the conduct of research on this topic. An important recommendation would be that the food industries inform on the food label, the possible effects of its consumption at this early period of life (gestation and lactation). Regarding the neural effects of Aspartame consumption over the pregnancy and lactation period, studies on the eventual negative consequences for both the mother and child are scarce. Because of that, Aspartame consumption by pregnant women should be restricted to only those pregnant women who are diabetic, and therefore have to use sweeteners. This restriction might help preserving the neurological health of the fetus. We believe that in the long term this systematic review may contribute to the development of preventive and therapeutic actions regarding the neural effects of aspartame.

References

1. ASPARTAME INFORMATION CENTER. Products. Available in: http://www.aspartame.org/aspartame_products.html.

2. FDA (FOOD AND DRUG ADMINISTRATION). Food additives permitted for direct addition to food for human consumption; aspartame. Federal Register, v.61, p.33654-56, 1996.

3. MATTES, R. D.; POPKIN, B. M. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms1–3. American Journal Clinical Nutrition, v.89, p.1–14, 2009.

4. HUMPHRIES, P.; PRETORIUS, E.; NAUDÉ, H. Direct and indirect cellular effects of aspartame on the brain. European Journal of Clinical Nutrition, v.62, p.451–462, 2008.

5. CARDELLO, H. M. A. B.; SILVA, M. A. A. P.; DAMÁSIO, M. H. Avaliação tempo-intensidade de doçura e amargor de aspartame e ciclamato/sacarina em equivalência à sacarose em altas concentrações. Curitiba: Boletim Centro de Pesquisa de Processamento de Alimentos-CEPPA, v. 19, n. 2, p. 391-410, jul./dez. 2001.

6. CÂNDIDO, L. M. B.; CAMPOS, A. M. Alimentos para fins especiais: dietéticos. São Paulo: Varela, 1996. p. 115-258.

7. SAMUNDSEN, J.A. Has aspartame an aftertaste? J. Food Sci., v. 50, p. 1510-1502, 1985.

8. THOMSON, D.M.H.; TUNALEY, A. A reappraisal of the use of multidimensional scaling to investigate the sensory characteristics of sweeteners. J. Sensory Stud., v. 2, p. 215-230, 1987.

9. BELL, J. High intensity sweeteners: a regulatory update. Food Technology. Chicago, v. 47, n. 11, p. 136, 1993.

10. NELSON, A. L. Carbohydrate Polymers. Sweetners: Alternative, v. 44, n. 2, p. 172, 2001.

11. JACOB, S. E; STECHSCHULTE, S. Formaldehyde, aspartame, and migraines: a possible connection. Dermatitis, v.19, n.3, p. E10-1, 2008.

12. SUN-EDELSTEIN, C.; MAUSKOP, A. Foods and supplements in the management of migraine headaches. The Clinical Journal of Pain, v.25, n.5, p.446-52, 2009.

13. BLUMENTHAL, H. J.; VANCE, D. A. Chewing gum headaches. Headache, v. 37, p.665–666, 1997.

14. CAMFIELD, P. R.; CAMFIELD, C. S.; DOOLEY, J. M.; GORDON, K.; JOLLYMORE, S.; WEAVER, D. F. Aspartame exacerbates EEG spike-wave discharge in children with generalized absence epilepsy. Neurology, v.42, p.1000–1003, 1992.

15. ESHEL, Y.; SAROVA-PINHAS, I. Aspartame and seizures. Neurology, v.43, p.2154–2155, 1993.

16. GABY, A. R. Natural Approaches to Epilepsy. Alternative Medicine Review, v. 12, n.1, 2007.

17. MORTELMANS, L. J.; VAN L. M.; DE CAUWER, H. G.; MERLEVEDE, K. Seizures and hyponatremia after excessive intake of diet coke. European Journal of Emergency Medicine, v.15, n.1, p.51, 2008.

18. TOLLEFSON, L.; BARNARD, R. J. An analysis of FDA passive surveillance reports of seizures associated with consumption of aspartame. Journal of the American Dietetic Association, v.92,

n.5, p.598– 601, 1992.

19. WALTON, R. G. Seizure and mania after high intake of aspartame. Psychosomatics, n.27, p.218–220, 1986.

20. VITOLO,Marcia Regina(ed.) Nutrição: da gestação ao envelhecimento. Rio de Janeiro:Ed.Rubio,2008.

21. DRAKE, M. E. Panic attacks and excessive aspartame ingestion. Lancet, v.2, p.631, 1986.

22. CHRISTIAN, B..; MCCONNAUGHEY., K.; BETHEA, E..; BRANTLEY, S.; COFFEY, A.; HAMMOND, L.; HARRELL, S.; METCALF, K.; MUEHLENBEIN, D.; SPRUILL, W.; BRINSON, L.; MCCONNAUGHEY, M. Chronic aspartame affects T-maze performance, brain cholinergic receptors and Na^+,K^+-ATPase in rats. Pharmacology Biochemistry and Behavior, v.78, p.121–127, 2004.

23. LAU, K.; MCLEAN, W. G.; WILLIAMS, D. P.; HOWARD, C. V. Synergistic Interactions between Commonly Used Food Additives in a Developmental Neurotoxicity Test. Toxicological sciences, v.90, n.1, p.178–187, 2006.

24. BANDYOPADHYAY, A.; GHOSHAL, S.; MUKHERJEE, A. Genotoxicity Testing of Low-Calorie Sweeteners: Aspartame, Acesulfame-K, and Saccharin. Drug and Chemical Toxicology, v.31, p.447–457, 2008.

25. ALSUHAIBANI, E. S. In Vivo Cytogenetic Studies on Aspartame. Comparative and Functional Genomics, Volume 2010, Article ID 605921, 4 pages doi:10.1155/2010/605921.

26. LIM, U.; SUBAR, A. F.; MOUW, T.; HARTGE, P.; MORTON, L. M.; STOLZENBERG-SOLOMON, R. CAMPBELL, D.; HOLLENBECK, A. R.; SCHATZKIN, A. Consumption of Aspartame-Containing Beverages and Incidence of Hematopoietic and Brain Malignancies. Cancer Epidemiology, Biomarkers & Prevention, v.15, n.9, 2006.

27. SOFFRITTI, M.; BELPOGGI, F.; ESPOSTI, D. D.; FALCIONI, L.; BUA, L. Consequences of Exposure to Carcinogens Beginning During Developmental Life. Basic & Clinical Pharmacology & Toxicology, v.102, 118–124, 2008.

28. VENCES-MEJÍA, A.; LABRA-RUÍZ, N.; HERNÁNDEZ-MARTÍNEZ, N.; DORADO-GONZÁLEZ,V.; GÓMEZ-GARDUÑO, V.; PÉREZ-LÓPEZ, I.; NOSTI-PALACIOS, R.; CAMACHO-CARRANZA, R.; ESPINOSA-AGUIRRE, J. J. The effect of aspartame on rat brain xenobiotic-metabolizing enzymes. Human & Experimental Toxicology, v.25, p.453-459, 2006.

29. BERGSTROM, B. P.; CUMMINGS, D. R.; SKAGGS, T. A. Aspartame decreases evoked extracellular dopamine levels in the rat brain: An in vivo voltammetry study. Neuropharmacology, v.53, p.967-974, 2007.

9- O alcoolismo como fator de risco em comportamentos violentos entre parceiros íntimos

Alcoholism as a risk factor for violent behavior among intimate partners

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Resumo

O presente estudo teve como objetivo a investigação dos principais fatores associados à expressão do comportamento violento entre parceiros íntimos em sujeitos atendidos em um centro de dependência quimica. Foram entrevistados 95 pacientes, entre eles homens (N=64) e mulheres (N=31). Para o levantamento de dados, foram utilizados um questionário sócio-demográfico, dois instrumentos de rastreio para o consumo de álcool, o Cut down Annoyed Guilty Eye-opener Questionnaire (CAGE), o The Alcohol Use Disorders Identification Test- AUDIT e uma escala para aferir o tipo e a gravidade da violência expressa pelos pacientes, a Escala Tática de Conflitos (CTS2). Os resultados apontaram vários fatores que quando associados ao uso inadequado do álcool, poderiam favorecer a expressão do comportamento, entre os quais, destacam-se: histórico pessoal, convivência social, presença de transtorno mental, baixa escolarização e desorganização familiar. Os agressores entrevistados não estabeleceram uma relação causal entre álcool e violência. Entretanto, relataram que a violência exercida contra o parceiro era mais grave quando o mesmo se encontrava sob efeito do álcool. Espera-se que este estudo possa fomentar reflexões na área em questão bem como estimular a criação e implementação de políticas públicas que favoeçam a detecção e a intervenção em sujeitos em situação de risco (vítima ou perpetrador).

Palavras-chave: Álcool, Comportamento violento, Violência doméstica.

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Abstract

The present study aimed to investigate the main factors associated with the expression of violent behavior among intimate partners in subjects attended at a chemical dependency center. 95 patients were interviewed, among them men (N = 64) and women (N = 31). A sociodemographic questionnaire, two screening instruments for alcohol consumption, the Cut down Annoyed Guilty Eye-opener Questionnaire (CAGE), the Alcohol Use Disorders Identification Test (AUDIT) and a scale for To assess the type and severity of violence expressed by patients, the Tactical Conflict Scale (CTS2). The results point to several factors that, when associated with inappropriate alcohol use, could favor the expression of behavior, among which the following stand out: personal history, social coexistence, presence of mental disorder, low schooling and family disorganization. The aggressors interviewed did not establish a causal relationship between alcohol and violence. However, they reported that violence against their partner was more severe when they were under the influence of alcohol. It is hoped that this study may foster reflections in the area in question as well as stimulate the creation and implementation of public policies that favor the detection and intervention in subjects at risk (victim or perpetrator).
Key words: Alcohol, Violent behavior, Domestic violence.

Introdução

O uso do álcool é relatado na literatura científica dos primórdios aos tempos atuais. Atualmente, seu abuso e dependência vem se configurando como uma grande ameaça à saúde pública, acarretando prejuízos nos mais variados setores pessoais e da sociedade[1,2].

A Síndrome da Dependência Alcoólica (SDA), é conceituada como um transtorno crônico que se institui ao longo da vida, não sendo considerado como um transtorno estático[3]. É um fenômeno onde se interage mutuamente fatores biológicos, psicológicos e sociais, que determina de certa forma, a maneiro como o sujeito irá estabelecer sua relação com o álcool [3,4].

Nesse processo de aprendizagem, o sujeito experimentará a tolerância, quando necessita de doses cada vez maiores para sentir os efeitos da substância e a abstinência. Quando a ingestão do álcool ocorre apenas com o objetivo de minimizar os efeitos desagradáveis que a ausência do álcool traz, observa-se então, o surgimento da dependência[3,4].

Pesquisas atuais vêm relacionando o abuso e dependência do álcool à episódios de violência, acidentes, comprometimento físico, psicológico e social [5-9]. Farmacologicamente falando, o álcool se constitui como um depressor do sistema nervoso central afetando de forma direta e indireta diversos sistemas e mecanismos cerebrais. Inicialmente quando consumido, o álcool provoca euforia e desinibição, à medida que a

concentração aumenta na corrente sanguínea, o sujeito, apresenta alterações na percepção de si e dos demais, acarretando em erros no julgamento e condutas impróprias, favorecendo então, a expressão do comportamento violento [8,9].

Apesar da forte associação do abuso do álcool e comportamento violento, ainda parece equivocado aponta-lo como causa unidirecional à agressividade [10-12], uma vez que boa parte dos sujeitos que utilizam álcool, não se tornem violentos e não apresentem o que se considera fatores de riscos situacionais, entre os quais podemos apontar, de acordo com a literatura, provocação de terceiros, situações de ameaça real ou interpretada, frustração, pressão social para o comportamento violento, entre outros [13-15].

Diante dessa problemática, o presente estudo teve como objetivo, investigar e descrever os principais fatores de risco associado ao uso do álcool que possam favorecer o comportamento violento numa população com diagnosticada com SDA, principalmente, no que diz respeito às violências exercidas na esfera intrafamiliar, que se encontravam em tratamento em um centro de dependência química.

Método

A presente pesquisa se configurou em um estudo transversal. Foi realizada no Núcleo de dependência química (NEDEQ) do Hospital das Clínicas da Universidade Federal de Pernambuco (HC- UFPE) com sujeitos que buscaram atendimento no período de outubro de 2012 e fevereiro de 2013. O mesmo objetivou estimar a prevalência e os fatores associados à violência intrafamiliar em portadores da Síndrome da dependência alcóolica (SDA)

Para o levantamento de dados, foram utilizados um questionário sócio demográfico, dois instrumentos de rastreio para o consumo de álcool, o Cut down Annoyed Guilty Eye-opener Questionnaire (CAGE), o The Alcohol Use Disorders Identification Test- AUDIT e uma escala para aferir o tipo e a gravidade da violência expressa pelos pacientes, a Escala Tática de Conflitos (CTS2). Para verificar a associação entre as variáveis foram utilizados os testes de Qui-quadrado e exato Fisher.

Resultados

A população participante do estudo (N=95) foi composta por 64 homens (67%), e 31 mulheres (33%), com predominância da faixa etária que compreende entre 30 a 39 anos em idade. No variável estado civil, encontrou-se que 72% dos homens e 84% das mulheres referiam estar em situação marital.

Mais da metade dos homens e mulheres entrevistados apresentam tempo de estudo igual ou superior a oito anos. Em relação ao consumo de risco, avaliado através do AUDIT com pontuação superior a 8 pontos, 100% da amostra total referiu realizar, os mesmos resultados foram encontrados na escala CAGE, onde duas ou mais respostas

são utilizadas como ponto de corte para averiguar a presença da dependência. Os dados relatados, podem ser observados na tabela 1.

Tabela 1: Distribuição das características sócio demográficas dos pacientes atendidos no NEDEQ no período de outubro de 2012 a fevereiro 2013.

	Homens (N=64)		Mulheres (N=31)	
	N	FREQ	N	FREQ
Idade				
18-29	17	27%	9	29%
30-39	18	28%	12	39%
40-49	13	20%	10	32%
>50	16	25%	0	0%
Ocupação				
Autônomo (a)	17	27%	14	45%
Empregado(a)	29	45%	10	32%
Desempregado(a)	10	16%	7	23%
Aposentado (a)	8	12%	0	0%
Escolaridade				
≤ 8 anos de estudo	24	37,5%	12	39%
9 ou mais anos de estudo	40	62,5%	19	61%
Estado Civil				
Vivendo com companheiro (a)	46	72%	26	84%
Vivendo sem companheiro (a)	18	28%	5	16%
Consumo de risco de álcool				
Sim (AUDIT ≥ 8)	0	0	0	0%
Não (AUDIT < 8)	64	100%	31	100%
Dependência de álcool				
Sim (CAGE ≥ 2)	0	0	0	0%
Não (CAGE < 2)	64	100%	31	100%

Ao investigar a relação do histórico positivo para uso de álcool na família de origem dos participantes e o consumo do álcool pelo mesmo, foi encontrado associação estatisticamente significante entre os homens, entretanto, observa-se perceber que a porcentagem encontrada nas mulheres se encontra alta. Os dados obtidos demonstra-

ram que o consumo do álcool presente no histórico familiar exerce forte influência em relação ao uso do mesmo pelos participantes.

Presenciar violência motivada pelo álcool em algum momento da vida, principalmente na família de origem, também foi um fator significante, se configurando em um fator de risco para a expressão do comportamento violento atual. A significância foi demonstrada tanto quando cruzamos os dados desconsiderando a variável sexo (P =0,0139) e também quando analisamos separadamente conforme tabela 2.

Tabela 2: Relação entre presenciar episódios de violência motivada pelo álcool e comportamento violento expresso atualmente.

			N	FREQ	Valor de P
Gênero	Homens	Presenciar violência motivada pelo álcool	57	89%	P= 0.0131*
		Comportamento violento atual	64	100%	
	Mulheres	Presenciar violência motivada pelo álcool	31	100%	
		Comportamento violento atual	31	100%	

*P< 0,05 pelo teste exato de Fisher

O início precoce (antes dos 18 anos) do uso do álcool em 86¨% homens (N=55) e 87% mulheres (N=27) também demonstrou significância em ambos os sexos (P= 0,0029), resultado semelhante em quem possuía mais de 10 anos de consumo alcóolico.

O instrumento utilizado neste estudo para medir a violência praticada entre os parceiros íntimos foi a escala de tática de conflitos (CTS2). A mesma possibilitou a identificação de 3 tipos de violência de acordo com a natureza da ação e também situações e injúria que é configurada como condutas que resultam em lesão física no parceiro.

As respostas foram organizadas de acordo com a ocorrência de pelo menos uma das categorias propostas pela escala. Desta maneira, foi possível verificar a prevalência dos comportamentos violentos praticados tanto pelos usuários como pelos parceiros de acordo com as vivências relatadas na entrevista pelo usuário.

De acordo com as mulheres (N=31), todas haviam expresso ao menos uma vez a agressão psicológica, o mesmo tipo de conduta foi adotada por todos os seus respectivos parceiros. Em relação aos homens, 90% (N=58) adotaram essa conduta enquanto 78% de suas parceiras (N=50) fizeram o mesmo. A violência física foi praticada por

84% das mulheres (N=26) e sofridas por 94% dos seus parceiros (N=29). Em relação aos homens, a referida conduta foi observada 8% dos usuários (N=51) e sofridas por 60% de suas companheiras (N=39).

Cerca de 3% das mulheres (N=1) admitiram ter exercido violência sexual ao menos uma vez na vida. Em contrapartida afirmaram terem sofrido pelo menos uma dessas agressões em 29% dos casos pelos seus companheiros (N=9), configurando-se na única modalidade de violência na qual houve um relato de maior vitimização das mulheres quando comparado aos índices de perpetração dessa violência pelas mesmas. Dados estes, semelhantes a perpetuação no caso dos usuários (N=16) que adotaram essa conduta em 25% dos casos, sofrendo apenas 1,5% praticado por suas companheiras (N=1).

Tabela 3: Prevalência dos tipos de violências praticadas e sofridas pelos usuários e seus parceiros.

		Tipo de Violência	Praticado pela (a) usuário (a)		Praticada pelo(a) parceiro (a)		
			N	FREQ	N	FREQ	Valor de P
Gênero	Homens	Violência psicológica	58	90%	50	78%	P<0.0001*
		Violência física	51	80%	39	60%	P<0.0001*
		Violência sexual	16	25%	1	1,5%	P<0.0001*
		Injúria	54	17%	45%	14	P<0.0001*
	Mulheres	Violência psicológica	31	100%	31	100%	-
		Violência física	26	84%	29	94%	P= 0,0174*
		Violência sexual	1	3%	9	29%	P<0.0001*
		Injúria	17	54%	14	45%	P<0.0001*

*P< 0,05 pelo teste exato de Fisher

Em relação à presença do álcool nos episódios de violência, todas as mulheres e 97% dos homens (N=63) afirmaram estares sob o efeito do mesmo. Os usuários consideraram que a gravidade da violência exercida e sofrida entre o casal, era maior na presença do álcool, a afirmação foi desta associação foi relatada por 98% dos homens (N=62) e por todas as mulheres. Dos participantes entrevistados, 93,5% das mulheres (N=29) e homens (N=60), consideraram sua relação com o álcool prejudicial, relatando

que já foram orientados por familiares, amigos e médicos a buscarem tratamento para a dependência alcóolica.

Ao serem questionados a respeito sobre a influência exercida pelo álcool no comportamento que poderiam estar associados aos episódios de violência, os principais motivos relatados pelas mulheres foram& irritação (80% n=25), sentimento de ser traída pelo parceiro ou pessoas próximas (58% N=18), falta de apoio (32% N=10) entre outros motivos (13% N=4). O mesmo se repetiu nas respostas fornecidas nas entrevistas realizadas nos homens& irritação (90% n=58), sentimento de ser traída pelo parceiro ou pessoas próximas (47% N=30), falta de apoio (31% N=20) entre outros motivos (12,5% N=8). Todas as variáveis mostraram significância estatística quando calculadas juntas ou separadamente conforme tabela 4.

Tabela 4: Relação entre a violência sofrida na infância e a praticada atualmente

			Violência sofrida na infância		Violência praticada atualmente		
			N	FREQ	N	FREQ	Valor de P
Gênero	Homens	Violência Psicológica	43	67%	58	90%	P= 0.0020*
		Violência Física	39	61%	51	80%	P= 0.0326*
		Violência Sexual	2	3%	16	25%	P= <0.0001*
	Mulheres	Violência Psicológica	25	81%	31	100%	P= 0.0240*
		Violência Física	24	77%	26	84%	P= 1.0000
		Violência Sexual	3	9%	1	3%	P= 0.6124

*P< 0,05 pelo teste exato de Fisher

Outra variável relacionada ao histórico de abusos sofridos na infância foi estatisticamente significante quando comparados à violência exercida contra os filhos, demonstrando que indivíduos abusados na infância, tendiam a repetir os mesmos comportamentos nos filhos, podendo gerar um ciclo vicioso onde caberia intervenções com o objetivo de intervir sobre o mesmo, evitando então, possíveis expressões comportamentos violentos intrafamiliares futuramente, conforme tabela 5.

Tabela 5: Influência do álcool no comportamento dos usuários relatado pelos mesmos.

		Influência do álcool no comportamento			
			N	FREQ	Valor de P
Gênero	Homens	Irritado	58	90%	P= 0,0276*
		Traído	30	47%	P=< 0,0001*
		Falta de apoio	20	31%	P=< 0,0001*
		Outros	8	12,5%	P=< 0,0001*
	Mulheres	Irritado	25	80%	P= 0,0240*
		Traído	18	58%	P=< 0,0001*
		Falta de apoio	10	32%	P=< 0,0001*
		Outros	4	13%	P=< 0,0001*

*P< 0,05 pelo teste exato de Fisher

Discussão

Este trabalho constatou que o uso nocivo e a dependência do álcool juntamente a fatores como abuso na infância, histórico familiar positivo para o uso de álcool, frequência e padrão do consumo alcóolico, uso de múltiplas substâncias e o fato de presenciar violências motivadas pelo uso do álcool, contribuíram para a expressão atual do comportamento violento intrafamiliar.

Identificar os principais fatores associados ao comportamento violento em portadores da síndrome da dependência alcóolica, se constitui numa importante ferramenta para o desenvolvimento de estratégias de intervenção com o objetivo de reduzir estes comportamentos que causam impactos negativos nas famílias e na qualidade de vida de seus membros.

Apesar da grande associação entre consumo de álcool e violência doméstica entre diferentes profissionais tanto da área de saúde, judicial e social, ainda não foi possível estabelecer como esses fatores interagem e influenciam esses comportamentos [16]. O álcool, funcionaria como um catalisador na expressão do comportamento violento. Autores concordam que o fenômeno possui etiologia multifatorial [16,17].

Através dos levantamentos realizados pelas entrevistas, nota-se que a maior parte dos participantes referiu crescer em um ambiente violento, nos quais presenciavam episódios de violência motivados ou não pelo uso de álcool, tanto entre a família de

origem quanto por vizinhos. Apesar de esses dados demonstrarem significância estatística para o comportamento atual, não podemos afirmar este fator como determinante, entretanto, devemos levar em consideração que as experiências vivenciadas neste contexto, poderiam funcionar como um tipo de facilitador à aprendizagem deste tipo de comportamento.

Ser vítima indireta da violência conjugal onde os protagonistas são as figuras de referência possibilita a vivência de um mundo imprevisível, inseguro e assustador, promovendo a manifestação de sintomas de ansiedade, de evitação e agressividade [17-21].

Além de desestabilizar a vida emocional dos filhos, as experiências de violências domésticas vivenciadas[19,20], poderiam acionar estratégias de sobrevivência desajustadas, associando-se a outros aspectos como baixa autoestima, medo e revolta, que dificultam o sujeito a regularem suas emoções, favorecendo níveis elevados de reatividade comportamental [20,21].

Quando não há intervenção com o objetivo de interromper estes casos de conduta, a ocorrência da interiorização de que os comportamentos vivenciados em casa podem aparentar como a melhor forma de lidar com os problemas pode ser maior, favorecendo, portanto, a transgeracionalidade destes comportamentos, tornando a criança mais suscetível a estados afetivos variáveis, imprevisíveis e negativos [18-20].

Quando há a vivência na infância e/ou adolescência de situações familiares de violência, percebemos que a aprendizagem por parte do agressor sobre um determinado estilo de relacionamento, é repetida em suas vítimas, tanto em relações assimétricas, demonstradas com a violência castigo e com o terrorismo íntimo ou no contexto de relações simétricas onde encontramos a violência agressão ou o controle violento mútuo [19,20] .Fato observado em nosso estudo entre a relação sobre presenciar violência e exercê-la atualmente e corroborado por Almeida (2009) [22].

Em relação às manifestações de violência doméstica, observa-se de acordo com os relatos dos participantes, as mulheres demonstraram maiores níveis e gravidade de agressões que seus companheiros, com exceção à violência sexual e física, provavelmente por encontrar-se em desvantagem em relação à utilização da força física. Em uma pesquisa conduzida por Bhona (2011) [23], onde foi utilizada a mesma escala deste estudo para violência entre o casal, verificou-se que houve corroboração em relação às manifestações de violência perpetradas pelas mulheres. No entanto, na referida pesquisa, as mulheres foram mais agressivas fisicamente, em nosso estudo presente estudo, contudo, apesar de apresentarem este comportamento mais prevalente que os homens em situação semelhante, as mesmas foram mais agredidas fisicamente por seus parceiros.

Em relação às situações de injúria tiveram maior frequência quando as mulheres foram as responsáveis pelas lesões em 54% das ocorrências. Os homens teriam provo-

cado danos à saúde das parceiras em aproximadamente 51% dos casos. Bácskai , Czobor, Gerevich (2011)[24], acreditam que as mulheres sentem menos inibição em relação a agressões físicas em suas relações íntimas do que os homens e tendem a subestimar menos que os homens os atos de violência por elas praticados. Resultados estes que contribuem para a compreensão dos dados analisados

As prevalências encontradas para violência psicológica nesta investigação apontaram 100% das mulheres e 90% dos homens como perpetradores. Estes resultados encontraram-se elevados quando comparados aos achados de Reichencheim et al. (2006)[25] em levantamento realizado em 16 capitais brasileiras (média de 78.3% de agressão psicológica). Nessa mesma pesquisa, foram encontradas, em média, prevalência de violência física praticada por mulheres em torno de 20%, e praticada por homens de aproximadamente 15%, valores bem inferiores aos encontrados neste estudo. Contudo, vale salientar que Reichencheim et al. (2006) [25] investigaram um número inferior de comportamentos de violência ao utilizarem uma versão reduzida da CTS2.

No que tange aos motivos que originaram os episódios de violência, os participantes atribuíam a responsabilidade ao estado de embriaguez em que se encontravam. Afirmando que a presença do álcool, tornavam as agressões mais graves, os mesmos referiam que se sentiam mais irritados e os motivos mais citados pelos mesmos enquanto motivadores das discussões foram: o uso do álcool, ciúmes, falta de apoio, discussões sobre os filhos e interferência familiar e questões acerca das dificuldades financeiras do casal, dados semelhantes foram encontrados no estudo de Almeida (2009), indicando que apenas o álcool não foi o único causador dos episódios violentos.

Ainda que as prevalências nos homens sejam mais elevadas, a tendência na redução das diferenças mostra-se bastante preocupante. As mulheres são mais vulneráveis aos efeitos e prejuízos do álcool quando comparada aos homens [24]. Tanto para fatores biológicos, absorvendo 30% a mais de álcool que o homem e também pelo fato da dificuldade que o organismo feminino tem de metabolizar o álcool por ter mais gordura e menos água que o masculino. O homem possui uma quantidade duas vezes maior de enzima desidrogenase, que protege o fígado dos efeitos maléficos do álcool. Como também a fatores relacionados à violência associada ao consumo de bebidas alcóolicas, onde a vulnerabilidade da mulher à violência é bem maior, mesmo que ela não ingira álcool. Estudos relacionados à violência entre parceiros íntimos revelam que os casos de violência entre os parceiros estão associados a relatos de homens alcoolistas frente ao relato de mulheres alcoolistas [26,27].

Apesar do rigor metodológico adotado para a condução da pesquisa, alguns pontos não conseguiram ser relevados em sua totalidade. Algumas variáveis como cor da pele e religião, poderiam ter sido abordadas, o que facilitaria a compreensão de algumas variáveis que se mostraram controversas. Entrevistar e aplicar as escalas no parceiro

dos participantes poderia nos revelar e possibilitar o entendimento do funcionamento da dinâmica familiar. Esta participação poderia auxiliar na verificação dos pontos concordantes e discrepantes nas respostas dos parceiros e participantes, mesmo não se configurando como objetivo da pesquisa, uma vez que a relevância se encontra na percepção do paciente sobre sua relação com o álcool.

Apesar da boa adesão aos objetivos e possuírem o padrão de respostas rápidas, o grande número de questões e extensão da entrevista pode ter despertado algum tipo de desinteresse ou desconforto. O que pôde ser observado quando os pacientes foram submetidos à escala CTS2, apesar das respostas serem dicotômicas (SIM/NÃO), a mesma apresentava algumas questões delicadas e difíceis para alguns participantes assumirem adotar alguns tipos de comportamentos quando questionados.

Os estudos transversais são muito utilizados para a investigação e prevalência de condições, o que possibilita melhorar o planejamento das ações de saúde e gerar hipóteses de novos estudos. Entretanto, este tipo de estudo não permite a observação ao longo do tempo em relação a redução da ingestão e padrão do consumo alcoólico como a diminuição dos episódios violentos, avaliando desta forma o estado e não a continuidade.

Conclusão

No que tange o comportamento violento, os fatores associados mais frequentes encontrados em usuários de álcool, foram: histórico familiar positivo para o uso de álcool, ter presenciado violência associada ao álcool em algum momento do desenvolvimento, abuso infantil, tanto nas esferas física, psicológica quanto sexual, uso de múltiplas substâncias, padrão e tempo de consumo, demonstrando que os prejuízos estão de certa forma interligada ao tempo de uso.

Os achados neste estudo reforçam a hipótese de que sofrer e ser exposto à violência ao longo do desenvolvimento, facilita a expressão do comportamento violento na vida adulta. De acordo com o relato dos participantes, as agressões são mais violentas com a presença do álcool.

Os mesmos acreditam que quando estão alcoolizados, acabam perdendo a noção da intensidade da força física, bem como, o controle sobre o que é dito para humilhar e agredir verbalmente o parceiro. Uma vez que o álcool diminuiria a regulação do comportamento. Entretanto, para afirmar este fato no estudo, seria necessária uma descrição mais detalhada dessas lesões e agressões a fim de comparar a violência sem a influência do álcool.

Referências

1. Almeida, R M M; Pasa, G G; Scheffer, M. Álcool e violência em homens e mulheres. Psicol. reflex. Crit. 2009.22(2)& 252-260.

2. Elbogen EB; Johnson SC. The intricate link between violence and mental disorder: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Arch Gen Psychiatry. 2009 Feb.66(2): 152-61,

3. Zaleski, Marcos; Pinsky, Ilana; Laranjeira, Ronaldo; Ramisetty-Mikler, Suhasini; Caetano, Raul. Intimate partner violence and alcohol consumption. Rev. Saúde Pública.2010.44(1)& 53-59.

4. Easton CJ; Sacco KA; Neavins TM; Wupperman P; George TP. Neurocognitive performance among alcohol dependent men with and without physical violence toward their partners& a preliminary report. Am J Drug Alcohol Abuse; 2008.34(1): 29-37.

5. Buckley L; Sheehan M; Chapman R. Adolescent protective behavior to reduce drug and alcohol use, alcohol-related harm and interpersonal violence. J Drug Educ. 2009,39(3): 289-301.

6. Duke NN, Pettingell SL, McMorris BJ, Borowsky IW. Adolescent violence perpetration: associations with multiple types of adverse childhood experiences. Pediatrics. 2010 Apr;125(4):e778-86.

7. Gadoni-Costa, LM; Zucatti, APN; Dell'Aglio, DD. Violência contra a mulher: levantamento dos casos atendidos no setor de psicologia de uma delegacia para a mulher. Estud. psicol. (Campinas). 201128(2): 219-227.

8. Herrera Paredes, JM; Ventura, CAA. Alcohol consumption and domestic violence against women: a study with university students from mexico. Rev. Latino-Am. Enfermagem; 2010. 18(spe): 557-564,

9. Monras M, Aparicio A, Lopez JA, Pons I. Prevalence of alcohol consumption related disorders in a prison population convicted of crimes against road safety. Actas Esp Psiquiatr. 2011 May-Jun;39(3):163-7.

10. Fulkerson JA, Pasch KE, Perry CL, Komro K. Relationships between alcohol-related informal social control, parental monitoring and adolescent problem behaviors among racially diverse urban youth. J Community Health. 2008 Dec;33(6):425-33.

11. Hines DA; Straus MA . Binge drinking and violence against dating partners: the mediating effect of antisocial traits and behaviors in a multinational perspective. Aggress Behav; 2007 Sep-Oct.33(5): 441-57.

12. Cork A; Ferns T. Managing alcohol related aggression in the emergency department (Part II). Int Emerg Nurs. 2008 Apr 16(2): 88-93.

13. Calafat, amador; Kronegger, Luka; Juan, Montse; Angels Duch, Mari Angels; Kosir, Matej. Influence of the friends' network in drug use and violent behaviour among young people in the nightlife recreational context . Psicothema (Oviedo); 2011.23(4)& 544-551.

14. Castro, ML; Cunha, SS; Souza, Delma P O. Comportamento de violência e fatores associados entre estudantes de Barra do Garças, MT. Rev. Saúde Pública; 2011. 45(6): 1054-1061.

15. Holder HD. Alcohol and violence: a complex nexus of drinking environment and drinking pat-

tern. Addiction; 2008 Jan.103(1): 78-9.

16. Sprunger JG, Eckhardt CI, Parrott DJ.Anger, problematic alcohol use, and intimate partner violence victimisation and perpetration.Crim Behav Ment Health. 2015 Dec 10;25(4):273-86.

17. Karasov AO, Ostacher MJ.Alcohol and the law.Handb Clin Neurol. 2014;125:649-57

18. Kaufmann VG, O'Farrell TJ, Murphy CM, Murphy MM, Muchowski P.Alcohol consumption and partner violence among women entering substance use disorder treatment.Psychol Addict Behav. 2014 Jun;28(2):313-21.

19. Harford TC, Yi HY, Grant BF.Associations between childhood abuse and interpersonal aggression and suicide attempt among U.S. adults in a national study.Child Abuse Negl. 2014 Aug;38(8):1389-98.

20. Lee C, Cronley C, White HR, Mun EY, Stouthamer-Loeber M, Loeber R.Racial differences in the consequences of childhood maltreatment for adolescent and young adult depression, heavy drinking, and violence.J Adolesc Health. 2012 May;50(5):443-9

21. McNaughton Reyes HL, Foshee VA, Bauer DJ, Ennett ST.Heavy alcohol use and dating violence perpetration during adolescence: family, peer and neighborhood violence as moderators.Prev Sci. 2012 Aug;13(4):340-9.

22. Almeida, MV. Violência conjugal e álcool& (In)existência de uma relação causal?. Dissertação (Mestrado)- Universidade de Coimbra, 2009.

23. BHONA. FMC. Violência doméstica e consumo de álcool entre mulheres: um estudo transversal por amostragem na cidade de Juiz de Fora - MG - Universidade Federal de Juiz de Fora -Dissertação (Mestrado. 2011).

24. Bácskai e, czobor p, gerevich J.Gender differences in trait aggression in young adults with drug and alcohol dependencecompared to the general population. Prog Neuropsychopharmacol Biol Psychiatry. 2011 Jul 1;35(5)&1333-40.

25. Reichenheim ME, et al. Violence and injuries in Brazil: the effect, progress made, and challengesahead. Lancet, Jun 4;377(9781):1962-75, 2011

26. Dang C, Hispard E, Chariot P.Experience of violence perpetration and victimization in alcohol-dependent patients hospitalized for alcohol withdrawal.J Forensic Leg Med. 2012 Oct;19(7):402-6.

27. Klimkiewicz A, Jakubczyk A, Wnorowska A, Klimkiewicz J, Bohnert A, Ilgen MA, Brower KJ, Wojnar M.Violent behavior and driving under the influence of alcohol: prevalence and association with impulsivity among individuals in treatment for alcohol dependence in Poland.Eur Addict Res. 2014;20(3):151-8.

10 - Fatores associados a tentativas de suicídio em portadores da síndrome da dependência alcoólica: Uma revisão

Factors associated with suicide attempts in patients with alcohol dependence syndrome: A review

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Resumo

O comportamento suicida tem sido reconhecido como um problema crescente entre em portadores da síndrome da dependência alcóolica (SDA). Autores referem que o uso do álcool juntamente com outros fatores de riscos facilitaria tentativas de suicídio nesta população. A presente revisão teve como objetivo investigar os fatores associados às tentativas de suicídio em sujeitos portadores da síndrome da dependência alcoólica. Para a construção da revisão, foi realizada uma busca nas principais bases de dados eletrônicas PubMed, BVS, e Lilacs, por artigos publicados nos períodos de janeiro de 2010 a setembro de 2015, utilizando os seguintes descritores: "Suicide attempts and alcoholism", "alcoholism", "suicide attempts and risk factors". Foram encontrados, 161 artigos publicados, porém, apenas 21 foram aproveitados por contemplarem os critérios de inclusão do estudo. Os resultados apontaram vários fatores que quando as-

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sociados ao uso nocivo ou dependência do álcool, poderiam favorecer a expressão do comportamento suicida, entre os quais, destacam-se: presença de depressão, histórico pessoal, abuso na infância, impulsividade, histórico de suicídio na família e desorganização familiar.
Palavras-chave: Tentativa de suicídio e alcoolismo, Alcoolismo, fatores de risco

Abstract

Suicidal behavior has been recognized as a growing problem bearers of alcohol dependence syndrome (SDA). Authors state that the use of alcohol along with other risk factors facilitate suicide attempts in this population. This review aimed to investigate the factors associated with suicide attempts in the subjects with alcohol dependence syndrome. For the construction of the review, a search was conducted in the main electronic databases PubMed, BVS, and Lilacs, for articles published in the period January 2010 to September 2015 using the following keywords: "Suicide attempts and alcoholism", "alcoholism", "suicide attempts and risk factors". Were found, 161 published articles, but only 21 were availed by contemplating the inclusion criteria of the study. The results pointed out several factors that when combined with abuse or dependence on alcohol, could favor the expression of suicidal behavior, among which are: presence of depression, personal history, childhood abuse, impulsivity, suicide of a family history and family disorganization.
Key Words: Suicide attempts and alcoholism, alcoholism risk factors

A utilização do álcool pelos seres humanos vem sendo relatado por pesquisadores de tempos mais remotos aos dias atuais[1]. O consumo ao longo do tempo, produz tolerância, onde o sujeito precisa de doses cada vez maiores até significativa dependência física, originando diversas repercussões negativas em sua saúde física, mental, impactando em relacionamentos interpessoais e laborais. O mesmo, se configura como uma substância de fácil acessibilidade, baixo valor e aceitabilidade social, o seu uso muitas vezes é incentivado e encorajado. [1,2]

A Síndrome da Dependência Alcoólica (SDA), caracteriza-se como um transtorno constituído ao longo da vida, tornando-se desta forma, crônico. É definido como um estado psíquico e físico devido à ingestão repetitiva do álcool, havendo desta forma a compulsão de ingestão de bebidas alcoólicas [3].

Um problema que vem sendo reconhecido de forma cada vez mais frequente, são as tentativas de suicídio e suicídio nesta população[1,2]. O uso nocivo de álcool e a dependência alcoólica associam-se frequentemente a outros transtornos psiquiátricos, maior exposição a riscos, como também ao comportamento suicida[3]. Estudos que verificaram o nível de alcoolemia no sangue de vítimas de mortes violentas, apresentaram em grande parte, resultados positivos, principalmente em mortes prematuras devido

a acidentes, homicídios, suicídios e suicídios indeterminados[4-6]. Os sangues analisados mostravam níveis de concentração alcoólica superior ao permitido por lei, representando um importante fator de risco para todos os tipos de mortes violentas. [7-9].

A literatura demonstra que o uso abusivo de álcool e drogas associados a transtornos mentais, principalmente sintomatologia depressiva, tem sido importante preditor em tentativas de suicídio, indicando que a depressão em pacientes alcoolistas precede as tentativas de suicídio na maioria dos casos. Entretanto, ainda não há um perfil de risco estabelecido para tentativa de suicídio em pacientes alcoolistas portadores de transtornos mentais, acreditando-se ainda na interação de fatores que facilitariam a ocorrência desse tipo de comportamento [10, 11].

Um estudo conduzido por Thornberry [12] (2010), verificou as repercussões que maus tratos na infância traria na vida adulta, onde constatou que dos 907 participantes de sua pesquisa, 72% apresentavam comportamento violento, problemas com álcool, sintomas depressivos e tentativas de suicídio, dados esses, corroborados por vários estudos[13-17], que objetivavam identificar os fatores relacionados a vivências traumáticas na infância com tentativas de suicídio e na idade adulta [15-23]

Em vista a problemática exposta, o presente estudo teve como objetivo, verificar na literatura atual, quais os principais fatores associados a tentativas de suicídio em portadores da SDA.

Método

Esta pesquisa é um estudo de corte transversal; quanto à abordagem: qualitativa; quanto aos objetivos: exploratória e descritiva; quanto ao nível de investigação: básica, podendo tornar-se aplicada, após coleta de dados e maior aprofundamento futuro; quanto aos procedimentos de coleta: bibliográfica.

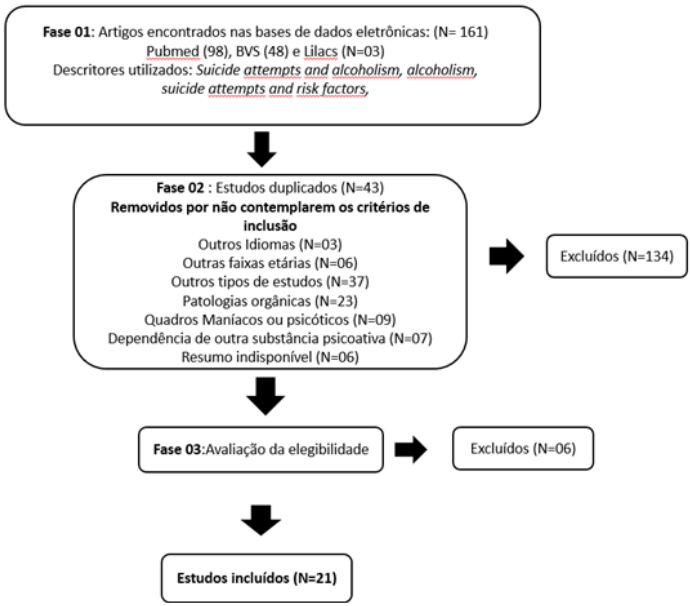
Nesse sentido, realizou-se uma revisão integrativa da literatura disponível nos principais bancos de dados da PubMed, BVS (Biblioteca Virtual de Saúde), e Lilacs. Os artigos coletados foram publicados nos últimos cinco anos, ou seja, compreendidos no período de Janeiro de 2010 a setembro de 2015. Os descritores utilizados nas bases de dados em inglês foram: "Suicide attempts and alcoholism", "alcoholism", "suicide attempts and risk factors".

A presente revisão incluiu artigos que cumpriam os seguintes critérios: a) Pesquisas originais b) Pesquisa com seres humanos c) Com texto em inglês, português, espanhol e alemão d) Sujeitos com idade entre 18-65 anos e) pesquisa com sujeitos que cumpriam os critérios diagnósticos para uso nocivo ou dependência alcoólica. Foram excluídos da amostra, estudos que a) Não apresentavam resumos disponíveis b) Outros idiomas c) Estudo com crianças ou adolescentes d) Outros estudos que não demonstravam relevância a temática investigada e) Patologias orgânicas d) Quadros maníacos ou

psicóticos, uma vez que a natureza dos sintomas poderiam mascarar os fatores investigados e) Dependência de outra substância psicoativa que não fosse o álcool.

Do total da busca, foram obtidos 161 artigos, dos quais apenas 21 foram aproveitados, por serem pertinentes e relevantes ao objetivo do presente artigo, conforme fluxograma abaixo:

Figura 1. Fluxograma e critérios de seleção e inclusão dos artigos.



Resultados

Como já mencionado, a busca localizou um total de 161 artigos dos quais apenas 21 puderam ser aproveitados neste estudo. A grande maioria dos estudos foram transversais, seguidos por retrospectivos, prospectivos, descritivos e comparativos. A número de participantes variavam em função do serviço/centro em que as informações eram coletadas. Os instrumentos utilizados, foram em sua grande totalidade, entrevistas estruturadas [19,24-29], escalas para a verificação de comorbidades psiquiátricas [18,22,25-31] questionários para avaliação do uso problemático com o álcool e outras drogas [18,30-36], entrevistas sobre experiências infantis[19-23] e em quase 90%, escalas para avaliação do risco ou tentativas de suicídio, conforme a tabela 1:

Tabela 1: Características dos estudos incluídos na revisão da literatura

Autor, Ano	Tipo de estudo	Amostra	País do estudo	Instrumentos utilizados
Reyes-Tovilla et al.. 2013 [24]	Comparativo	144	México	Escala de ideação suicida
Ortiz-Gómez et al. 2014 [30]	Transversal	57	EUA	Entrevista neuropsiquiátrica Mini-internacional, questionários avaliativos sobre consumo de substâncias, depressão e tentativas de suicídio, e a escala de Holmes e Rahe para a avaliação de estresse.
Jakubczyk et al. 2014 [18]	Transversal	364	Polônia	Dados demográficos, avaliação de histórico familiar em transtorno mental, histórico de tentativas de suicídio, abuso sexual e físico na infância e na idade adulta e gravidade dos problemas com álcool.
Kubiak et al. 2013 [24]	Transversal	124	Polônia	Entrevista estruturada.
Desouches et al. 2011 [33]	Retrospectivo	200	Argentina	Registros epidemiológicos conduzidos por profissionais do Departamento de Psiquiatria do Hospital Posadas, contendo informações gerais sobre a tentativa de suicídio e consumo de substâncias psicoativas

Singh et al. 2013[25]	Comparativo	218	Índia	Entrevista e administração de Patient Health Questionnaire (PHQ)
Czyz et al. 2013 [26]	Descritivo	165	Web	Entrevista estruturada e uma pergunta aberta sobre os motivos que o levam a não procurar ajuda profissional.
Hung et al. 2013ª [19]	Prospectivo	194	Taiwan	Entrevista estruturada e questionário para avaliação experiências adversas na infância
Garcia -Rabago et al. 2010 [27]	Comparativo	106	Colômbia	Entrevista estruturada
Hung et al. 2013b [20]	Transversal	175	Taiwan	Questionários sobre a história da Saúde da Família
Mackril e Hesse, 2012 [21]	Transversal	344	Dinamarca	Entrevistas sobre experiências na infância
Huang et al. 2012 [22]	Transversal	196	EUA	Entrevista clínica estruturada para o DSM-IV - Transtornos do Eixo I e Childhood Trauma Questionnaire (CTQ) para verificação de traumas na infância
Klimkiewicz, et al. 2012 [28]	Comparativo	113	Polônia	Entrevista
Matsumoto, et al. 2012 [31]	Transversal	1420	Japão	Um questionário de auto-relato sobre idade, sexo, tipos de substâncias ilícitas, depressão e comportamento suicida atual

Yaldizli, et al. 2010 [36]	Transversal	1863	EUA	Questionário padronizado
Vyssoki, et al. 2011 [34]	Retrospectivo	116	Áustria	Questionário para avaliação da distribuição temperamental. As dimensões sobre a dependência de álcool foram avaliadas utilizando uma entrevista estruturada informatizada.
Randall, et al. 2013 [32]	coorte prospectiva	107	Canadá	Testes de associação implícitas que avaliaram pensamentos de morte, suicídio e automutilação. Também foram preenchidos pelos pacientes: Inventário de Desesperança de Beck, escala de impulsividade Barratt, a escala CAGE para alcoolismo, e o teste de despistagem de drogas de abuso
Hashimoto e Ashizawa. 2012 [35]	Transversal	64	Japão	Preenchimento de dados sócio demográficos, participação no AA, histórias familiares sobre dependência de álcool, período de abstinência, e fenômenos suicidas. Foram também, questionados sobre seu comportamento antes e depois de se tornar membros de AA

Demirbas et al. 2011 [33]	Retrospectivo	142	Turquia	Escala de ideação suicida, Inventário de Depressão de Beck, Inventário de Desesperança de Beck, Escalas de Estado/Traço de Ansiedade de Spielberger, e a Expressão de Raiva Traço-Estado
Bedi et al. 2011[23]	Retrospectivo	2559	EUA	Banco de dados de um estudo com família sobre maus tratos na infância
Hamdan et al. 2011 [29]	Transversal	468	EUA	Entrevista estruturada, o Composite International Diagnostic Interview (CIDI), além disso, foram avaliadas depressão, ansiedade, desesperança, impulsividade e hostilidade auto referida, adversidades na primeira infância e comportamento suicida em parentes de primeiro e segundo grau.

No que se refere aos principais fatores de riscos relacionados às tentativas de suicídio em sujeitos que fazem uso nocivo ou são portadores da síndrome da dependência alcóolica, foi construída um tabela (tabela 2) com a finalidade de apresentar os dados de forma sucinta e objetiva.

Tabela 2: Objetivos dos estudos e fatores de riscos associados as tentativas de suicídio

Autor, Ano	Objetivo do estudo	Fatores associados	Conclusão
Reyes-Tovilla et al.. 2013 [24]	Identificar as diferenças entre os pacientes com tentativa de suicídio impulsiva e pacientes com tentativa de suicídio premeditada.	Maior escolaridade e menor gravidade foram encontrados em pacientes com tentativas impulsivas O consumo de álcool e uso de cannabis estiveram presentes em pacientes que haviam premeditado uma tentativa.	Existem diferenças clínicas entre os indivíduos que realizaram uma tentativa de suicídio impulsiva daqueles que haviam premeditado.
Ortiz-Gómez et al. 2014 [30]	Determinar os fatores psicológicos e sócio-demográficos associados a depressão e tentativas de suicídio em sujeitos em reabilitação para consumo de drogas.	Depressão e abuso de álcool e outras drogas.	O uso de substâncias na família em associação à depressão e dependência de álcool funcionou como fator de risco, ressaltando a necessidade de ações destinadas a prevenir vícios no ambiente doméstico.

Jakubczyk et al. 2014 [18]	Investigar associações entre a história de abusos sexuais na infância e tentativas de suicídio em uma amostra de pacientes dependentes de álcool.	Mulheres jovem, maior gravidade da dependência de álcool, menor apoio social, e pior situação econômica. Além disso, houve uma associação significativa entre a história de tentativas de suicídio e histórico de transtorno mental familiar, tentativas de suicídio na família, a história de abuso sexual na infância, bem como história de abuso físico.	Histórico de abuso sexual na infância é um fator de risco significativo em tentativas de suicídio em indivíduos dependentes de álcool.
Kubiak et al. 2013 [24]	Analisar, os principais fatores associados à tentativa de suicídio por overdose de drogas no mês anterior.	Eventos estressantes interpessoais. A grande maioria deles estavam associados aos relacionamentos com cônjuges ou parceiros.	Eventos estressantes no mês anterior à tentativa de suicídio associados a relacionamentos interpessoais conturbados estariam associados as tentativas de suicídio.

Desouches et al. 2011 [33]	Analisar o uso de substâncias psicoativas em pacientes internados em um Hospital Geral com o diagnóstico de tentativa de suicídio e definir o perfil de comportamento suicida.	Associação entre o consumo de álcool, maconha e cocaína foi encontrada no sexo masculino, álcool e sedativos no sexo feminino.	Substâncias psicoativas encontram-se associadas à tentativas de suicídio.
Singh et al. 2013[25]	Validar relatório anterior referente ao alto índice de suicídios e avaliar traços psicológicos.	Gênero feminino, Depressão, ansiedade, abuso de álcool e transtorno alimentar como compulsão alimentar e Bulimia nervosa.	Depressão e gênero feminino foram apontados como fatores significativos em tentativas de suicídio na população estudada, ao passo que o abuso de álcool embora citado, não foi um aparentou ser um fator significativo.

Czyz et al. 2013 [26]	Descrever as barreiras auto-relatadas na busca de atendimento profissional entre os estudantes universitários que estão apresentam elevado risco de suicídio.	O estigma foi mencionado por apenas 12% dos alunos. Houve diferenças notáveis com base no sexo, raça e gravidade da depressão e abuso de álcool.	Intervenções visando alcançar os sujeitos com risco de comportamento de risco suicida elevado devem ser particularmente sensíveis a estas barreiras comumente descritas.
Hung et al. 2013ª [19]	Avaliar a associação entre história de experiências adversas na infância e tentativas de suicídio.	Depressão vida, uso de drogas ilícitas, e a gravidade da dependência do álcool, abuso físico na infância, divórcio dos pais .	Há uma relação direta entre adversidades na infância e tentativas de suicídio entre pacientes alcoólatras do sexo masculino.
Garcia -Rabago et al. 2010 [27]	Identificar os fatores de risco associados às tentativas de suicídio de baixa letalidade e tentativas de suicídio de alta letalidade.	As taxas de tentativas mais elevadas ocorreram no grupo de alta letalidade, mas apenas dois fatores tiveram diferença estatística significativa: "viver sozinho" e "intoxicação por álcool horas antes, adulto jovem e sexo masculino.	"viver sozinho" e intoxicação alcoólica demostrou diferenças estatisticamente significativas.

Hung et al. 2013b [20]	Identificar a associação entre exposição de adversidades na infância e propensão a tentativas de suicídio futuras	Entre os participantes, 48 tinham história de tentativa suicídio e 156 relataram adversidades na infância.	Histórico de abuso e exposição a adversidades na infância alcançou o melhor poder preditivo para uma tentativa de suicídio futura.
Mackril e Hesse, 2012 [21]	Explorar a associação entre o comportamento suicida em pais com problemas de abuso de álcool e comportamento suicida em sua prole.	Comportamento suicida parental, abuso de álcool, Ameaças de suicídio.	O estudo salienta a importância de abordar e avaliar a história da família com comportamento suicida e suas repercussões em sua prole.
Huang et al. 2012 [22]	Analisar a prevalência da exposição a traumas na infância em pacientes submetidos desintoxicação alcoólicas.	Abuso emocional abuso físico, abuso sexual, negligência emocional e negligência física.	Observou-se altos índices comportamentos suicidas, abuso de álcool e comorbidades psiquiátricas em sujeitos expostos a traumas na infância.

Klimkiewicz, et al. 2012 [28]	Comparar os relatos sobre tentativas de suicídio durante um episódio de binge drink com os de tentativas de suicídio durante a sobriedade relativa.	Sexo masculino, idade mais jovem, maior gravidade de dependência de álcool e a tentativa impulsiva.	Entre os pacientes em tratamento para dependência de álcool que fez um suicídio tentativa, a tentativa mais séria era susceptível de ter sido impulsiva cometida por homens durante um episódio de binge drink.
Matsumoto, et al. 2012 [31]	Identificar fatores de risco para o suicídio no transtorno do uso de substâncias psicoativas e examinar as diferenças entre gêneros entre esses pacientes.	Depressão atual foi associada com ideação suicida grave em pacientes do sexo feminino.	Idade mais jovem e sexo feminino estavam intimamente associada com ideação suicida grave.
Yaldizli, et al. 2010[36]	Determinar os fatores de risco para tentativa de suicídio em um subgrupo de pacientes com história de dependência de álcool ou abuso e sintomas depressivos.	Sintomas depressivos, experiências adversas durante o consumo de álcool, experiências de abuso de droga.	Os fatores de risco preditivos para a tentativa de suicídio nesta população foram o abuso ou dependência de álcool associados a sintomas depressivos.

Vyssoki, et al. 2011 [34]	Avaliar o impacto dos traços temperamentais em pacientes dependentes de álcool sobre o curso da doença.	Temperamento depressivo e dependência do álcool aumentaram a probabilidade de tentativas de suicídio.	Temperamento depressivo e ansioso, parecem ser preditores negativos na dependência do álcool.
Randall, et al. 2013 [32]	Identificar a relação entre pensamentos implícitos sobre a morte, suicídio e auto-mutilação com a ocorrência de auto-mutilação no futuro.	Depressão, ansiedade, impulsividade e abuso de álcool	A existência e interação de comorbidades acarretam maior probabilidade de comportamento de auto-mutilante
Hashimoto e Ashizawa. 2012 [35]	Descrever a redução do risco de suicido na dependência do álcool em pacientes que participavam do Alcoólicos Anônimos (AA)	Histórias familiares sobre dependência de álcool, período de abstinência, e fenômenos suicidas, foram retrospectivamente perguntado antes e depois de se tornar membros de AA.	A participação no grupo AA causou uma diminuição significativa no risco de fenômenos suicidas em dependência do álcool.

Demirbas et al. 2011 [33]	Avaliar as relações entre estilos de expressão raiva, estado e traço de ansiedade - em pacientes dependentes de álcool coma probabilidade, de suicídio.	Houve correlações significativas entre a probabilidade de suicídio em depressão, alto nível de raiva, baixo nível de desesperança.	Ansiedade, desespero e depressão, aumentaram a probabilidade tentativa de suicídio em pacientes dependentes de álcool.
Bedi et al. 2011[23]	Descrever a associação entre abuso sexual na infância, depressão e tentativa de suicídio.	Risco significativo associado ao abuso sexual na infância, depressão e transtorno de estresse pós-traumático, e comportamento suicida em homens e mulheres.	Abuso sexual na infância, depressão e transtorno de estresse pós-traumático aumentariam os riscos de tentativas de suicídio.
Hamdan et al. 2011 [29]	Investigar a correlação entre comportamento suicida parentes árabes selecionados por conta da alta taxa de comportamento suicida.	Tentativas de suicídio, depressão, ansiedade, desesperança, impulsividade e hostilidade, adversidades na primeira infância e comportamento suicida em parentes de primeiro e segundo grau.	Foram encontradas associações significativas entre pensamentos e comportamentos suicidas, presença de história familiar de suicídio e transtornos mentais. Além disso, impulsividade e hostilidade também foram significativamente associados com tendências suicidas.

Um estudo conduzido por Klimkiewicz e cols28.(2012), ao comparar a letalidade da tentativa do suicídio, destaca que a mesma seria mais grave quando associada a comportamento impulsivo e intoxicação alcoólica por binge drink.

Binge Drinking é definido internacionalmente como o padrão de consumo de 4 ou mais doses em uma ocasião 28,. No Brasil, este fenômeno é semelhante ao beber pesado em um curto espaço de tempo. Zalesky (2010)37 aponta que este padrão de consumo, encontra-se fortemente associado a maiores problemas físicos, mentais e sociais do que padrões de consumo que se aproximam da dependência.

Esses autores 28,29,37, acreditam que a intoxicação alcoólica aguda funcionaria como uma espécie de gatilho para pensamento suicidas e tentativas entre os sujeitos com outros fatores de riscos associados, aumentando a impulsividade e influenciando o potencial de letalidade da tentativa

Outro fator constantemente apontado, é a presença de sintomatologia depressiva e outras comorbidades psiquiátricas. Dos 57 participantes do estudo de Ortiz-Gómez e cols.30 (2014) 68,4% dos pacientes tinham depressão maior, destes, 75,4% tinham histórico de abuso de substâncias, sobretudo o álcool. A depressão, foi sem dúvida o transtorno mais prevalente quando comparada a quadros de ansiedade e estresse pós-traumático 23, 29,34, 36. Não foram incluídos nesse estudo quadros maníacos ou psicóticos pois, por conta da natureza dos sintomas os comportamentos investigados poderiam ser mascarados ou alterados por conta da própria psicopatologia inerente aos mesmos.

Apesar de ser considerado um depressor do sistema nervoso central influenciando a maioria dos sistemas neuroquímicos, quando consumido, o álcool, provoca desinibição e euforia podendo desencadear comportamentos inadequados predispondo então à violência. Acredita-se que este fenômeno é originado devido às propriedades psicoativas encontradas no mesmo, causando no decorrer do uso sérios comprometimentos neuropsicológicos, incluindo déficit de atenção, alterações cognitivas, distorção da percepção levando a um julgamento inadequado e equivocado das situações vivenciadas, aumentando e estimulando comportamentos violentos, hetero e autodirigidos. 38, 39

Estudos relacionados a avaliações neuropsicológicas como o de Cunha e Novaes (2004) 39, relatam inúmeros comprometimentos cognitivos decorrentes do uso abusivo do álcool, principalmente na função executiva, que envolve a capacidade de resolver problemas, antecipar consequências e modificar estratégias, observando o próprio comportamento, comparando-o com o planejamento inicial. Achados semelhantes, são encontrados em pacientes diagnosticados com depressão. Outros sintomas cognitivos relacionados ao abuso do álcool frequentemente relatados por alguns pesquisadores são: euforia, irritabilidade, labilidade afetiva, prejuízo no julgamento, diminuição da atenção, sonolência, lentificação psicomotora, redução do nível da consciência, podendo levar ao coma 39, 40

É sabido que, por ser uma substância depressora, o álcool reduz a concentração de serotonina principalmente em regiões responsáveis pela modulação do comportamento. A redução da transmissão desse neurotransmissor em conjunto com a diminuição do mesmo (provocado pela ingestão alcoólica), típica em pacientes deprimidos, contribuem então, na expressão da agressividade e impulsividade. 40

A consistência desses dados, levantaram a hipótese de que a agressividade, a impulsividade, bem como outros achados psicopatológicos pudessem estar relacionados ao comportamento suicida. Hipóteses estas, corroboradas em estudos de neuroimagem. 41

Heeringen (2010) 42, através de uma revisão sistemática, destacou que pacientes portadores da síndrome da dependência do álcool, apresentaram alterações volumétricas na substância cinzenta, hipometabolismo na região frontal (responsável pelas funções mentais superiores como planejamento, controle da impulsividade e execução) além de baixo processamento em atividades cognitivas. Resultados semelhantes foram encontrados em pacientes com depressão e histórico de tentativas de suicídio, corroborando a hipótese de que ambos os transtornos compartilham a mesma fisiopatologia.

Outro fator frequentemente associado ao risco aumentado para tentativas de suicídio, é o abuso infantil, seja psicológico, físico ou sexual. Ao realizar um levantamento sobre os principais fatores associados às tentativas de suicídio, Hamdan (2011) 29, afirma que há associações significativas entre pensamentos e comportamentos suicidas, presença de história familiar de suicídio e transtornos mentais. Esses dados foram substanciados com outros estudos que demonstravam: uso de substâncias na família 30,33 abusos 18 ou exposição a comportamentos violentos entre parentes 19,20 , ameaças ou tentativas de suicídio feitas pelos pais 21 além da presença de transtornos psiquiátricos na família 20,21,22,29, funcionariam como fortes preditores de tentativas de suicídio nesta população.

História de tentativas prévias de suicídio, parecem ser fatores de risco comuns a várias populações como forte preditor para o risco para suicídio completo em alguns estudos 18,20,21,28,29,34, 43,44. . Estima-se que existem cerca de dez tentativas para cada ato consumado, quatro tentativas não conhecidas para cada uma registrada 43,44. Sendo consideradas muitas vezes como a sexta causa de incapacitação, devido às tentativas más sucedidas, ou seja, sem a consumação do ato.

No que tange ao gênero, em mulheres são relatados maiores números de tentativas do que no sexo oposto. Homens tendem a utilizar de métodos mais letais, enquanto mulheres se utilizam de métodos em que, quando há o socorro em tempo hábil, o quadro pode ser revertido, como por exemplo, intoxicação por álcool e drogas e auto envenenamento.20,21,22, 25,31. Apenas um estudo não encontrou correlação entre abuso de álcool em tentativa de suicídio em mulheres25, no entanto, pelo fato do estudo ter sido realizado na Índia onde, a grande maioria das doutrinas religiosas condenem o ato

de beber, acredita-se que o número de abstêmios seja muito alto e assim a associação esteja enviesada.

A grande maioria dos estudos, no entanto, demonstrou uma forte associação de transtornos mentais, sobretudo os de humor depressivo e abuso de substância, principalmente o álcool e histórico de abuso infantil como fatores que aumentariam a propensão à comportamentos suicidas. Apesar de serem considerados comórbidos, ainda não foi possível estabelecer qual seria transtorno de base (se a SDA favoreceria o surgimento de transtornos depressivos ou, os transtornos depressivos levariam à SDA). Acredita-se que as alterações emocionais, disfunções no processamento cognitivo e a baixa modulação da impulsividade, estariam relacionados ao comportamento adicto, depressivo e suicida.

Todavia, estudos com pacientes em tratamento/reabilitação, vem se mostrando muito promissores. Hashimoto e Ashizawa (2012)35, apontaram uma significativa diminuição nas tentativas de suicídio em pacientes que apresentavam esses fatores de risco. No entanto, não são todos os sujeitos que conseguem ter acesso a tratamentos adequados. Czyz e cols (2013) 26 apontam que algumas populações com risco elevado, defrontam-se com barreiras ao buscarem atendimento especializado, como o estigma e preconceito, por exemplo. Muitas pessoas ainda acreditam que a tentativa de suicídio é um comportamento de livre escolha do sujeito, que é uma manifestação da sua autonomia ou uma tentativa de manipulação ou chamar a atenção. Mas isso é equivocado; é um problema de saúde mundial, em geral, a expressão final de um estado mental deprimido, produzido normalmente por uma doença que é possível de tratar.

Conclusão

Apesar dos levantamentos realizados, percebe-se o quão complexo é identificar a gênese do comportamento suicida. Dentre os fatores de riscos mais relatados, encontram-se: Presença de algum transtorno psiquiátrico, quase sempre de humor, abuso de álcool e outras drogas. Salienta-se, no entanto, que apesar da grande correlação, esses fatores parecem ser necessários, mas não suficiente para o desenvolvimento do comportamento suicida. Verificou-se que a associação desses fatores em conjunto a: histórico de abusos na infância, impulsividade, hostilidade, baixa tolerância à frustração, tentativas prévias, história de suicido na família, desorganização familiar, solidão, desesperança, apresentaram forte predição às tentativas de suicídio.

Referências

1. Coelho. B M. A influência da comorbidade entre depressão e transtorno por uso de álcool nos comportamentos suicidas no Epidemiológica de São Paulo Estudo de Área de Captação, Brasil. Rev. Bras. Psiquiatr. vol.32 no.4 São Paulo dez 2010.

2. Hakansson a, Bradvik l, Schlyter f, Berglund m. Factors associated with the history of at-

tempted suicide. Crisis. 31(1):12-21, 2010.

3. Laranjeira, R. . Apresentação: Álcool: Da saúde pública à comorbidade psiquiátrica. Rev. Bras. Psiquiatr, 26(1), 1-2. 2004.4. Freitas EA; Mendes ID; Oliveira LC . Alcohol consumption among victims of external causes in a university general hospital. Rev Saude Publica;42(5): 813-21, 2008 Oct.

4. Ray JG, Moineddin R, Bell CM, Thiruchelvam D, Creatore MI, Gozdyra P, Cusimano M, Redelmeier DA. Alcohol sales and risk of serious assault. PLoS Med. 2008 May 13;5(5):e104.

5. McKinney CM, Caetano R, Rodriguez LA, Okoro N. Does alcohol involvement increase the severity of intimate partner violence? Alcohol Clin Exp Res. 2010 Apr;34(4):655-8.

6. Palmstierna T; Olsson D. Violence from young women involuntarily admitted for severe drug abuse. Acta Psychiatr Scand;115(1): 66-72, 2007 Jan.

7. Paula, C M. C. de; Ruzzene, M A. M; Martinis, B S. Blood alcohol concentration and violent deaths. Medicina (Ribeirão Preto);41(1): 24-29, jan.-mar. 2008. tab, graf.

8. Pulay AJ, Dawson DA, Hasin DS, Goldstein RB, Ruan WJ, Pickering RP, Huang B, Chou SP, Grant BF. Violent behavior and DSM-IV psychiatric disorders: results from the national epidemiologic survey on alcohol and related conditions. J Clin Psychiatry. 2008 Jan;69(1):12-22.

9. King, a l s; Nardi, a e; Cruz, M S. Suicide risk in alcohol-dependent patient with depression. J Bras Psiquiatr; 55(1): 70-73, jan.-mar. 2006.

10. Bolton JM; et al. Exploring the correlates of suicide attempts among individuals with major depressive disorder: results from the national epidemiologic survey on alcohol and related conditions. Source: J Clin Psychiatry, 69 (7): 1139-1149, July 2008.

11. Thornberry TP, Henry KL, Ireland TO, Smith CA. The causal impact of childhood-limited maltreatment and adolescent maltreatment on early adult adjustment. J Adolesc Health. 2010 Apr;46(4):359-65. Epub 2010 Jan 4.

12. Centers for Disease Control and Prevention (CDC). Alcohol and suicide among racial/ethnic populations - 17 states, 2005-2006. MMWR Morb Mortal Wkly Rep. 2009 Jun 19;58(23):637-41.

13. Ocampo R, Bojorquez I, Cortés M. Substance use in suicides in Mexico: results of the Epidemiological Surveillance System of Addictions, 1994-2006. Salud Publica Mex. 2009 Jul-Aug;51(4):306-13. Spanish.

14. Walton MA; Chermack ST; Shope JT; Bingham CR; Zimmerman MA; Blow FC; Cunningham RM.Effects of a brief intervention for reducing violence and alcohol misuse among adolescents: a randomized controlled trial. JAMA;304(5): 527-35, 2010 Aug 4.

15. Chermack ST; Murray RL; Walton MA; Booth BA; Wryobeck J; Blow FC. Partner aggression among men and women in substance use disorder treatment: correlates of psychological and physical aggression and injury. Drug Alcohol Depend;98(1-2): 35-44, 2008 Nov 1.

16. Colbert SJ; Krause N. Witnessing violence across the life course, depressive symptoms, and alcohol use among older persons. Health Educ Behav;36(2): 259-77, 2009 Apr.

17. Jakubczyk A, Klimkiewicz A, Krasowska A, Kopera M, Sławińska-Ceran A, Brower KJ, et al. History of sexual abuse and suicide attempts in alcohol-dependent patients. Child Abuse Negl. 2014 Sep;38(9):1560-8.

18. Hung GC, Huang MC, Yip PS, Fan HF, Chen YY. Association between childhood adversities and suicide attempts among alcoholic inpatients in Taiwan. J Stud Alcohol Drugs. 2013a Jul;74(4):559-64.

19. Hung GC, Caine ED, Fan HF, Huang MC, Chen YY. Predicting suicide attempts among

treatment-seeking male alcoholics: an exploratory study. Suicide Life Threat Behav. 2013 Aug;43(4):429-38

20. Mackrill T, Hesse M. Suicide behavior in parents with alcohol abuse problems and suicide behavior in their offspring-adult offspring and counselor perspectives. Nord J Psychiatry. 2012 Oct;66(5):343-8.

21. Huang MC, Schwandt ML, Ramchandani VA, George DT, Heilig M. Impact of multiple types of childhood trauma exposure on risk of psychiatric comorbidity among alcoholic inpatients. Alcohol Clin Exp Res. 2012 Jun;36(6):1099-107

22. 22. Bedi S, Nelson EC, Lynskey MT, McCutcheon VV, Heath AC, Madden PA, et al. Risk for suicidal thoughts and behavior after childhood sexual abuse in women and men. Suicide Life Threat Behav. 2011 Aug;41(4):406-15.

23. Kubiak M, Musikowska B, Sein Anand J. Recent life events preceding suicide attempt by drug overdose. Przegl Lek. 2013;70(8):551-4.

24. Singh PK, Singh RK, Biswas A, Rao VR. High rate of suicide attempt and associated psychological traits in an isolated tribal population of North-East. J Affect Disord. 2013 Nov;151(2):673-8.

25. Czyz EK, Horwitz AG, Eisenberg D, Kramer A, King CA. Self-reported barriers to professional help seeking among college students at elevated risk for suicide. J Am Coll Health. 2013;61(7):398-406.

26. García-Rábago H; Sahagún-Flores JE; Ruiz-Gómez A; Sánchez-Ureña GM; Tirado-Vargas JC; González-Gámez JG. Factores de riesgo, asociados a intento de suicidio, comparando factores de alta y baja letalidad. / [Comparing high- and low-lethality factors regarding attempted suicide-associated risk factors].Rev Salud Publica (Bogota); 2010 Oct.12(5): 713-21,

27. Klimkiewicz A, Ilgen MA, Bohnert AS, Jakubczyk A, Wojnar M, Brower KJ. Suicide attempts during heavy drinking episodes among individuals entering alcohol treatment in Warsaw, Poland. Alcohol Alcohol. 2012 Sep-Oct;47(5):571-6.

28. Hamdan S, Melhem N, Orbach I, Farbstein I, El-Haib M, Apter A, et al. Risk factors for suicide attempt in an Arab kindred. J Affect Disord. 2011 Jul;132(1-2):247-53.

29. Ortíz-Gómez LD, López-Canul B, Arankowsky-Sandoval G. Factors associated with depression and suicide attempts in patients undergoing rehabilitation for substance abuse. J Affect Disord. 2014 Dec;169:10-4.

30. Matsumoto T, Matsushita S, Okudaira K, Naruse N, Cho T, Muto T, et al. Sex differences in risk factors for suicidality among Japanese substance use disorder patients: association with age, types of abused substances, and depression. Psychiatry Clin Neurosci. 2012 Aug;66(5):390-6

31. Randall JR, Rowe BH, Dong KA, Nock MK, Colman I. Assessment of self-harm risk using implicit thoughts. Psychol Assess. 2013 Sep;25(3):714-21

32. Demirbas H, Ilhan IO, Dogan YB. Assessment of the mode of anger expression in alcohol dependent male inpatients. Alcohol Alcohol. 2011 Sep-Oct;46(5):542-6.

33. Vyssoki B, Blüml V, Gleiss A, Friedrich F, Kogoj D, Walter H, et al. The impact of temperament in the course of alcohol dependence J Affect Disord. 2011 Dec;135(1-3):177-83.

34. Hashimoto S, Ashizawa T. Does participating in AA decrease the risk for suicide in alcohol dependence?. Nihon Arukoru Yakubutsu Igakkai Zasshi. 2012 Dec;47(6):308-16

35. Yaldizli O; Kuhl HC; Graf M; Wiesbeck GA; Wurst FM. Risk factors for suicide attempts in patients with alcohol dependence or abuse and a history of depressive symptoms: a subgroup analysis from the WHO/ISBRA study. Drug Alcohol Rev; 2010 Jan. 29(1): 64-74,

36. ZALESKI, Marcos; et al. Intimate partner violence and alcohol consumption. Rev. Saúde Públi-

ca; 2010 44(1): 53-59,.

37. HIGGINS E. S.; GEORGE M.S. Neurociências para prática clínica: a fisiopatologia do comportamento e da doença mental. Porto Alegre: Artmed, 2010.

38. CUNHA, P. J., & NOVAES, M. A. . Avaliação neurocognitiva no abuso e dependência do álcool: Implicações para o tratamento. Revista Brasileira de Psiquiatria, 26(1), 23-27. 2004

39. MARLBERGIER, A.; AMARAL R. A. Transtornos relacionados com o uso de substâncias psicoativas. Em: Teixeira L. A.; Caramelli P. Neurologia cognitiva e do comportamento. Rio de Janeiro, Revinter, Pp 402-413,2012.

40. Corrêa H, Barrero SP. Suicídio: uma morte evitável. São Paulo: Atheneu; 2006.

41. HEERINGEN, K V. Functional brain imaging suicidal patientes. Psychiatria Danubina, Vol. 22, suppl 1, 2010

42. Gairin I, House A, Owens D. Attendance at the accident and emergency department in the year before suicide: retrospective study. Br J Psychiatry. 2003;183:28-33.

43. Pandey GN. Biological basis of suicide and suicidal behavior. Bipolar Disord. 2013:15(5):524-41.

11 - From Claude Bernard to Homer Smith: the evolution of renal function and research

Frederico Fazan[1]

Milieu interieur

In the late 19th century, Claude Bernard, the father of modern physiology, stated that the medium in which we live is not the atmosphere that surrounds us, but the blood and body fluids that bath our organs, tissue, and cells. Bernard described this medium as a micro-universe elaborately isolated from the external world and protected from vicissitudes by several physiological mechanisms that work towards maintaining its volume, physical and chemical properties. The partial pressures of oxygen and carbon dioxide, the concentrations of nutrients and catabolites, the temperature, hydrogenionic concentration, the osmotic pressure, the concentration of several cations and anions and the volume of this internal medium are kept in a precise and narrow limit of normality through the function and interconnectivity of our organs.

The complexity of life and the "necessity" of the internal medium

It is thought that the selective pressure that created the necessity of an internal medium was the gain in complexity and multicellularity of the primitive forms of life. The unicellular life that rose in the aqueous medium, in order to stay alive, executed cellular metabolism; that is, acquired useful metabolites from the medium and at the same time excreted useless or potentially dangerous metabolites into the medium. This exchange with the primitive ocean, infinitely bigger than the organism, could never change the effective concentrations of the ocean. However, as life grew in complexity and size, and with non-axial growth, some cells began to lose direct contact with the ocean. Now, the process of acquiring and excreting substances from these

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cells could effectively change the interstitial fluid (the fluid immediately between the cells).

Hence, the transport generated through simple diffusion could no longer satisfy the metabolic demand of the cells distant from the medium. This generated selective pressure favoring the emergence of a system that could transport the medium into close contact with these cells, or later, a fluid that traverses the cells and that represented the ocean. The vascular system arises. This system offered transport of metabolites through drag or convection, favoring the exchange of the distant cells with this internal medium satisfying efficiently the metabolic demand even if they were not in direct contact with the external medium.

We will now leap hundreds of millions of years and arrive in our recent mammal vascular system. Now, we have a system of closed ducts that do not directly come in contact with the external medium. Furthermore, this medium that surrounds us is dry, and life as we know always needs water. In fact, our organism is made of around 70% water separated in specific compartments, and the blood that runs through our vascular system is still a representative of the external medium. With a closed vascular system, however, the transition from an aqueous environment to a dry one, there was again selective pressure for the enhancement of the systems that regulate the internal medium (that had already arisen long ago).

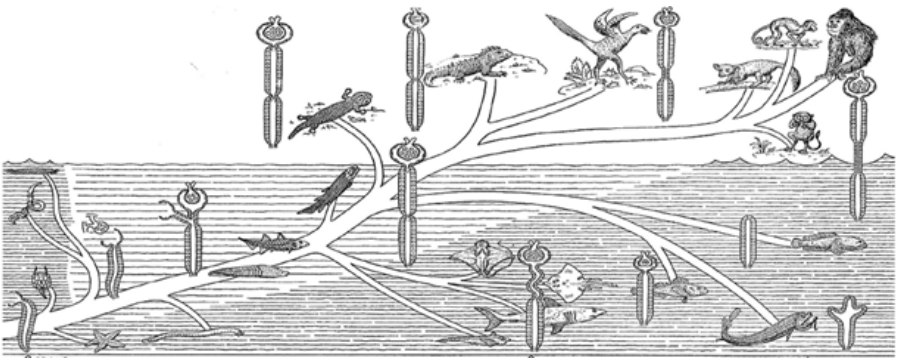


Figure 1: Representation of the evolution of the nephron with the conquer of land. Modified from: (Chevalier 2017).

The kidneys provide the ultimate role in the regulation of the concentration of the final products of metabolism, as well as the regulation of the osmotic pressure, volume, and ionic composition of the internal medium. The transition from the aqueous environment to the land generated more efficient mechanism of urinary concentration, represented by the increased loop length and the differentiation of the proximal and distal portions of the nephron 1.

Claude Bernard also suggested that as the living organisms gained complexity, it was necessary to maintain a more sensitive and delicate medium, therefore, it was necessary to maintain it more effectively. Hence, it is possible to assume that it is because of our blood's composition, and recognizing that we have this blood because of our renal function, that we achieved physiological liberty and independence from water medium. That's why it is possible to say that environmental freedom can only happen because of enhanced kidney function.

Renal function and the stationary state theory

The kidneys are commonly described as excretory organs. The attribution of this limited role, however, is unjust. They are organs that keep the composition and volume of the internal medium. The excretory function is a consequence of the regulatory function. This fact can be demonstrated in the following example: consider two individuals – one with normal renal function and the other with chronic and long-lasting bilateral kidney disease (CKD). Suppose that both individuals ingest identical quantities of protein, carbohydrates, and fat. Also, both will ingest the same amount of sodium and potassium and also high and identical volumes of water. How will the urine of both these individuals differ? The answer is: there will be no difference 2.

Both will excrete the same amount of nitrogen, sodium, and potassium. If the water ingestion is high, the urine volume will be the same. The individual with kidney disease can excrete some proteins, red blood cells and cylinders; however, the main products of excretion will be exactly the same.

Why, then, should the clinician worry about the patient with chronic kidney disease? Because the unhealthy kidney is deficient in maintaining the composition, and in a lesser degree, the volume of the internal medium. The patient with CKD will have 10 times more nitrogen in the blood in the form of urea, the potassium concentration in the blood can be up to 2 times as high (bringing possible fatal consequences). If the ingestion of salts is restricted, the continuous depletion can lead to a rapid decrease in blood pressure with much higher intensity when compared to the normal individual.

The idea that normal excretion can lead to such differences in the internal medium is not intuitive. Therefore, it is absolutely necessary to use the concept of the stationary state. It is this state that defines how a solute will behave in a determined situation in terms of concentration, especially if it is a consequence of the relation between ingestion and excretion. For that, we can make a hydraulic analysis: consider the internal medium as a sink with a faucet that constantly fills up the tank and the drain that constantly drains the water as represented in Figure 2A. If the faucet is constantly open, representing the regular intake of absorbable solute, the total volume of the sink rises, but not indefinitely. The volume will rise until the filling rate equalizes with the

drain rate (that will rise because of the rise in hydraulic pressure by the water column created). By doing this, a new stationary state is formed, where both the filling and draining rate are equal and the total volume of the sink is higher (Figure 2B).

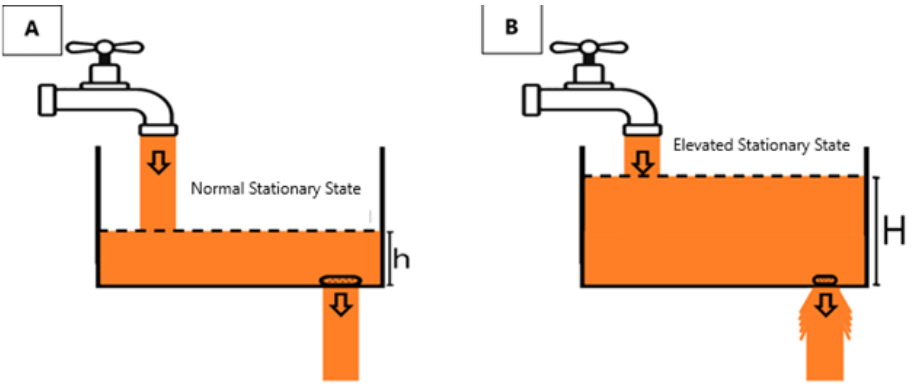


Figure 2: Representative image of the stationary state in a hydraulic model. (A) Normal Stationary State. (B) Elevated Stationary State. The filling and draining flows are equal in both situations.

Roughly, the same thing occurs in the patient with CKD. The mechanisms of internal medium control are compromised such that a new stationary state is formed, from that point forward, the excretion is normal, and the internal medium is pathologically higher.

How to study renal function?

In humans, the kidneys filter around 120 mL per minute, totalizing 170 liters of filtered every day. Therefore, the glomerular filtration rate is one of the most important ways of studying renal function. However, this process occurs in the inside of the kidneys, in the filtering structure of the nephron: the glomeruli. Hence, for many years there was no method of studying kidney function accurately. After all, mere urine concentration does not necessarily reveal the kidneys health as we examined previously. It was only at the end of the 1930s that Homer Smith introduced and popularized the clearance method, providing a non-invasive method of studying glomerular filtration rate, renal plasmatic flow and tubular transport [3].

The clearance method consists in comparing the excreted charge with the filtered charge of determined solute. Smith, brilliantly noticed that if the determined solute is freely filtered, not reabsorbed nor secreted in the tubules, then the filtered charge should be numerically the same as the excreted charge.

When using the unit of charge, it means the amount in terms of mass. In order to obtain this unit, the concentration of a determined substance is multiplied by the volume in which it is contained. Hence:

$$\boldsymbol{Concentration}\ \left[\frac{mass}{\cancel{Volume}}\right] * \cancel{\boldsymbol{Volume}} = \boldsymbol{Charge}\ [mass]$$

Dimensional analysis of the charge of determined substance

Therefore, to obtain the excreted charge of determined substance, simply multiply its urinary concentration to the urinary volume. The same for filtered charge: multiply the serum concentration of a determined substance (that is equal to the early filtered) to the filtration rate. Hence, clearance calculus follows:

$$Excreted\ Charge = Filtered\ Charge$$

$$Ux * V = Px * GFR$$

Where:
Ux = Urinary concentration of the solute X (mEq/L)
V = Urinary Flow (mL/min)
Px = Serum concentration of the solute X (mEq/L)
GFR = Glomerular Filtration Rate (mL/min)

It is important to notice that it is only possible to determine the glomerular filtration rate using the clearance method if the substance's excreted charge is equal to the filtered charge. Therefore, again, the substance must be freely filtered, not reabsorbed nor secreted by the tubules. The vast majority of the substances that come in contact with the nephron do not qualify for GFR estimation because they are either reabsorbed or secreted. However, a protein metabolite naturally produced in our muscle metabolism does: creatinine.

Therefore, it is possible to say that creatinine clearance informs the glomerular filtration rate. The urinary creatinine can be detected in the urine and in the serum by the colorimetric reaction of Jaffe, with alkaline picrate.

It is possible to determine the clearance of any substance, but it is desirable that the substance has some specific characteristic that could give useful information about the nephron's function.

In addition to creatinine, the clearance of para-aminohippuric acid (PAH) also informs an important measurement: renal plasma flow. The characteristic that makes the clearance of PAH useful is its high tubular secretion. Because of that, all of the PAH that reaches the kidney is filtered and the remaining PAH is totally secreted into the tubules from the peritubular capillaries. Because of this, the excretion charge is equal to the renal plasmatic flow.

The concept of clearance can still be very abstract, so follow the text along with Figure 3 below. The term clearance comes from the word "clear", that is, to free or take out. The clearance unit is in mL per minute and it means in a few words, the volume of serum that is cleared from determined substance over time. Let's analyze the creatinine and PAH clearance to visualize this process.

The illustration in Figure 3 represents the glomerulus associated with the afferent and efferent renal arteries connected with the peritubular capillaries. The Bowman's capsule is immediately below the glomerulus and is connected with a short representation of the tubules. The plasmatic flow is around 600 mL/min, represented by 5 squares of 120 mL. These squares of plasma contain creatinine (black dots inside the squares) dissolved. When the 600 mL arrive in the glomerulus, only 120 mL (1 square) is filtered (the filtration fraction is around 1/5 – 20%). Since creatinine is freely filtered, all the creatinine is carried along with the 120 mL of filtered to the inside of the capsule. Since creatinine is not reabsorbed nor secreted, the filtered charge is equal to the excreted charge at the end of the nephron, and the 120 mL of water is reabsorbed and returned to the plasma. Therefore, creatinine clearance is 120 mL per minute. Same as saying that 120 mL of plasma is "cleared" from creatinine per minute.

We can also analyze the PAH clearance in Figure 4. The same filtration process occurs, as PAH is freely filtered. However, all the remaining PAH in the peritubular capillaries are secreted into the tubules. Therefore, all the plasma cleared from PAH is equal to the amount of plasma that reached the nephron. Because of this, we can say that PAH clearance is the plasma flow rate (600 mL/min). Same as saying that 600 mL of plasma is "cleared" from PAH per minute.

The clearance method created by Homer Smith also offers the possibility of stu-

Figure 3: Schematic illustration of Creatinine and PAH Clearance. The logic behind clearance method for GFR and RPF calculations

dying the solute handling of the tubules through the fractional excretion calculations [4]. This measure represents the total excreted charge compared to the filtered charge of determined substance. Therefore, the value represents a percentage of excretion in relation to the filtered charge as demonstrated by the following equation:

$$FEx = \frac{Excreted\ Charge\ of\ x}{Filtered\ Charge\ of\ x}$$

$$FEx = \frac{Ux * V}{Px * GFR}$$

Where:
FEx = Fractional Excretion of Solute X (au)
Ux = Urinary Concentration of Solute X (mEq/L)
V = Urinary Flow (mL/min)
Px = Plasmatic Concentration of Solute X (mEq/L)
RFG = Glomerular Filtration Rate (mL/min)

Since (Ux learance:

$$FEx = \frac{Clearance\ x}{Creatinine\ Clearance}$$

In normal conditions, the fractional excretion of sodium (FENa) is less than 1%, as the nephron reabsorbs over 99% of this solute. Hence, if the FENa increases, it can be linked to tubular injury leading to lower absorptive capacity as occurs in the renal ischemia-reperfusion injury (Figure 5).

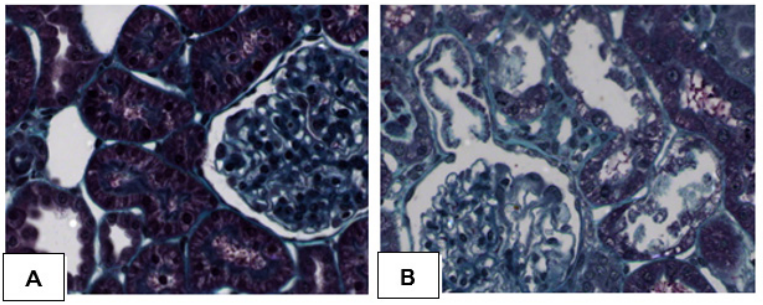


Figure 4: Segments of rat renal cortex dyed with Tricromium of Masson. (A) Healthy kidney. (B) Acute tubular necrosis (ATN) induced by 45 min of arterial ischemia through the occlusion of the renal artery with micro-clamps. The ATN is characterized by the presence of cells inside the tubules, lumen dilation, loss of the brush border and lowered cell adhesion to the basal membrane [5].

The FENa of Animal "A" was around 0.46% whereas the animal "B" was nearly 30%. This represents the loss of efficiency in epithelial transport across the tubule. This analysis was done in the acute phase of ischemia, before regeneration or scaring of the kidney.

The Kidney and Nutrition: peculiarities of potassium homeostasis

Potassium is the main intracellular cation. Approximately 98% of all the body's potassium is present inside our cells, making this compartment the main potassium storage in the body. Adequate concentrations of potassium are fundamental for several intracellular functions such as protein synthesis, nucleic acid polymerization, maintaining cell volume and pH, and also the transmembrane functions such as providing gradient and maintaining the resting membrane potential.

The cell membrane is highly permeable to potassium (around 20 times more than sodium). The potassium concentration in the extracellular fluid (ECF) is 3.5 - 4.5 mEq/L, whereas the intracellular fluid (ICF) is around 140 mEq/L. Potassium is 35 times more concentrated in the ICF when compared to ECF, and it is with purpose. This gradient provides an equilibrium across the membrane of around -91 mV estimated by Nernst Equation. This value is very close to the real resting membrane potential of the excitable cells (-70 to -80 mV). This demonstrates the important role that potassium has in the electrogenesis and maintenance in the resting membrane potential.

But after all, why is potassium, and not other cations, the most abundant in the intracellular space of the living organisms?

This is a very important question. After all, the heritage that the species obtain during the selective evolution occurs without a specific goal. Even if it seems otherwise, evolution does not have the intention to satisfy any particular purpose. Therefore, it is safe to assume that either intracellular potassium is advantageous in some way for life, or that it is a very strong heritage from our ancestors – or both.

Yuri V. Natochin's Theory

Today, the most recognized theory involving this question is based on geological and biochemical evidence that suggests that the origin of life began in an ocean quite different from the ocean that exists today. This so-called "primitive ocean" was much richer in potassium, and the early life that began to form, the replicators, preserved this potassium-rich environment as compartmentalization occurred. Hence, when the first compartmentalized replicators rose, they sequestered part of the ocean with themselves, and all processes necessary for life were adapted in this potassium-rich medium. It is speculated that the potassium concentration in the ICF relates to the primitive ocean,

where life began. In fact, protein synthesis is more efficient in a potassium-rich fluid and recent findings in deep rocks from distant geologic eras show that potassium was much more present in the ocean when compared to today 6,7.

How do the kidneys respond to potassium overload?

The adequate potassium intake for an adult is defined as 4,7 g (120 mmol) per day 8. In this dose, several beneficial effects have been shown such as the reduction in systemic arterial pressure, attenuation of renovascular hypertension and reduced renin release. In addition, potassium has an evident diuretic effect and is able to reduce the hypertensive effect of sodium ingestion. However, a recent study has shown that over 20 countries in Europe and Asia do not meet the adequate recommended intake for potassium 9,10. In this case, increasing potassium intake is desirable.

It is true that fruits, in general, have a high potassium content. Normally the first way of increasing potassium intake that comes to mind is by eating bananas. A banana weighing 100 grams has around 350 mg of potassium. However, other foods can be a much higher source of potassium in our diets such as soya (1700 mg of potassium per 100g), Brazilian nut (660 mg of potassium per 100g) and pork meat (425 mg of potassium per 100g).

Reaching the daily recommended intake of potassium is necessary. However, if an individual focuses his diet into potassium-rich foods, the 4.7 g/day is easily reached and overcome.

Elevated potassium concentrations in the blood (>5.0 mEq/L) characterize hyperkalemia. This condition is a serious electrolyte problem with possibly fatal consequences. It is important to say, however, that in individuals with healthy kidneys, it is virtually impossible to achieve severe hyperkalemia from natural food sources because of the efficiency of the body's internal medium control. There are very few reports of fatal hyperkalemia induced by oral ingestion, and all of them are related to potassium supplementation overdose in the form of salt or tablet 11.

In situations of intense and acute overload artificially produced in the laboratory, the fractional excretion of potassium can reach up to 180%. This means that on top of the 100% that was filtered, there was intense secretion of potassium into the tubules. In fact, the urinary potassium charge is normally a consequence of secretion. This shows the immense ability of kidneys to control the internal medium – which is why hyperkalemia from ingestion of natural foods is so rare. The main control site for potassium is the final portion of the nephron that contains the connective and collecting ducts. In this region, two cells present potassium secretion capacity: principal and intercalated cells (Figure 5). The principal cell expresses epithelial sodium channels (ENaC) and potassium channels (ROMK and Maxi-K) whereas the intercalated cell expresses potassium/hydrogen exchangers. Increasing the expression of these channels may increase potassium secretion into the tubule.

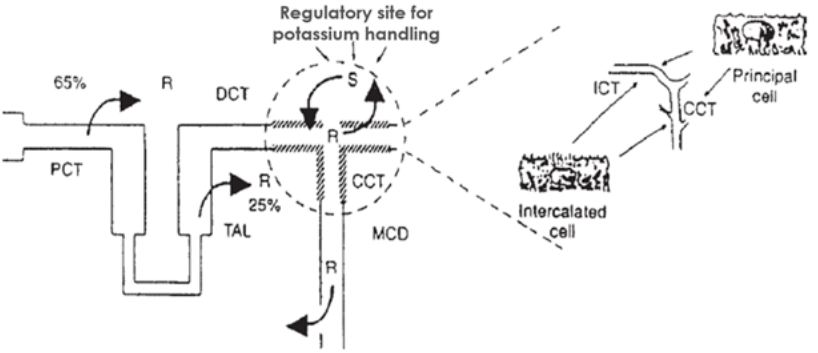
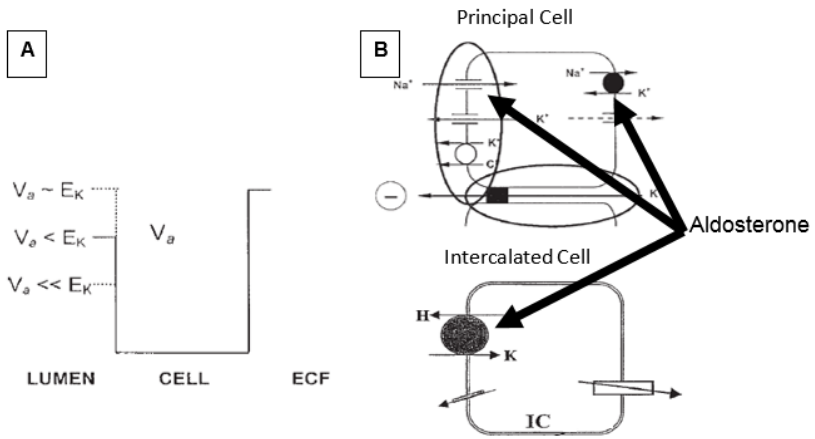


Figure 5: Representative illustration of the nephron and the main site for potassium handling. Modified from: (Giebish 2001)

Curiously, the ENaC is not directly influenced by the rise in extracellular potassium concentration. They are expressed when there is aldosterone present interacting with the intracellular mineralocorticoid receptor (MR). The rise in extracellular potassium induces aldosterone release by depolarizing the zona glomerulosa cells from the adrenal gland. Finally, the ENaC expression lead by potassium overload increases sodium reabsorption in the collecting duct, increasing the transepithelial asymmetry and favoring potassium secretion from the ROMK and Maxi-K channels as represented in Figure 6. Aldosterone also increases the Sodium/Potassium ATPase (pump), increasing transepithelial sodium transport.



Fig**ure 6:** (A) Representation of the tranepithelial potential between the ECF and tubule lumen across the cell. The rise in sodium reabsorption increases potassium peak into the lumen. (B) Main responsible cells for potassium handling in the distal nephron and the main aldosterone acting channels. Modified from : (Giebisch 2001).

Another important mechanism that the kidneys use in order to increase potassium clearance is through the intense urine flow. Potassium overload increases the urine output by mechanisms not totally elucidated. Additionally, potassium also induces the expression of ROMK and Maxi-K Channels [13]. These combined alterations potentialize the potassium secretion in a situation of potassium overload.

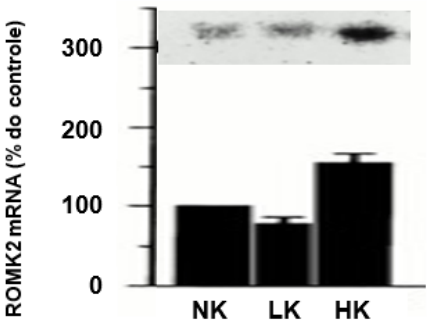


Figure 7: Regulation of mRNA for ROMK2 by the ingestion of potassium. Northern for ROMK2 in the intact medula of rats subjected to normal (NK) low (LK) and high (HK) Potassium Intake Modified from: (Wald et al. 1998).

References

1. Chevalier, R. L. Evolutionary Nephrology. Kidney Int. Rep. 2, 302–317 (2017).
2. Pitts, R. F. Physiology of the kidney and body fluids : an introductory text. (Year Book Medical Publishers, 1963).
3. Giebisch, G. Homer W. Smith's contribution to renal physiology. J. Nephrol. 17, 159–165 (2004).
4. Smith, H. W. The Kidney: Structure and Function in Health and Disease. (Oxford University Press, 1951).
5. Jablonski, P. et al. AN EXPERIMENTAL MODEL FOR ASSESSMENT OF RENAL RECOVERY FROM WARM ISCHEMIA. Transplantation 35, 198 (1983).
6. Dubina, M. V. et al. Potassium Ions are More Effective than Sodium Ions in Salt Induced Peptide Formation. Orig. Life Evol. Biosph. 43, 109–117 (2013).
7. Mulkidjanian, A. Y. & Galperin, M. Y. On the origin of life in the zinc world. 2. Validation of the hypothesis on the photosynthesizing zinc sulfide edifices as cradles of life on Earth. Biol. Direct 4, 27 (2009).
8. Campbell, S. Dietary Reference Intakes: Water, Potassium, Sodium, Chloride, and Sulfate. Clin. Nutr. Insight 30, 1 (2004).
9. Mierlo, L. A. J. van, Greyling, A., Zock, P. L., Kok, F. J. & Geleijnse, J. M. Suboptimal Potassium Intake and Potential Impact on Population Blood Pressure. Arch. Intern. Med. 170, 1501–1502 (2010).

10. Yi, S. S. et al. Highlighting the ratio of sodium to potassium in population-level dietary assessments: cross-sectional data from New York City, USA. Public Health Nutr. 17, 2484–2488 (2014).

11. Wetli, C. V. & Davis, J. H. Fatal Hyperkalemia From Accidental Overdose of Potassium Chloride. JAMA 240, 1339–1339 (1978).

12. Giebisch, G. Renal potassium channels: Function, regulation, and structure. Kidney Int. 60, 436–445 (2001).

13. Wald, H., Garty, H., Palmer, L. G. & Popovtzer, M. M. Differential regulation of ROMK expression in kidney cortex and medulla by aldosterone and potassium. Am. J. Physiol.-Ren. Physiol. 275, F239–F245 (1998).

12 - Hypertension, Diet and Nerve Function

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Hypertension

Hypertension is a chronic systemic disease defined by persistent elevated arterial pressure. Four scaled categories of blood pressure (BP) were described for adults as follows: a) normal (systolic blood pressure (SBP) < 120 mmHg and diastolic blood pressure (DBP) < 80 mmHg), b) elevated (SBP between 120-129 mmHg and DBP < 80 mmHg), c) stage 1 hypertension (SBP between 130-139 mmHg and DBP between 80-89 mmHg) and d) stage 2 hypertension (SBP > 140 mmHg and DBP >90 mmHg) (1).

According to the World Health Organization (WHO), high blood pressure causes 7.5 million deaths, about 12.8% of the total of all deaths per year (2). In that study, the overall prevalence of raised blood pressure in adults aged 25 and over was described as around 40% in 2008. Across the WHO regions, the prevalence of raised blood pressure was highest in Africa, where it was 46% for both sexes combined (2). They showed that the lowest prevalence of raised blood pressure was in the Americas, being 35% for both sexes. Men in this region had a higher prevalence than women (39% for men and 32% for women) (2). In all WHO regions, men have a slightly higher prevalence of raised blood pressure than women. This difference was only statistically significant in the Americas and Europe (2).

Hypertension is currently considered one of the most important risks for cardiovascular disease: first, because of its high prevalence. Second, because it has a relationship with fatal and non-fatal cardiovascular events, being this relationship continuous, positive and independent of other factors. Although considered as a silent

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disease, hypertension causes the heart to exert a greater effort than normal and ends up compromising the functioning of not only the heart itself but also other organs. High blood pressure is a public health problem that affects men and women worldwide. In Brazil alone, one in five individuals suffers from the disease, reaching a number of more than 30 million people (3).

Hypertension and Diet

It is known that approximately 50% of hypertensive patients are salt sensitive and its exaggerated use is associated with an increased risk of hypertension. Salt sensitivity of BP has been associated with an increased risk of hypertension, cardiovascular disease, and premature death (4-6). People who consume a diet with less salt have a lower prevalence of hypertension and blood pressure does not rise with age. Salt restriction at 6 g / day produces a mean drop in SBP of 2 to 8mmHg (7-10). Increased sodium intake has been observed in populations with low socioeconomic level. Thus, the association between hypertension and low socioeconomic level must take the diet into consideration.

On the other hand, potassium intake seems to be inversely related to high blood pressure (11,12). A higher level of potassium might blunt the effect of sodium on BP (1,13), with the lower sodium-potassium ratio being associated with a lower level of BP compared to corresponding levels of sodium or potassium alone (1,14). Moreover, epidemiological studies suggest that a lower sodium-potassium ratio may result in a reduced risk of cardiovascular disorders as compared with the pattern for corresponding levels of either cation on its own (1,15).

The consumption of alcohol can have either beneficial or detrimental effects on the heart function and cardiovascular system, depending on the amount consumed. Low-to-moderate amounts of ethanol intake are associated with improvements in cardiac function and vascular health (16). But, when ethanol is chronically consumed in large amounts, it may act as a toxin to the heart and vasculature. Estimates of the contribution of alcohol consumption to population incidence and prevalence of hypertension vary according to the level of intake. Alcohol consumption has a two-phase effect on BP. Small quantities lower BP levels, probably due to the vasodilator effect. However, the chronic and continuous use of ethanol causes levels of pressure to increase and also decreases the effectiveness of antihypertensive drugs (17,18). The potential mechanisms involved in increasing BP associated with excessive alcohol consumption are: stimulation of the sympathetic nervous system, stimulation of endothelin, stimulation of the renin-angiotensin-aldosterone system, insulin resistance and cortisol stimulation; inhibition of vasodilatory substances, depletion of calcium and magnesium, increased intracellular calcium in vascular smooth muscle and increased acetaldehyde (17,18).

Therefore, it is recommended to limit the consumption of alcoholic beverages to 20 to 30g of ethanol per day for men and 10 to 20g for women (18).

There is a known established relation between body weight and high BP as well as a direct relationship between overweight/obesity and hypertension (19). Other studies have identified (19,20) a direct relationship between body mass index and BP being continuous and almost linear, with no evidence of a specific threshold (21,22). There is an estimation (1) that obesity may be responsible for about 40% of hypertension or even higher (78% in men and 65% in women) (19). It also has been demonstrated for a young age that being obese continuously or acquiring obesity was associated with a relative risk of 2.7 for developing hypertension (23). Becoming normal weight reduced the risk of developing hypertension to a level similar to those who had never been obese.

Interestingly, excess weight gain is associated with marked sodium retention and expansion of extracellular fluid volume. Obese subjects usually have an increase in plasma renin activity, plasma angiotensinogen, angiotensin-converting enzyme activity, and plasma angiotensin II levels. Angiotensin II plays a significant role in stimulating sodium reabsorption, impairing renal-pressure natriuresis, thus causing hypertension in obesity (21).

Hypertension and the Nervous System

It is widely accepted that hypertension accounts for approximately 40% of stroke deaths and about 25% of coronary heart disease deaths. Hypertension can compromise the cerebral microcirculation in various ways (24), ranging from functional changes impairing vasomotor capacity, modifying blood flow transiently, to severe physical damage resulting from conditions like thrombosis (25). The multiple negative effects on the brain vasculature due to hypertension have devastating consequences for auto--regulation of cerebral blood flow. Local disturbances in this flow lead to brain lesions affecting important white-matter tracts causing complete and/or incomplete infarcts, microbleeds and white-matter hyperintensities (26).

Hypertension-associated cerebral microbleeds are typically located in basal ganglia, thalamus, brain stem, and cerebellum (27). Diffuse white-matter damage or leukoaraiosis is also attributable to high systolic blood pressure and indicates a reduction in white-matter density (28). White-matter lesions evolve from a combination of demyelination, lacunar infarcts, and axonal loss and are not only associated with ischemic and hemorrhagic stroke but also with dementia, especially with deficits in motor speed and executive functions (29).

The peripheral nervous system is also injured in hypertension but, despite a large amount of literature on how the central nervous system is affected negatively by high

blood pressure, the involvement of the peripheral nervous system has been much less explored. It was no early than two decades ago that the first experimental studies on nerve impairment in hypertension were published (24,30). These authors described structural changes in the interfascicular arteries and to a lesser extent, intrafascicular arteries were also affected on a similar way of the brain arteries (24). Major findings were luminal narrowing and thickening of the wall, suggesting that hypertension may represent a risk factor for reduced blood flow and ischemia of peripheral nerves (24). Myelinated fibers alterations were also described such as reduction of the myelin area in larger fibers (thinning of the large myelinated fibers) and a decrease of large myelinated fibers and an increase of smaller size nerve fibers (30).

Afterward, several experimental studies were published suggesting that hypertension can indeed cause neuropathy. For all these animal studies, the spontaneously hypertensive rats (SHR) were used for being the closest model of human essential hypertension (31) and the most extensively used for investigation of hypertensive target-organ damage and its treatment (32).

Autonomic, sensory and motor nerves were morphologically investigated in SHR and a mild neuropathy was observed in all nerves. The aortic depressor nerve (a sensory nerve with important autonomic functions for being the afferent arm of the baroreflex) shows axonal atrophy for the myelinated fibers (33) and a reduced number of the unmyelinated fibers (34). It also has a disrupted relation between the myelin sheath thickness and the axonal size (35) suggesting impairment of the conduction velocity of the myelinated fibers in SHR. For the vagus nerve (36) (an autonomic nerve) and the sural nerve (37) (a sensory nerve), myelinated fibers diameter and G ratio (relation between the axonal diameter and total fiber diameter – degree of myelination) were reduced in the large fibers. The small myelinated fibers appeared to be reduced in number. Moreover, endoneural vessels showed wall thickening and reduced lumen as described earlier (24). Interestingly, no gender differences were reported for the morphological alterations despite that male SHR have significantly higher arterial pressure compared to females (36,37). When essentially motor nerves were investigated (phrenic and recurrent laryngeal nerves), it was possible to distinctly describe alterations for small and large myelinated fibers, since the bimodal fiber distribution is a morphometric characteristic of these nerves (38-42). For the phrenic nerve, myelinated fibers histograms clearly demonstrated a reduction in the frequency of smaller fibers in SHR (41). On the other hand, SHR myelinated fibers were generally larger on average, especially in males, as was the average area of the myelin sheath (41). Average G ratio of the myelinated fibers was generally smaller on SHR (41). Likewise, the G ratio histograms for SHR showed a higher frequency of fibers with G ratios smaller than 0.4 (41). In the recurrent laryngeal nerves, there was a reduction of fiber size, more evident

on the axon, associated with a reduction of the small myelinated fibers percentage in animals with high blood pressure (42). Also, 20-week-old animals showed a significant reduction of the blood vessel percentage of occupancy compared to younger ages (42). Thus, it is important to mention that there is enough experimental evidence of neuropathy due to hypertension and that this neuropathy has both components: axonal and demyelinating.

Hypertension and Nerve Function

Despite the morphological descriptions of a mild neuropathy in several functional distinct peripheral nerves (sensory, motor and autonomic) as described above, very little is known about how these morphological alterations affect nerve function in hypertension. Motor nerve conduction velocity was found reduced in patients with arterial blood pressure above 120 mmHg (43) and the authors suggested a subclinical peripheral neuropathy due to hypertension. Interestingly, the reduction of nerve conduction velocity paralleled elevation of blood pressure (44), which is in accordance to our morphological results for the phrenic nerves (males with higher blood pressure had more severe nerve alterations than females with small blood pressure vales) (41).

More recently, experimental studies have further investigated nerve conduction velocity in SHR. Gregory et al. (2012) (45) described that female SHR rats showed progressively developing nerve conduction velocity deficits with aging and attenuated axonal radial growth in the sciatic and sural nerves, without myelinated fiber loss or any change in fiber density. Another study used male SHR (32) and showed also a slowed sensory and motor conduction velocity in hind limb nerves. In our recent study (unpublished data) we investigated motor and sensory conduction velocity of nerves from male and female SHR, comparing different ages in both genders. Sensory and motor conduction velocity increased with age from 5 up to 20 weeks old animals in both genders. Motor conduction velocity was not different in males and females and reached a plateau from 20 to 40 weeks of age. On the other hand, sensory conduction velocity was slower in females but continued to increase from 20 to 40 weeks of age while for males it decreased in this period. This is in line with our previous results (37,41) of a small fiber neuropathy (sensory fibers being more affected) that follows the increase in blood pressure (higher in males).

Thus, there is clinical and experimental evidence of the existence of a so-called "hypertensive neuropathy", independently of other associated diseases, that clinicians, cardiologist, and neurologists must be aware of and should investigate in hypertensive patients.

References

1. Whelton PK, Carey RM, Aronow WS, Casey Jr. DE, Collins KJ, Himmelfarb CD, DePalma SM, Gidding S, Jamerson KA, Jones DW, MacLaughlin EJ, Muntner P, Ovbiagele B, Smith Jr. SC, Spencer CC, Stafford RS, Taler SJ, Thomas RJ, Williams Sr. KA, Williamson JD, Wright Jr.JT, 2017, ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. JACC 71, DOI: 10.1016/j.jacc.2017.11.006.

2. www.who.int/gho/ncd/risk_factors/blood_pressure_prevalence_text/en/

3. http://www.socesp.org.br/blogdocoracao/2018/04/23/hipertensao-atinge-mais-de-30-milhoes-de-pessoas-no-brasil/

4. Morimoto A, Uzu T, Fujii T, Nishimura M, Kuroda S, Nakamura S, Inenaga T, Kimura G. Sodium sensitivity and cardiovascular events in patients with essential hypertension.Lancet. 1997; 350:173.

5. Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. Hypertension. 2001; 37(2 pt 2):429–432.

6. Barba G, Galletti F, Cappuccio FP, Siani A, Venezia A, Versiero M, Della Valle E, Sorrentino P, Tarantino G, Farinaro E, Strazzullo P. Incidence of hypertension in individuals with different blood pressure salt-sensitivity: results of a 15-year follow-up study.J Hypertens. 2007; 25:1465–1471.

7. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005; 365:217-223.

8. Elliott P. Observational studies of salt and blood pressure. Hypertension. 1991; 17(suppl I):3–8.

9. National Institutes of Health. National Heart Lung and Blood Institute. The DASH Diet. NIH 1999; publication 99-4062.

10. Frost CD, Law MR, Wald NJ. By how much does dietary salt reduction lower blood pressure? II. Analysis of observational data within populations. BMJ. 1991; 302:815–818.

11. Mente A, O'Donnell MJ, Rangarajan S, McQueen MJ, Poirier P, Wielgosz A, Morrison H, Li W, Wang X, Di C, Mony P, Devanath A, Rosengren A, Oguz A, Zatonska K, Yusufali AH, Lopez-Jaramillo P, Avezum A, Ismail N, Lanas F, Puoane T, Diaz R, Kelishadi R, Iqbal R, Yusuf R, Chifamba J, Khatib R, Teo K, Yusuf S; PURE Investigators. Association of Urinary Sodium and Potassium Excretion with Blood Pressure. N Engl J Med. 2014; 371:601-611. DOI: 10.1056/NEJMoa1311989.

12. Strazzullo P, D'Elia L, Kandala NB, Cappuccio FP. Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. BMJ. 2009; 339:b4567.

13. Rodrigues SL, Baldo MP, Machado RC, Forechi L, Molina MCB, Mill JG. High potassium intake blunts the effect of elevated sodium intake on blood pressure levels. JASH. 2014; 8:232-238

14. Sanada H, Jones JE, Jose PA. Genetics of salt-sensitive hypertension. Curr Hypertens Rep. 2011; 13(1):55-66. doi: 10.1007/s11906-010-0167-6.

15. Cook NR, Obarzanek E, Cutler JA, Buring JE, Rexrode KM, Kumanyika SK, Appel LJ, Whelton

PK. Joint Effects of Sodium and Potassium Intake on Subsequent Cardiovascular DiseaseThe Trials of Hypertension Prevention Follow-up Study. Arch Intern Med. 2009; 169(1):32-40. doi:10.1001/archinternmed.2008.523.

16. Gardner JD, Mouton AJ. Alcohol Effects on Cardiac Function. Compr Physiol. 2015; 5:791-802.

17. Cushman WC, Izzo JL, Black HR. Alcohol use and blood pressure. Hypertension Primer, Council on High Blood Pressure Research, American Heart Association 1999; 263-265.2nd edition.

18. Xin X, He J, Frontini MG, Ogden LG, Motsamai OI, Whelton PK. Effects of Alcohol Reduction on Blood Pressure: A Meta-Analysis of Randomized Controlled Trials. Hypertension. 2001; 38:1112-17.

19. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation. 1983; 67:968–977.

20. Huang Z, Willett WC, Manson JE, Rosner B, Stampfer MJ, Speizer FE, Colditz GA. Body weight, weight change, and risk for hypertension in women. Ann Intern Med. 1998; 128:81–88.

21. Hall JE. The kidney, hypertension, and obesity. Hypertension. 2003; 41:625–633.

22. Jones DW, Kim JS, Andrew ME, Kim SJ, Hong YP. Body mass index and blood pressure in Korean men and women: the Korean National Blood Pressure Survey. J Hypertens 1994; 12:1433–1437.

23. Juonala M, Magnussen CG, Berenson GS Venn A, Burns TL, Sabin MA, Srinivasan SR, Daniels SR, Davis PH, Chen W, Sun C, Cheung M, Viikari JS, Dwyer T, Raitakari OT. Childhood adiposity, adult adiposity, and cardiovascular risk factors. N Engl J Med. 2011; 365:1876–1885.

24. .Sabbatini M, Vega JA, Amenta F. Peripheral nerve vascular changes in spontaneously hypertensive rats. Neurosci Lett. 1996; 217:85-88.

25. Feihl F, Liaudet L, Waeber B, Levy BI: Hypertension: a disease of the microcirculation? Hypertension. 2006; 48:1012–1017.

26. Meissner A. Hypertension and the brain: A risk factor for more than heart disease. Cerebrovasc Dis. 2016; 42:255-262. doi: 10.1159/000446082

27. Greenberg SM, Vernooij MW, Cordonnier C, Viswanathan A, Al-Shahi Salman R, Warach S, Launer LJ, Van Buchem MA, Breteler MM; Microbleed Study Group. Cerebral microbleeds: a guide to detection and interpretation. Lancet Neurol. 2009; 8: 65–174.

28. Verhaaren BF, Vernooij MW, de Boer R, Hofman A, Niessen WJ, van der Lugt A, Ikram MA. High blood pressure and cerebral white matter lesion progression in the general population. Hypertension. 2013; 61:1354–1359.

29. Wiseman SJ, Doubal FN, Chappell FM, Valdes-Hernandez MC, Wang X, Rumley A, Lowe GD, Dennis MS, Wardlaw JM. Plasma biomarkers of inflammation, endothelial function and hemostasis in cerebral small vessel disease. Cerebrovasc Dis. 2015; 40:157–164.

30. Tomassoni D, Traini E, Vitaioli L, Amenta F. Morphological and conduction changes in the sciatic nerve of spontaneously hypertensive rats. Neurosci Lett. 2004;362:131-135.

31. Trippodo NC, Frohlich ED. Similarities of genetic (spontaneous) hypertension. Man and rat. Circ Res. 1981; 48:309-319.

32. Nukada H, Baba M, Ogasawara S, McMorran D, Yagihashi S. Neuropathy in the spontaneously hypertensive rat: An electrophysiological and histological study. Muscle Nerve. 2016; 54:756-762. doi: 10.1002/mus.25098.

33. Fazan VPS, Fazan Júnior RF, Salgado HC, Barreira AA. Morphology of aortic depressor nerve myelinated fibers in normotensive Wistar-Kyoto and spontaneously hypertensive rats. J Auton Nerv Syst. 1999; 77:133-139.

34. Fazan VPS, Salgado HC, Barreira AA. Aortic depressor nerve unmyelinated fibers in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol. 2001; 280:H1560-1564.

35. Fazan VPS, Salgado HC, dos Reis GC, Barreira AA. Relation between myelin area and axon diameter in the aortic depressor nerve of spontaneously hypertensive rats. J Neurosci Methods. 2005; 148:130-136.

36. Licursi de Alcântara AC, Salgado HC, Sassoli Fazan VP. Morphology and morphometry of the vagus nerve in male and female spontaneously hypertensive rats. Brain Res. 2008; 1197:170-180. doi: 10.1016/j.brainres.2007.12.045.

37. Sanada LS, da Rocha Kalil AL, Tavares MR, Neubern MC, Salgado HC, Fazan VPS. Sural nerve involvement in experimental hypertension: morphology and morphometry in male and female normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). BMC Neurosci. 2012; 13:24. doi: 10.1186/1471-2202-13-24.

38. Gottschall J. The diaphragm of the rat and its innervation. Muscle fiber composition; perikarya and axons of efferent and afferent neurons. Anat Embryol. 1981; 161:405-417

39. Rodrigues Filho OA, Fazan VP. Streptozotocin induced diabetes as a model of phrenic nerve neuropathy in rats. J Neurosci Methods 2006; 151:131-138.

40. Lima NT, Fazan VP, Colafêmina JF, Barreira AA. Recurrent laryngeal nerve post-natal development in rats. J Neurosci Methods. 2007; 165:18-24.

41. Rodrigues AR, Ferreira RS, Salgado HC, Fazan VPS. Morphometric analysis of the phrenic nerve in male and female Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). Braz J Med Biol Res. 2011; 44:583-591.

42. Da Silva GA, Mendes VA, Genari AB, Castania JA, Salgado HC, Fazan VPS. Recurrent laryngeal nerve alterations in developing spontaneously hypertensive rats. Laryngoscope. 2016; 126:E40-7. doi: 10.1002/lary.25426.

43. Chaco J, Viskoper RJ. Hypertension and subclinical peripheral neuropathy. Angiology. 1971; 22:521-522.

44. Gregory JA, Jolivalt CG, Goor J, Mizisin AP, Calcutt NA. Hypertension-induced peripheral neuropathy and the combined effects of hypertension and diabetes on nerve structure and function in rats. Acta Neuropathologica, 2012; 124(4), 561–573.doi:10.1007/s00401-012-1012-6

13 - Neuroanatomia das emoções: do lobo límbico às redes neurais

Neuroanatomy of emotions: from the limbic lobe to brain networks

Rodrigo Coelho Marques[1], Larissa Vieira[2], Amaury Cantilino

Abstract

The scientific literature often uses terms such as "limbic structures" or "limbic system" to describe regions of the nervous system that participate in emotional processes. Nonetheless, these concepts have been thoroughly revised over the last 150 years and there is no clear consensual definition for which regions are actually "limbic" or otherwise. This chapter will summarize the evolution of the concept of a limbic system and discuss the main neural structures that are credited as taking part in the elaboration and perception of emotional states.

Keywords: Limbic system, emotions, large scale brain networks.

Introdução

Atualmente é comum encontrarmos na literatura termos como "sistema límbico" ou "estruturas límbicas", os quais se referem a regiões do sistema nervoso que possuem alguma participação na produção dos estados afetivos. Essa terminologia, apesar de bastante disseminada, não é consistente em sua definição e apresenta fragilidades conceituais importantes. Contudo, a noção de sistema límbico tem sido um paradigma importante para guiar a pesquisa neurocientífica, possuindo valor heurístico relevante. Este capítulo revisará a evolução teórica que ampara a definição desse sistema, assim como os principais achados relativos às estruturas individuais que o compõem.

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A história do sistema límbico

O primeiro uso registrado do termo límbico para designação anatômico de regiões cerebrais data de 1664, na obra de Thomas Willis. O médico inglês utiliza a nomenclatura cerebri limbus para descrever a região cortical que circunda o tronco cerebral. O termo limbo etimologicamente deriva do latim e significa orla, borda ou rebordo. Mais de dois séculos depois de Willis, em 1878, o consagrado neurologista francês Paul Broca realiza uma extensa investigação comparativa entre cérebros humanos e de outros mamíferos. Neste estudo, estabelece a distinção principal entre os dois como sendo o volume do lobo frontal e da região que chamou de grande lobe limbique – o grande lobo límbico. Na visão de Broca, essa região compreenderia principalmente as estruturas mediais dos hemisférios, que perderam sua dominância funcional para o lobo frontal, esse muito mais volumoso no humano do que em outras espécies. Provavelmente desconhecendo o uso do termo feito por Willis, Broca introduziria a nomenclatura que seria revisitada no século seguinte, dando origem à expressão "sistema límbico" (1,2).

Ainda no final do século XIX, Brown & Schäfer realizaram estudos de ablação experimental, observando que a lobectomia temporal bilateral modificava o comportamento de animais selvagens ferozes, tornando-os dóceis. Na década de 1930, Klüver & Bucy replicaram achados semelhantes em seus experimentos com macacos Rhesus, detalhando melhor as mudanças de comportamento e quais as regiões implicadas. A interação entre o córtex cerebral e estruturas subcortais na produção de estados afetivos foi aventada por Cannon (1927), postulando que, enquanto o diencéfalo enviaria estímulos para os núcleos da base motivando a efetivação de atos motores relacionados às reações emocionais, também transmitiria estímulos ao córtex, permitindo apreciação regulação dessas respostas(2,3).

Uma contribuição seminal para o estudo da neuroanatomia das emoções, construída sobre as regiões incluídas por Broca no "lobo límbico", viria através do circuito descrito por James Papez em 1937, o qual a partir de então levaria o seu nome. Papez propôs que as interconexões entre o hipotálamo, núcleo anterior do tálamo, hipocampo e o giro do cíngulo seriam o embasamento neural para a elaboração dos fenômenos afetivos. Uma parte desse circuito, envolvendo o hipotálamo, hipocampo e tálamo anterior, seriam responsáveis pela produção dos componentes autonômicos e neuroendócrinos. Outra alça de projeção provinda desse circuito alcançaria o giro do cíngulo via corpos mamilares, portanto acessando o córtex cerebral e resultando nos aspectos envolvendo as percepções e respostas conscientes derivadas dos afetos(3–5).

Posteriormente, autores como Yakovlev (1948) e MacLean (1952) passariam a aplicar o raciocínio da biologia evolucionista ao estudo dos neurocircuitos das emoções. Assim, começam a surgir detalhamentos de sub-regiões classificadas a partir de sua idade filogenética e características citoarquitetônicas. Para MacLean, o córtex do lobo

límbico seria composto principalmente de arquicórtex (giro denteado e hipocampo), paleocórtex (área olfativa), assim como os córtices de transição que circundam essas regiões. Além disso regiões neocorticais e subcorticais possuiriam forte conectividade com o lobo límbico e formariam um sistema anatomicamente abrangente e funcionalmente integrado que denominou "sistema límbico". Este incluiria também a área piriforme, toda a formação hipocampal, os giros paraesplenial, cigulado e subcaloso, amígdalas, ínsula anterior, polo anterior do córtex temporal, núcleos septais, hipotálamo, epitálamo e alguns dos núcleos da base. Essas estruturas estariam anatomicamente organizadas em três agrupamentos evolutivamente coerentes, formando sub-sistemas com predomínio de funções instintivas típicas de diferentes espécies de vertebrados, denominadas por MacLean como reptiliano (funções de agressividade, dominância e territorialidade baseadas em regiões mesencefálicas e prosencefálicas basais), paleomamífero (regiões subcorticais, dienfefálicas e telencefálicas, correspondendo às regiões límbicas originais, envolvidas na produção das emoções, socialização e motivação) e neomamífero (composto sobretudo por neocórtex e relacionada com as funções cognitivas, linguagem e capacidade de racionalidade) (2,5,6).

Coube ainda a Nauta (1971) ampliar a visão anatômica do sistema límbico, demonstrando que sua abrangência engloba mais regiões do córtex pré-frontal (especialmente medial), assim como estruturas mesencefálicas onde residem núcleos monoaminérgicos. A expansão sugerida por Nauta e explorada por autores como Mesulam e Nieuwenhuys situa as estruturas límbicas em um sistema bastante amplo, estendendo-se caudalmente para incluir a substância cinzenta periaquedutal, núcleos da ponte, núcleos parabraquiais e os núcleos dorsais do nervo vago. A conexão dessas regiões com áreas motoras, incluindo o cerebelo, formaria uma extensa rede modulatória para mediar o input emocional com o output efetor, permitindo um grau de controle voluntário sobre os desfechos comportamentais (3,7,8).

Nesse modelo, chamado por alguns de "sistema límbico expandido", dois grandes compartimentos se destacam. Um compartimento ventral, que revolve em torno da atividade amigdaliana e órbitofrontal, integrando ainda as vias de gratificação e comportamento motivado, e um compartimento dorsal, centrado na atividade do hipocampo e córtex cingulado posterior, composto ainda pelas demais estruturas do circuito de Papez pelo córtex pré-frontal dorsolateral. O compartimento dorsal estaria implicado na formação de memórias declarativas e na capacidade auto-reflexiva da consciência. Esse esquema prevê que o compartimento ventral seria a origem de projeções ascendentes para o córtex cerebral, como uma forma de input de processamento emocional mais primário e inconsciente. Ao se integrar com o compartimento dorsal, esse input passaria a surgir como elementos conscientes suscetíveis à percepção do indivíduo. Em direção contrária, circuitos córtico-subcorticais originados no compartimento dorsal

atuariam como uma alça modulatória sobre a atividade do compartimento ventral. Um dos principais pontos de interação entre os dois compartimentos seria o córtex orbitofrontal e o córtex cingulado anterior (4).

Os modelos de sistema límbico são fundamentados sobretudo pelo conhecimento de neuroanatomia adquirido ao longo do século XX. Contudo, com o advento de técnicas de neuroimagem funcional, uma leitura complementar à abordagem estrutural passa a ser relevante, sobretudo a partir da década de 1990. O estudo da atividade de regiões cerebrais a partir dessas técnicas revelou a existência das chamadas redes neurais de larga escala (large scale brain networks), que essa designação por serem sistemas que abrangem várias regiões em pontos distantes do encéfalo. As regiões componentes de uma rede atuam de modo coordenado e possuem conectividade preferencial entre si. Em relação à neuroanatomia das emoções três redes seriam mais relevantes, de certa forma se superpondo ao conceito anatômico de sistema límbico expandido: a rede de saliência, a rede modo-padrão e a rede executiva central (4).

A rede de saliência é composta por estruturas que participam da regulação emocional e do comportamento motivado. Uma de suas regiões chave é o giro do cíngulo anterior, área que funciona como uma âncora dos dois componentes da rede de saliência: o primeiro envolvendo córtex órbitofrontal, ínsula anterior e amígdalas, e um segundo baseado nos circuitos de recompensa e motivação dopaminérgicos fronto-estriatais. Essa rede funcional se assemelha ao compartimento ventral do sistema límbico expandido (4,9,10).

A rede modo-padrão (default-mode network) é um sistema caracterizado por reduzir a atividade de seus componentes quando a atenção é dirigida para o meio externo durante a execução de tarefas. Inclui principalmente regiões mediais, como o córtex pré-frontal medial, giro do cíngulo posterior, precúneo e lobo temporal medial. As estruturas da rede modo-padrão demonstram atividade durante tarefas de simulação mental e de reconhecimento de informações direcionadas para o Self, como considerar pontos de vista diferentes, imaginar cenários, atribuir qualidades a si mesmo ou reconhecer comentários direcionados para si. É conceitualmente semelhante ao compartimento dorsal do sistema límbico expandido (4,9).

Já a rede executiva central consiste em circuitos frontoparietais (córtex pré-frontal dorsolateral - córtex parietal lateral). Essa rede apresenta atividade durante a realização de tarefas cognitivas, agindo como uma unidade de controle. A conectividade entre o córtex pré-frontal dorsolateral, cíngulo anterior e córtex parietal é crucial para a manutenção e manipulação de informações ativas, permitindo resolução de problemas e tomada de decisão. Sua atividade costuma recrutar vários componentes das funções executivas, sobretudo a memória de trabalho. Em parte, possui algumas das atribuições do compartimento dorsal do sistema límbico expandido (9).

Essas redes atuam dinamicamente, de forma coordenada, influenciando-se mutuamente. As redes de modo padrão e executiva central parecem atuar de forma antagônica, a partir da mediação da rede de saliência. Assim, a atenção e o comportamento se voltariam ao espaço subjetivo interno ou externo, ligando ou desligando as redes modo-padrão ou executiva central, a depender da relevância de certos estímulos para cada indivíduo, sob orquestração da rede de saliência. A integração harmônica da atividade entre estas redes é fundamental para um funcionamento psíquico adaptativo (9).

Principais estruturas anatômicas relacionadas às emoções

Restam ainda grandes lacunas para uma compreensão mais precisa acerca do funcionamento do sistema límbico. A despeito das enormes controvérsias sobre sua definição e sobre as estruturas que o compõem, existe certa concordância na literatura a respeito de algumas estruturas básicas. O conhecimento destas estruturas e da forma como elas se conectam e interagem entre si em redes neurais de larga escala é capaz de nos dar uma boa ideia do seu funcionamento e das evidências já consolidadas a respeito do tema (11).

As estruturas límbicas circundam as superfícies basilares e mediais dos hemisférios cerebrais que margeiam os ventrículos laterais, compondo um arco em forma de "C" (12). Elas serão capazes de ligar emoções e impulsos a conteúdos mentais, coordenando atividades autonômicas, hormonais e imunes e o processamento cognitivo para este fim. Faremos uma breve revisão das principais estruturas funcionalmente envolvidas com o sistema límbico (sistema nervoso autônomo, hipotálamo, amígdala, nucleus accumbens, sistema septo-hipocampal, ínsula, giro do cíngulo, córtex pré-frontal) e das principais redes neurais de larga escala a ele relacionadas (rede de saliência, rede modo-padrão e a rede executiva central) (7,13).

Sistema nervoso autônomo

A divisão autonômica do sistema nervoso periférico tem como principal função o controle homeostático do corpo através de sua influência sobre a musculatura lisa presente nas vísceras, nos vasos sanguíneos, nas glândulas e sobre o músculo cardíaco. Através de receptores especializados, as vísceras são capazes de captar e enviar informações homeostáticas, via neurônios aferentes, a regiões límbicas, pré-frontais e hipotalâmicas, que mediarão as respostas autonômicas a serem conduzidas aos órgãos efetores.

O sistema nervoso autônomo é dividido em um componente simpático e outro parassimpático. Em linhas gerais, o simpático prepara o corpo para situações de perigo e estresse (respostas de luta, fuga ou paralisia), tendo a noradrenalina como principal neurotransmissor. Sua atividade sobre a glândula suprarrenal exerce importante papel no controle da produção de hormônios reguladores do estresse, como por exemplo, o

cortisol. O parassimpático controla as funções vitais quando o corpo se encontra em repouso (descanso, digestão), tendo a acetilcolina como principal neurotransmissor. Várias das alterações somáticas fundamentais para as respostas emocionais são derivadas de respostas autonômicas: sudorese, rubor facial, frequência cardíaca, motilidade gastrintestinal, diâmetro da pupila, dentre muitas outras (14–16).

Hipotálamo

O hipotálamo é uma região situada acima da hipófise, localizando-se ventralmente ao diencéfalo, nas imediações do terceiro ventrículo. Exerce um papel central dentre as estruturas límbicas, uma vez que age como pivô das influências telencefálicas sobre regiões mesencefálicas, como a substância cinzenta periaquedutal. Estra estrutura regula o sistema nervoso autônomo, notadamente através de sua ação sobre a hipófise, e participa do controle sobre a ingesta hídrica, comportamento alimentar, comportamento de defesa e comportamento reprodutor (14–16).

Amígdala

A amígdala consiste numa estrutura complexa, composta por vários núcleos inter-relacionados, que desempenha um papel central na integração de informações sensoriais, sobretudo de estímulos perigosos, a respostas afetivas comportamentais e fisiológicas. Esta estrutura localiza-se medial e profundamente nos pólos dos lobos temporais, abaixo da camada cortical, recebendo aferências extensas, provindas do bulbo olfatório e de áreas neocorticais associativas polimodais, através de duas vias principais. A primeira, importante para a produção de respostas instintivas e imediatas, está implicada na transmissão menos refinada de estímulos, que chegam à amígdala pela via talâmica. A segunda, mais lenta, acrescida de uma etapa cortical, recebe estímulos com maior grau de processamento, propiciando maior compreensão contextual da informação emocional. Esta última via torna-se mais relevante à medida que o aprendizado emocional se aprimora e possibilita respostas mais refinadas e com maior grau de deliberação (14,15,17,18).

A amígdala apresenta importantes projeções para o hipocampo, essenciais para a integração dos significados emocionais às memórias, e extensamente influentes nos processos de fixação e consolidação mnêmicos. Outras importantes áreas de projeção amigdaliana incluem regiões neocorticais, como o córtex orbitofrontal, envolvido nas respostas socialmente relevantes e no controle das respostas agressivas; e o giro cingulado posterior, envolvido na apreciação consciente da ansiedade. Conexões com outras estruturas como o hipotálamo, o tálamo, o núcleo septal, o núcleo accumbens e regiões mesencefálicas, como a substância cinzenta periaquedutal contribuem, de diferentes formas, para o controle das respostas somáticas afetivas. (15,16,18).

Nucleus accumbens

O nucleus accumbens, também denominado estriado ventral, integra uma via de projeções dopaminérgicas que têm origem na área tegmentar ventral do mesencéfalo e seguem até regiões do córtex cerebral. O nucleus accumbens relaciona-se com a motivação e o reforço comportamental, sendo ativado por diferentes estímulos biologicamente relevantes, que direcionam o comportamento do indivíduo ao seu favor. Essa região pode ser compreendida como uma interface límbico-motora, que regula a intensidade, a duração e a capacidade seletiva (valoração) da atividade direcionada. As alças mesocorticais, que ligam o nucleus accumbens ao córtex pré-frontal, possiblitam a influência top-down do sistema dopaminérgico motivacional, assim como a interferência bottom-up dos aspectos motivacionais em processos cognitivos corticais. A área tegmentar ventral também possui projeções para a amígdala e o hipocampo (14,19).

Sistema septo-hipocampal

A região septal possui rica conectividade bidirecional com o hipocampo, sendo muitas vezes abordada, por este motivo, em conjunto com essa estrutura (sistema septo-hipocampal). O septo envia projeções de núcleos colinérgicos e gabaérgicos para o hipocampo via fímbria do fórnix, e recebe projeções gabaérgicas e glutamatérgicas hipocampais. Em contraste com o hipocampo, relacionado fundamentalmente com funções mnêmicas, o septo tem implicações diretas com os estados emocionais. O sistema septo-hipocampal possui importante papel na elaboração do medo e da ansiedade, modulando reações de sobressalto e congelamento diante de estímulos, respostas agressivo-defensivas diante de situações ameaçadoras e reações de medo. Lesões nesta região podem tanto exacerbar e tornar inapropriadas reações de medo e defesa, como reduzir estados de estresse e ansiedade, a depender dos núcleos septais lesados (14).

Ínsula

A ínsula é uma região cortical localizada profundamente ao suco lateral, entre o córtex sensitivo posterior e o córtex motor anterior. Sua visão a partir da perspectiva lateral do cérebro encontra-se ocultada pelo lobo temporal. Esta região recebe amplas aferências de regiões sensitivas corticais e talâmicas e projeta suas eferências para o córtex motor e o lobo temporal. A ínsula conecta-se, ainda, reciprocamente, com regiões parietais, da gânglia basal e outras estruturas límbicas, como o giro cingulado e os córtices pré-frontais orbitofrontais e mediais. Desta forma, a ínsula desempenha importante papel na consciência interoceptiva, relativa a estados internos do corpo, contribuindo para a percepção dos fenômenos emocionais, notadamente através de sua conectividade com o córtex pré-frontal medial e o giro do cíngulo. Juntamente com a

amígdala, está envolvida processamento dos estados ansiosos. Apesar de implicada na percepção de diferentes emoções, geralmente negativas, ela possui particular relação com a sensação de nojo ou repulsa, seja no sentido literal ou moral, com o apetite e com a percepção da dor (3,16,17,20).

Giro do cíngulo

Situado profundamente na fissura longitudinal, o giro do cíngulo ou giro cingulado consiste numa estrutura cortical que circunda o corpo caloso, formando um grande arco. Essa estrutura, extensamente conectada às demais áreas do sistema límbico, desempenha papel central na mediação entre as emoções e as funções cognitivas, por um lado, e as respostas motoras, por outro.

Existem várias formas de se dividir o giro cingulado, de acordo com as especificidades de suas áreas. Tradicionalmente, divide-se o giro do cíngulo, de forma mais simplificada, em duas grandes porções: o córtex cingulado anterior e o córtex cingulado posterior, de acordo com seu posicionamento em relação ao giro central.

O córtex cingulado anterior pode, ainda, ser subdividido em duas porções principais: sua porção rostral ou subgenual, amplamente conectada a regiões límbicas, como amígdala, hipotálamo, ínsula e córtex orbitofrontal, e intimamente relacionada a funções emocionais do controle autonômico e do dor; e sua porção dorsal, conectada ao córtex pré-frontal, parietal e a áreas motoras, envolvendo-se no monitoramento de conflitos e planejamento de reações motoras, quando relacionados a estímulos emocionalmente salientes. Já o córtex cingulado posterior, conectado a regiões talâmicas e dos córtices frontal, temporal, parietal posterior e occipital, e ao hipocampo, está envolvido na orientação visuoespacial em resposta a estímulos somatossensoriais, na percepção consciente de memórias associadas a estados emocionais, na evocação de memórias autobiográficas, relacionando-se às atividades mentais em que a atenção se volta ao espaço psíquico "interno", em contraposição ao ambiente externo (14–16).

Córtex pré-frontal

O córtex pré-frontal localiza-se na porção rostral do lobo frontal, anteriormente ao córtex pré-motor. Graças a esta região, os humanos destacaram-se dos demais primatas, ao alcançar a sofisticação do pensamento contextual e do comportamento direcionado por objetivos. O córtex pré-frontal caracteriza-se como o grande centro cerebral do processamento integrativo heteromodal, exercendo uma influência hierárquica sobre as demais regiões cerebrais. Por este motivo, essa estrutura possui amplas conexões córtico-corticais e córtico-subcorticais, participando não só dos circuitos límbicos, como também das redes sensoriais, motoras e executivas cerebrais.

O córtex pré-frontal pode ser dividido, grosseiramente, em três sub-regiões ana-

tomofuncionais: orbitofrontal, medial e lateral. O córtex orbitofrontal relaciona-se à apreciação de emoções pessoais ou de outrem, baseada em recompensas positivas ou negativas para a seleção de comportamentos adequados e inibição de comportamentos não desejáveis. O córtex medial, por sua vez, está implicado na atividade cerebral introspectiva, de auto-reflexão e avaliação social. Já nas regiões laterais, se dão processos cognitivos diversos, relacionados às funções executivas e memória de trabalho (7,15,16).

Conclusão

A evolução do conceito de sistema límbico acompanhou os avanços da neurociência, incorporando aos conceitos anatômicos evidências de medidas funcionais do sistema nervoso. Atualmente, várias definições diferentes existem na literatura, havendo falta de consenso sobre exatamente quais regiões anatômicas integram esse sistema. Contudo, o conceito de sistema límbico segue como um importante marco teórico no estudo da relação do sistema nervoso com o comportamento humano, fundamentando vários ramos de pesquisa sobre a neurociência das emoções.

Referências

1. Pessoa L, Hof PR. From Paul Broca's great limbic lobe to the limbic system. J Comp Neurol [Internet]. 2015 Dec 1;523(17):2495–500. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26103943

2. . Mega MS, Cummings JL, Salloway S, Malloy P. The limbic system: an anatomic, phylogenetic, and clinical perspective. J Neuropsychiatry Clin Neurosci. 1997;9(3):315–30.

3. Morgane PJ, Mokler DJ. The limbic brain: Continuing resolution. Neurosci Biobehav Rev [Internet]. 2006 Jan;30(2):119–25. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0149763405000965

4. Arciniegas DB. Emotion. In: Arciniegas DB, Anderson CA, Filley CM, Garcia TA, editors. Behavioral neurology and neuropsychiatry. Cambridge: Cambridge University Press; 2013.

5. Roxo MR, Franceschini PR, Zubaran C, Kleber FD, Sander JW. The Limbic System Conception and Its Historical Evolution. Sci World J [Internet]. 2011;11:2427–40. Available from: http://www.hindawi.com/journals/tswj/2011/157150/

6. MacLean PD. Some psychiatric implications of physiological studies on frontotemporal portion of limbic system (Visceral brain). Electroencephalogr Clin Neurophysiol [Internet]. 1952 Nov;4(4):407–18. Available from: https://linkinghub.elsevier.com/retrieve/pii/0013469452900734

7. Mesulam M-M. Principles of Behavioral and Cognitive Neurology. 2nd ed. New York: Oxford University Press; 2000. 540 p.

8. Nieuwenhuys R, Voogd J, van Huijzen C. The Human Central Nervous System [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2008. Available from: http://link.springer.com/10.1007/978-3-540-34686-9

9. Menon V. Large-scale brain networks and psychopathology: A unifying triple network model. Trends Cogn Sci [Internet]. 2011;15(10):483–506. Available from: http://dx.doi.org/10.1016/j.tics.2011.08.003

10. Menon V. Salience Network. In: Brain Mapping [Internet]. Elsevier; 2015. p. 597–611. Available from: http://linkinghub.elsevier.com/retrieve/pii/B978012397025100052X

11. Murphy FC, Nimmo-Smith I, Lawrence AD. Functional neuroanatomy of emotions: A meta-analysis. Cogn Affect Behav Neurosci. 2003;

12. Patterson DW, Schmidt LA. Neuroanatomy of the human affective system. Brain and Cognition. 2003.

13. Damasio AR. Toward a Neurobiology of Emotion and Feeling: Operational Concepts and Hypotheses. The Neuroscientist. 1995.

14. Roberto Lent. Neurociência da Mente e do Comportamento. 1st ed. Rio de Janeiro: Gen-Guanabara Koogan; 2008. 374 p.

15. Banich MT, Compton RJ. Cognitive Neuroscience. 2010;

16. Clark DL, Boutros NN, Mendez MF. The Brain and Behavior And Behavior - An Introduction to Behavioral Neuroanatomy [Internet]. 3rd ed. Cambridge: Cambridge University Press; 2010. Available from: http://www.cambridge.org/9781107415324A009

17. Gazzaniga M, Heatherton T HD. Psychological Science. 5th ed. Sheri L. Snavely, editor. New York, London: W. W. Norton; 2016. 403-449 p.

18. LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci. 2000;

19. Salamone JD, Pardo M, Yohn SE, López-Cruz L, SanMiguel N, Correa M. Mesolimbic Dopamine and the Regulation of Motivated Behavior. In 2015. p. 231–57. Available from: http://link.springer.com/10.1007/7854_2015_383

20. Noël X, Brevers D, Bechara A. A triadic neurocognitive approach to addiction for clinical interventions. Front Psychiatry. 2013;4(DEC):1–14.

14 - Comparative studies on the effect of Oxytocin on memory: "Love hormone" to remember, "Love hormone" to forget

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Current studies indicate that oxytocin neuropeptide influence processes of perception, attention and learning that underlie social behaviour. There is a growing interest in the use of oxytocin as a treatment for memory-related psychological disorders and social cognitive disorders. Recent studies also have shown that the effect of oxytocin on social and cognitive functions greatly varies, even leading to reverse results. The factors that lead to the impacts of oxytocin and its underlying processes on such variabilities stay uncertain. In this mini-review, we intend to examine various theories regarding the effect of oxytocin on memory, along with the mechanisms which are proposed from human and animal experiments.

Oxytocin is predominantly being debated as "love hormone" or "cuddle hormone", because this hormone plays a crucial role in promotion of social bonding, feelings of love and well-being. Oxytocin is a small peptide that is mostly produced by the supraoptic and paraventricular nuclei of the hypothalamus. It is stored and secreted by the posterior pituitary gland. From the posterior pituitary, it is released into the bloodstream to act as a hormone and influence on body functions (1).

In 1906, Henry Dale discovered that a pregnant cat's uterus was contracted by extracts from the human posterior pituitary gland. He coined this name with the meaning of "fast birth" from the Greek names. For years, oxytocin just known as a crucial factors underlying childbirth, maternal behaviors, and, lactation (2).

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The essential role of oxytocin in maternal behaviors has been demonstrated in several researches. So that, after giving birth, female rats that received oxytocin antagonists did not show typical maternal behavior (3). By contrast, after infusing oxytocin into the cerebral spinal fluid, virgin female sheep showed maternal behavior towards foreign lambs (4).

Besides peripheral actions, oxytocin also has a neuromodulator and neurotransmitter actions in the brain. It has been observed that endogenous concentrations of oxytocin in the brain are as much as 1000 times greater than peripheral concentrations (5). The main source of oxytocin in the brain is the dendritic release of oxytocin from hypothalamus into the extracellular space, as well as direct projection from smaller parvocellular neurons in the paraventricular nucleus (that producing oxytocin) to other brain areas (6). Oxytocin receptors are expressed in spinal cord and many areas of the brain, including amygdala, suprachiasmatic nucleus, hippocampus, striatum, bed nucleus of stria terminalis and brainstem (7), which regulate motivations, emotions, reward processing and support mnemonic and executive functions (8). Several experiments have reported that oxytocin plays a key role in some behaviors, such as increasing of trust (9), empathy (10), social behavior (11) and reducing of anxiety (12), Sexual arousal (13), cognitive function (13) and interestingly anti-social states (fear, aggression, jealous and pride) (14–16).

One of the areas in which oxytocin plays an important role is its effect on learning and memory. However, the results of many studies and suggested mechanisms include numerous contradictions. Here, we compare the results of different human, animal studies and the proposed mechanisms to certify the effect of oxytocin in the form of three hypotheses; Impairing hypothesis, facilitates hypothesis and selective hypothesis.

Impairing hypothesis

Several studies showed the detrimental impacts of oxytocin on memory processes. In one study, Oxytocin impaired the performance of human memory in retaining a list of words (17). Oxytocin also decreased memory efficiency for the category of phrases related to reproduction associated with sex and baby care in an experiment with verbal stimuli. (18). Oxytocin was asserted to diminish the storage of verbal memory in humans (19).

Administration of high dosages of oxytocin to human subjects also showed that while learning did not appear to be impacted, subsequent recall (recalling 20 nonsense four-letter words and recognition of facial images earlier seen) was impaired (20). With regard to these detrimental effects on certain memory-related functions, oxytocin may be engaged in the forgetting of mother-related delivery pain (21).

Animal studies have also shown that oxytocin intaking in inhibitory avoidance tes-

ting after exercise significantly reduced memory efficiency in mice. While the use of an antagonist oxytocin receptor after practice, significantly enhanced their memory performance (22). Oxytocin also had a detrimental effect on the social memory in male rats, so that, rats that received oxytocin, were less likely to remember the mice that they had previously encountered. (23).

With regard to such researches, some scientists indicated that the detrimental effects of oxytocin on remembering past memories might help the situation for those who had an unpleasant memory of their lives (such as social anxiety, post-traumatic stress disorder (PTSD), and Etc.). No unique medicine was available to them. However, there was still a hurry of ambiguity about the accurate mechanism and its implementation.

Several studies have suggested that oxytocin caused to reduce the initial storage of information and the amount of storage (24). In this line, a series of studies also have proposed that reduction speed and performance in tasks after oxytocin administration was due to the sedative effects of oxytocin (25,26). Complementarily, it is suggested that oxytocin is more effective factor in occurring these changes by attenuating the cortisol levels and anxiety in response to physical stress (27). In humans, the observation of emotional visual stimuli was accompanied by activation of amygdala, but oxytocin significantly reduced this activation (28) and enhanced information processing in other brain areas (29).

Facilitates hypothesis

In fact, the above mentioned mechanisms did not cover the results of many other studies describing the role of oxytocin in facilitating recognition, learning, memory, and decision making (8,30). So that, after using oxytocin, the amount of particular personal retrieval of memory and its details increased in the autobiographical memory test (31). As well, oxytocin has also demonstrated that it can amplify the men's early memories of their mothers (32).

Several studies have shown that oxytocin had distinct effects on memory performance for social behavior (33). In particular, intranasal oxytocin administration could enhance recognition memory for faces (34), and this improvement was not related to gender, response biases or changes in mood (35).

In animal studies, oxytocin has also been shown to be important in memory and social recognition; In a research on mice, during their maternity that oxytocin level was naturally high, their spatial memory considerably improved (36). Oxytocin knock-out mice failed to recognize familiar conspecifics after repeated social exposures and social recognition (37). It is indicated that oxytocin was necessary for the normal development of social memory in mice (38). Oxytocin modulated reactions to fear by enhancing the maintenance of social memories. Rats that are genetically modified to

have a surplus of oxytocin receptors displayed a greater fear response to a previously conditioned stressor (39).

A dominant mechanism proposed that oxytocin enhanced the salience of emotional and social information in stimuli which led to a faster response because these cues were especially important to the survival and development of social behaviour (40). A related model claimed that oxytocin enhanced attention to emotional and social issues (41). On another hand, attention to social indicators was due to the interaction of oxytocinergic circuits with the dopaminergic system (42). It has also been shown that oxytocin could increase activity in VTA in response to social symptoms (43)

In particular, these contradictory results of oxytocin functions are important issues in determining oxytocin as an adjunct to psychosocial management. Since the use of oxytocin in some cognitive impairments may even increase the negative effects and worsen the defects. Therefore, a better understanding of the key role of oxytocin in cognitive functions regarding emotional and social concepts provides an opportunity for more effective interventions in learning disabilities and communication or management of psychological disorders.

Selective hypothesis

Based on contradictory findings in highlighting the increased or reduced effects of oxytocin on memory , other studies suggested that oxytocin administration in humans has led to improvement and also impairment in perception (44) and memory tasks (31,45). In this regard, in a study it was shown that the impacts of oxytocin on social stimuli (smile and angry faces) or non-social (coloured lights) were different. So that, oxytocin consumption could only facilitate socially reinforced learning and induced emotional empathy (10). In addition, oxytocin significantly and specifically improved the recognition of happy faces but it was not about anger, disgust, fear and sadness (35).

This dual effect of oxytocin has been discussed about inter/intra group behaviors, such as cooperation and competition within and between groups. Using oxytocin in humans not only induced a promoted in-group trust and collaboration, but also defensive and aggression toward competing out-groups (47) and it could promote human ethnocentrism (48).

Oxytocin moreover have been shown to modulate rodents' and monkeys' performance in memory tasks, and both increased and reduced effects of oxytocin on the cognitive performance and memory reported (49–53). Recent studies in animals indicated that the effects of oxytocin on the perception and evaluation of social stimuli greatly was dependent on the emotional/social valence of stimuli (54) and suggested that oxytocin context-dependently affected their behaviours (54). In our recent study that associated with the effects of oxytocin on macaque monkeys' memory performan-

ce, our results showed that the effect of oxytocin was dependent on the emotional content of stimuli. Thus, the oxytocin enhanced the adverse effects of negative stimuli on recalling, however, moderated this effect for positive stimuli. Our findings in monkeys did not support models suggesting a general effect of oxytocin in enhancing salience or deteriorating of attention to social stimuli. Instead, our results indicated that the cognitive effects of oxytocin was related to the emotional valence of contextual factors (55).

Results of several studies have demonstrated that oxytocin could enhance the social approach, intimacy and bonding by strengthening encoding through the recall of positive social information (45). It is appeared that the processing of positive and negative information has been affected by this hormone. In this regards, suggested that it may reduce the salience of potentially ambiguous and threatening social stimuli (46).

Despite all these results, oxytocin still showed an opposite effect in different tasks and individuals (56). Scientists indicated that oxytocin's influence on memory performance was related in part to the stimuli and type of memory test (18). Moreover, context- and person-dependent effects of oxytocin had an influence on memory performance (57). These inconsistencies of studies would probably be involvement of additional factors, including gene polymorphism, early life experiences as well as motivational status (58,59).

Taken together, in reviewing the studies, the general insights demonstrated the importance of oxytocin as an evolutionary hormone to create a group (family), improve social behaviors, reduce stress and create a good feeling in a group to keep that group together. On the contrary, it caused to produce anger, outgroup hatred, aggressive reactions to threaten outside the group. Perhaps this dual oxytocin behavior is part of its mission and necessity for modern human sociality and cognition.

References

1. Brownstein MJ, Russell JT, Gainer H. Synthesis, transport, and release of posterior pituitary hormones. Science (80-). 1980;207(4429):373–8.

2. Galbally M, Lewis AJ, IJzendoorn M van, Permezel M. The role of oxytocin in mother-infant relations: a systematic review of human studies. Harv Rev Psychiatry. 2011;19(1):1–14.

3. van Leengoed E, Kerker E, Swanson HH. Inhibition of post-partum maternal behaviour in the rat by injecting an oxytocin antagonist into the cerebral ventricles. J Endocrinol [Internet]. 1987 Feb [cited 2014 Dec 15];112(2):275–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3819639

4. Kendrick KM, Keverne EB, Baldwin BA. Intracerebroventricular oxytocin stimulates maternal behaviour in the sheep. Neuroendocrinology. 1987;46(1):56–61.

5. Baribeau DA, Anagnostou E. Oxytocin and vasopressin: linking pituitary neuropeptides and their receptors to social neurocircuits. Front Neurosci. 2015;9:335.

6. Knobloch HS, Grinevich V. Evolution of oxytocin pathways in the brain of vertebrates. Front Behav Neurosci. 2014;8:31.

7. Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. Nat Rev Neurosci. 2011 Sep;12(9):524–38.

8. MacDonald K, MacDonald TM. The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. Harv Rev Psychiatry. 2010;18(1):1–21.

9. Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E. Oxytocin increases trust in humans. Nature. 2005;435(7042):673–6.

10. Hurlemann R, Patin A, Onur OA, Cohen MX, Baumgartner T, Metzler S, et al. Oxytocin enhances amygdala-dependent, socially reinforced learning and emotional empathy in humans. J Neurosci. 2010 Apr 7;30(14):4999–5007.

11. Andari E, Duhamel J-R, Zalla T, Herbrecht E, Leboyer M, Sirigu A. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. Proc Natl Acad Sci U S A. 2010 Mar 2;107(9):4389–94.

12. Marazziti D, Dell'Osso B, Baroni S, Mungai F, Catena M, Rucci P, et al. A relationship between oxytocin and anxiety of romantic attachment. Clin Pract Epidemiol Ment Heal. 2006;2(1):28.

13. Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. Physiol Rev. 2001 Apr;81(2):629–83.

14. Shamay-Tsoory SG, Fischer M, Dvash J, Harari H, Perach-Bloom N, Levkovitz Y. Intranasal administration of oxytocin increases envy and schadenfreude (gloating). Biol Psychiatry. 2009;66(9):864–70.

15. Harari-Dahan O, Bernstein A. A general approach-avoidance hypothesis of oxytocin: accounting for social and non-social effects of oxytocin. Neurosci Biobehav Rev. 2014;47:506–19.

16. Beery AK. Antisocial oxytocin: complex effects on social behavior. Curr Opin Behav Sci. 2015;6:174–82.

17. Fehm-Wolfsdorf G, Born J, Voigt K-H, Fehm H-L. Human memory and neurohypophyseal hormones: opposite effects of vasopressin and oxytocin. Psychoneuroendocrinology. 1984;9(3):285–92.

18. Heinrichs M, Meinlschmidt G, Wippich W, Ehlert U, Hellhammer DH. Selective amnesic effects of oxytocin on human memory. 2004;83:31–8.

19. Bruins J, Hijman R, Van Ree JM. Effect of a single dose of des-glycinamide-[Arg8] vasopressin or oxytocin on cognitive processes in young healthy subjects. Peptides. 1992;13(3):461–8.

20. Ferrier BM, Kennet DJ, Devlin MC. Influence of oxytocin on human memory processes. Life Sci. 1980;27(24):2311–7.

21. Evans JJ. Oxytocin in the human--regulation of derivations and destinations. Eur J Endocrinol. 1997;137(6):559–71.

22. Boccia MM, Baratti CM. Involvement of central cholinergic mechanisms in the effects of oxytocin and an oxytocin receptor antagonist on retention performance in mice. Neurobiol Learn Mem. 2000;74(3):217–28.

23. Popik P, Vetulani J. Opposite action of oxytocin and its peptide antagonists on social memory in rats. Neuropeptides. 1991;18(1):23–7.

24. Bruins J, Hijman R, Van Ree JM. Effect of a single dose of des-glycinamide-[Arg 8] vasopressin or oxytocin on cognitive processes in young healthy subjects. Peptides. 1992;13(3):461–8.

25. Hess L, Votava M, Málek J, Kurzová A, Slíva J. Sedative effects of intranasal oxytocin in rabbits and rhesus monkeys. Physiol Res. 2016;65.

26. Uvnäs-Moberg K, Ahlenius S, Hillegaart V, Alster P. High doses of oxytocin cause sedation and low doses cause an anxiolytic-like effect in male rats. Pharmacol Biochem Behav. 1994;49(1):101–6.

27. Cardoso C, Ellenbogen MA, Orlando MA, Bacon SL, Joober R. Intranasal oxytocin attenuates the cortisol response to physical stress: a dose–response study. Psychoneuroendocrinology. 2013;38(3):399–407.

28. Petrovic P, Kalisch R, Singer T, Dolan RJ. Oxytocin attenuates affective evaluations of conditioned faces and amygdala activity. J Neurosci. 2008 Jun 25;28(26):6607–15.

29. Striepens N, Scheele D, Kendrick KM, Becker B, Schäfer L, Schwalba K, et al. Oxytocin facilitates protective responses to aversive social stimuli in males. Proc Natl Acad Sci U S A. 2012;109(44):18144–9.

30. Ebitz RB, Platt MM. An evolutionary perspective on the behavioral consequences of exogenous oxytocin application. Front Behav Neurosci. 2014;7:225.

31. Cardoso C, Orlando MA, Brown CA, Ellenbogen MA. Oxytocin and enhancement of the positive valence of social affiliation memories: an autobiographical memory study. Soc Neurosci. 2014;9(2):186–95.

32. Bartz JA, Zaki J, Ochsner KN, Bolger N, Kolevzon A, Ludwig N, et al. Effects of oxytocin on recollections of maternal care and closeness. Proc Natl Acad Sci. 2010;107(50):21371–5.

33. Savaskan E, Ehrhardt R, Schulz A, Walter M, Schächinger H. Post-learning intranasal oxytocin modulates human memory for facial identity. Psychoneuroendocrinology. 2008;33(3):368–74.

34. Rimmele U, Hediger K, Heinrichs M, Klaver P. Oxytocin makes a face in memory familiar. J Neurosci. 2009 Jan 7;29(1):38–42.

35. Marsh AA, Yu HH, Pine DS, Blair RJR. Oxytocin improves specific recognition of positive facial expressions. Psychopharmacology (Berl). 2010 Apr;209(3):225–32.

36. Tomizawa K, Iga N, Lu Y-F, Moriwaki A, Matsushita M, Li S-T, et al. Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. Nat Neurosci. 2003;6(4):384–90.

37. Ferguson JN, Aldag JM, Insel TR, Young LJ. Oxytocin in the medial amygdala is essential for social recognition in the mouse. J Neurosci. 2001;21(20):8278–85.

38. Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT. Social amnesia in mice lacking the oxytocin gene. Nat Genet. 2000;25(3):284.

39. Guzmán YF, Tronson NC, Sato K, Mesic I, Guedea AL, Nishimori K, et al. Role of oxytocin receptors in modulation of fear by social memory. Psychopharmacology (Berl). 2014;231(10):2097–105.

40. Bartz JA, Zaki J, Bolger N, Ochsner KN. Social effects of oxytocin in humans: Context and person matter. Trends Cogn Sci. 2011;15:301–9.

41. Vuilleumier P. How brains beware: neural mechanisms of emotional attention. Trends Cogn Sci. 2005;9(12):585–94.

42. Shamay-Tsoory SG, Abu-Akel A. The social salience hypothesis of oxytocin. Biol Psychiatry. 2016;79(3):194–202.

43. Groppe SE, Gossen A, Rademacher L, Hahn A, Westphal L, Gründer G, et al. Oxytocin influences processing of socially relevant cues in the ventral tegmental area of the human brain. Biol Psy-

chiatry. 2013;74(3):172–9.

44. Norman GJ, Cacioppo JT, Morris JS, Karelina K, Malarkey WB, Devries AC, et al. Selective influences of oxytocin on the evaluative processing of social stimuli. J Psychopharmacol. 2011 Oct;25(10):1313–9.

45. Guastella AJ, Mitchell PB, Mathews F. Oxytocin enhances the encoding of positive social memories in humans. Biol Psychiatry. 2008 Aug 1;64(3):256–8.

46. Di Simplicio M, Massey-Chase R, Cowen PJ, Harmer CJ. Oxytocin enhances processing of positive versus negative emotional information in healthy male volunteers. J Psychopharmacol. 2009;23(3):241–8.

47. De Dreu CKW, Greer LL, Handgraaf MJJ, Shalvi S, Van Kleef GA, Baas M, et al. The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. Science (80-). 2010;328(5984):1408–11.

48. De Dreu CKW, Greer LL, Van Kleef GA, Shalvi S, Handgraaf MJJ. Oxytocin promotes human ethnocentrism. Proc Natl Acad Sci. 2011;108(4):1262–6.

49. Boccia MM, Kopf SR, Baratti CM. Effects of a single administration of oxytocin or vasopressin and their interactions with two selective receptor antagonists on memory storage in mice. Neurobiol Learn Mem. 1998;69(2):136–46.

50. Lee S-Y, Park S-H, Chung C, Kim JJ, Choi S-Y, Han J-S. Oxytocin protects hippocampal memory and plasticity from uncontrollable stress. Sci Rep. 2015;5:18540.

51. McEwen B. Roles of vasopressin and oxytocin in memory processing. Vol. 50. Academic Press; 2004.

52. Parr LA. Intranasal oxytocin enhances socially-reinforced learning in rhesus monkeys. Front Behav Neurosci. 2014;8:278.

53. Parr LA, Mitchell T, Hecht E. Intranasal oxytocin in rhesus monkeys alters brain networks that detect social salience and reward. Am J Primatol. 2018;e22915. 54.

54. Ebitz RB, Watson KK, Platt ML. Oxytocin blunts social vigilance in the rhesus macaque. Proc Natl Acad Sci. 2013;110(28):11630–5.

55. Zarei SA, Sheibani V, Tomaz C, Mansouri FA. The effects of oxytocin on primates' working memory depend on the emotional valence of contextual factors. Behav Brain Res. 2019;362.

56. Pierce BH, Kensinger EA. Effects of emotion on associative recognition: valence and retention interval matter. Emotion. 2011;11(1):139.

57. Bakermans-Kranenburg MJ, van IJzendoorn MH. Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. Transl Psychiatry. 2013;3:e258.

58. Feeser M, Fan Y, Weigand A, Hahn A, Gärtner M, Aust S, et al. The beneficial effect of oxytocin on avoidance-related facial emotion recognition depends on early life stress experience. Psychopharmacology (Berl). 2014;231(24):4735–44.

59. . Luo S, Li B, Ma Y, Zhang W, Rao Y, Han S. Oxytocin receptor gene and racial ingroup bias in empathy-related brain activity. Neuroimage. 2015;110:22–31.

15 - Studies on new sweetening resource plant hydroponic Stevia rebaudiana Bertoni in Armenia

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Introduction

Stevia rebaudiana (Bertoni) is a herbaceous perennial plant of the Asteraceae family, native to Paraguay (South America). Stevioside, the major sweetener present in leaf and stem tissues of stevia, was first seriously considered as a sugar substitute in the early 1970s by a Japanese consortium formed for the purpose of commercializing stevioside and stevia extracts [1]. Diterpene glycosides produced by stevia leaves are many times sweeter than sucrose. They can be utilized as a substitute to sucrose [2]; they are natural sources of non-caloric sweetener and alternatives to the synthetic sweetening agents that are now available to the diet conscious consumers.

Classification of stevia

Stevia rebaudiana is one of the 950 genera of the Asteraceae family [3]. Although there are about 230 species in the genus, only S. rebaudiana gave the sweetest essence, while other species contain other biochemicals of interest [4].

Kingdom Plantae
Subkingdom Tracheobionta
Superdivision Spermatophyta
Division Magnoliophyta
Class Magnoliopsida

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Subclass Asteridae
Group Monochlamydae
Order Asterales
Family Asteraceae (Compositae formerly)
Subfamily Asteroideae
Tribe Eupatorieae
Genus Stevia
Species rebaudiana

Some other related species of Stevia rebaudiana are Stevia eupatoria, Stevia lemmonii Lemmon's stevia, Stevia micrantha annual stevia, Stevia ovata var. texana roundleaf candy leaf, Stevia plummerae Plummer's stevia, Stevia plummerae var. alba, Stevia rhombifolia Kunth, Stevia salicifolia willow-leaf dtevia, Stevia serrata sawtooth stevia, Stevia viscida viscid stevia, Stevia commixta, Stevia satureiaefilia, Stevia leptophylla, Stevia myriadenia, Stevia ophryphylla, Stevia selloi, Stevia nepetifolia, Stevia oligophylla, Stevia origanoides and Stevia triflora.

Botanical description

The inflorescence is loosely paniculate with the heads appearing opposite the bracts in irregular sympodial cymes. They are arranged in indeterminate heads. The flowers are small (1517 mm) and white [5] with pale purple throat corollas. The plant takes more than a month to pass through the various developmental flower stages and produce all its flowers [6].

Glycosides

Eight diterpene glycosides with sweetening properties have been identified in leaf tissues of stevia. These are synthesized, at least in the initial stages, using the same pathway as gibberellic acid, an important plant hormone [7]. The four major sweeteners are stevioside, rebaudioside-A, rebaudioside-C and dulcoside-A. According to Kinghorn [8] the sweetness of these compounds relative to sucrose are 210, 242, 30 and 30 times, respectively. The two main glycosides are stevioside, traditionally 510% of the dry weight of the leaves, and rebaudioside-A (Reb-A), 24%; these are the sweetest compounds. There are also other related compounds including minorglycos ides, such as rebaudioside-B, rebaudioside-C (12%), rebaudioside- D, rebaudioside-E, rebaudioside-F, dulcoside-A, dulcoside-C and steviolbioside, as well as flavonoid glycosides, coumarins, cinnamic acids, phenylpropanoids and some essential oils [9]. Among the components of stevia, one, called rebaudioside-A, is of particular interest because it has the most desirable flavour profile [10]. Stevioside traditionally makes up the ma-

jority of the sweetener (60-70% of the total glycosides content) and is assessed as being 110-270 times sweeter than sugar. It is also responsible for the bitter aftertaste, sometimes reported as a ''licorice'' taste. As well as sweetness, stevioside may have a lingering effect or certain degree of pungency, which is not appreciated by the majority of people, and which reduces its acceptability. Rebaudioside-A is usually present as 30-40% of total sweetener and has the sweetest taste, assessed as 180-400 times sweeter than sugar with no bitter aftertaste (licorice taste or lingering effect). The ratio of rebaudioside-A to stevioside is the accepted measure of sweetness quality; the more rebaudioside-A the better. If rebaudioside-A is present in equal quantities to stevioside, it appears that the aftertaste is eliminated. The minor glycosides are considered to be less sweet, 30-80 times sweeter than sugar [11]. The sweetening effect of these compounds is purely taste; they are undigested and no part of the chemical is absorbed by the body. There are reports of stevioside content (total glycosides) ranging between 4 and 20% on a dry weight basis, depending on the cultivaran d growing conditions [12]. The sweetening potency (sucrose1) of stevioside, rebaudioside-A, rebaudioside--B, rebaudioside-C, rebaudioside-D, rebaudioside-E, dulcoside-A and steviolbioside are 250-300, 350-450, 300-350, 50-120, 200-300, 520-300, 50-120 and 100-125, respectively. The essential oil composition of the aerial parts of five different Stevia rebaudiana genotypes cultivated in on the Tuscan coast (Italy) was examined by CC and GC/MS. Forty different components were identified and the main constituents in all studying samples were spathulenol (13.4-40.9%), caryophyllene oxide (1.3-18.7%), beta-caryophyllene (2.11-6.0%) and betapinene (5.5-21.5%) [13].

Biosynthesis of steviol glycosides

The steviol glycoside and gibberellin pathways diverge at kaurene. In stevia, kaurene is converted to steviol, the ''backbone'' of the sweet glycosides, then glucosylated or rhaminosylated to form the principle sweeteners. The precursor compounds are synthesized in the chloroplast, and from there are transported to the endoplasmic reticulum, Golgi apparatus and then vacuolated. The purpose of these compounds in the stevia plant is not yet clear, but their high concentration in the leaf and the conservation of the pathway within the species indicate that, at some point in evoluntionary time, their presence conferred significant advantage upon those individuals that possessed them. Recent experiments have shown that the early steps in steviol biosynthesis involve the plastid localized DXP pathway and not the mevalonate pathway [14]. Therefore, the first step in the steviol glycoside biosynthetic pathway is the formation of DXP from pyruvate and glyceraldehyde 3-phosphate by thiamine phosphate-dependent DXP synthase [15] is not necessarily the committed precursor of isopentenyl diphosphate (IDP) and that IDP and DMADP may arise from separate syntheses [16].

Isopentenyl diphosphate and DMADP are converted to geranylgeranyl diphosphate (GGDP) by GGDP synthase via three successive condensation reactions [17]. The two oxygenated functional groups of steviol, the C-19 carboxylate and the C-13 alcohol, provide attachment points forthe sugarside chains that determine the identity of the different glycosides. The C-13 alcohol is successively glucosylated, first yielding steviolmonoside then steviol-bioside, next the C-19 carboxylate is glucosylated, which forms stevioside [18]. The pathway terminates with the glucosylation of stevioside, which forms rebaudioside A.

Glycoside content in different plant parts

Plant organs contain different amounts of the sweet glycosides, which decline in the following order: leaves, flowers, stem, seeds and roots. Roots are the only organs that do not contain stevioside. The sweetness in the leaves is two times higher than that in inflorescence [5]. The fact that the highest stevioside content is found in the leaves suggests that they serve as the main tissue for both synthesis and primary accumulation of stevioside compounds. The largest amount of stevioside was found in the upper young, actively growing shoot sections, whereas the lowest senescent shoot sections exhibited the smallest amount of such compounds. During ontogeny, a gradual increase in the stevioside concentration was observed in both mature leaves and stems, and this process lasted to the budding phase at the onset of flowering [19], the lower, mature leaves of stevia have fewer glands per leaf surface area than the upper, younger leaves, i.e., there is a positive correlation between gland distribution density and steviol glycoside content. This argues in favor of possible increased accumulation, by 30 to 170% of glycosides, in upper young leaves as compared with lower senescent ones. Depending on the clone, the portion of the rebaudioside-A in the total glycosides content appeared to be increased as well. During ontogeny, little variation in the glycoside content is also found in roots. In these organs, from the vegetative phase to flowering, a gradual decrease in the glycoside content was observed. During the fruit development stage the levels of glycosides were found to revert to the initial level. However, in roots the total glycosides content never exceed 0.1% [20] demonstrated that the maximal content of stevioside in leaves is achieved during the formation of flower buds and it then gradually declines. All this information may indicate that the steviol glycosides are transported to generative organs. Similar results were obtained for ecdisteroids in Rhaponticum carthamoides, Ajuga reptans and Serratula coronata [21]. At the whole-plant level, steviol glycosides tend to accumulate in tissues as they age, so that older lower leaves contain more sweetener than younger upper leaves. Since, chloroplasts are important in precursor synthesis, those tissues devoid of chlorolphyll, such as roots and lower stems, contain no or trace amounts of glycosides. Once flowering is initiated, glycoside concentrations in the leaves start

declining. Stems of stevia plants contain little orno sweeteners, although it is suggested that they may contain some flavour enhancers, odourisers and other agents of potential use for improving foodstuffs or alcoholic beverages [7]. As stems mature and lose colour, any steviosides present dissipate. The structure, development and chemical content of stevia roots have also received attention, often associated with culturing procedures [22].

Environmental effect

The growth and flowering of stevia are affected by radiation, day length, temperature, soil moisture, and wind. Stevia is grown as a perennial crop in subtropical regions, including parts of the United States, and as an annual crop in mid to high latitude regions [23]. The results indicate that yield depends mainly on the genetic characters of the plant, the phenotypic expression of which is influenced by climatic and environmental factors [24]. Moreover, synthesis of terpenes is affected by climatic and environmental factors. Long-day conditions, as compared with short days, increase internode length, leaf area, and dry weight, and reduce the interval between the appearances of successive leaf pairs in S. rebaudiana. Total soluble leaf sugars, protein, and stevioside content are also augmented in both absolute and relative terms and the biosynthesis of steviol, the aglucone present in stevioside, is increased by 45%.

Phenotypic variability

In the wild populations of Stevia rebaudiana, there is great variation in phenotype and leaf analysis. The collections made as part of the various breeding and selection research programs have invariably included a range of genotypes and selections of plants with distinct levels of steviosides in their leaves. Shock [25] planted 200 lines for survival testing and screened 17 lines for productivity. The stevioside content of leaves can vary substantially (4-16%) between individual plants, even after a selection program has been continued for some time [26].

Breeding methods

Germplasm Introduction, Collection and Conservation

Germplasm is a very important material for the improvement of crops. Introduction of germplasm from one area to another continues to be an important activity for breeding, particularly in developing countries. It is generally used as source of superior genes and increasing genetic diversity in the germplasm for breeding programmes. Institutions around the world that have undertaken research and/or appraisal studies on stevia have collected seed and plant material from Paraguay in its wild, natural environment. The rationale behind seed collection is to conserve genes and not genotypes, since, in stevia, due to heterozygosity, no genotype is true breeding [27].

Future prospects

Rebaudioside-A is of particular interest among the glycosides produced in the leaves of stevia because it has the most desirable flavour profile, while stevioside is responsible for aftertaste bitterness. Improved genotypes with a high content of rebaudioside-A with respect to other glycosides (like stevioside) need to be developed, as the Food and Drug Administration has approved rebaudioside-A with 95% purity. Further research and development need to be carried out to improve stevia's potential as a crop by developing improved varieties with higher yield and quality through plant breeding methods and biotechnological approaches.

Hydroponic stevia cultivated in Armenia
Analytical methods, performers and collaborating organizations

Ecologically pure, high-quality raw material of Stevia (with a high content of biologically active substances), grown in Nagorno-Karabakh was harvested from August to October 2016. Stevia used in this study was botanically authenticated and voucher specimens (2779 A) were deposited in the Herbarium of Institute of Hydroponics (Experimental Hydroponic Station, outdoor hydroponic station with 60-100 m^2 vegetation surface area (feeding scaffolds with 12-20 repetitions). All indices of safety of Stevia dry leaves and Stevia-based food are defined in Republican Veterinary and Phytosanitary Laboratory Services Center State non-commercial Organization (SNCO) (Ministry of Agriculture of the Republic of Armenia) (Yerevan, Erebuni 12, reg. N2780, 20.01.2014) and fully comply with the decision of the RA Government on "The approval of the technical regulation of the requirements for juices and juice products and the repeal of the decision N744 of the RA Government of 26 June 2009".

The content of dry matter, aflatoxin B1, pesticides, nitrogen, protein, toxic elements (Pb, As, Cd, Hg) in leaf dry matter of Stevia have been determined in Narek CJSC scientific research company. The aspartat aminotransferase (AST)/alanin aminotransferase (ALT) (De-Ritis) ratio is an easily applicable blood test. Biochemical markers AST and ALT were used to assess the protective action of Stevia. The average value of ALT of normal rats was 10,42±0,8. Administration of Stevia at dose of 20mg/kg raised this value to 17,23±1,93. The average value of AST of normal rats was 10,02±0,7. Administration of Stevia raised this value to 17,19±2,76. De Ritis ratio was 0.99 in comparison to the initial level (0.96), which indicates the absence of toxic effects following administration of ecologically pure hydroponic Stevia rebaudiana Bertoni [28].

At the Institute of Biotechnology of the National Academy of Sciences, the content of diterpene glycosides (stevioside, rebaudioside, steviol, rebaudioside "C", dulcoside "A") and organic acids have been determined (Figure 1). The works have been done by Ph.D. V. Ghochikyan [29].

The high quality of stevioside is impaired by its residual bitterness and taste. They may be removed by modification of stevioside in the reaction of intermolecular transglycosylation, catalyzed by various enzymes, during which other carbohydrates are attached at positions C13 and C19. It is the number of carbohydrate units at these positions that determines the degree of sweetness of steviol.

Cyclodextrin glucanotranseferase (CGTases, EC 2.4.1.19) produced by mesophilic, thermophilic, alcalophylic and halophylic bacilli have been applied for the transglycosylation of stevioside and rebaudioside A in the presence of starch as a donor. Cyclodextrin glucanotranseferase produced by Bacillus stearothermophilus B-5076 and Bacillus macerans Bio 4m are the most efficient. The method can be applied for the transglycosylation Stevia extract directly without any preliminary purification of individual compounds.

It was shown that CGTases produced by extremophilic microorganisms are effective biocatalysts. Optimum temperature and pH of these enzymes were 45°C and pH 6.5–7.5, respectively. The optimum stevioside-to-CD ratio and total concentration of dry matter for the synthesis of the best-tasting product were 1 : 1 (w/w) and 11.6%, respectively [30]

Figure 1. Stevia Powder without calories

Cultivation of Stevia rebaudiana Bertoni in different conditions Ararat Valley (Armenia)

Hydroponic experimental station in Ararat Valley

Outdoor hydroponic station with 60-100 m^2 vegetation surface area

(feeding scaffolds with 12-20 repetitions)

Land area with 100 m^2 vegetation surface

Hydroponic vegetation vessels with 7 repetitions, each with 1 m^2 vegetation surface
Trenched membranous greenhouse with 21 m^2 vegetation area

Figure 2. Cultivation of Stevia rebaudiana Bertoni in open-air hydroponic conditions (Institute of Hydroponics, Armenia)

Artsakh

Askeran region, village Khanabad, land area belonging to "Mary" LLC with 1000 m^2 vegetation surface and 100 m^2 room conditions (Individual monopolists/farmers (Askeran, Martakert, Martuni, Stepanakert, Hadrut regions).

Biological definition of stevia rebaudiana bertoni

Table1. Stevia phenophases in test conditions (2013 - 2017)

Characterization and Phenophases	Ararat valley		Foothill	Nagorno-Karabagh Republic (village Khanabad)
	Soil	hydroponics		
Field planting	May I and II ten-day period	May I and II ten-day period	May I and II ten-day period	May I and II ten-day period
Booshing	June II and III ten-day period	June II ten-day period	June III ten-day period	June II ten-day period
Active growth period	July-September	June-October	July-September	July-September
Budding	August I ten-day period	In early August	August II ten-day period	August I ten-day period
Flowering (bisexual remontant)	August II ten-day period	August I ten-day period	In early October	September II ten-day period
Seeding	November II ten-day period	In early November	In early November (not massive)	November II ten-day period

Table 2. Stevia's biometric measurements (bud development stage) and the yield (dried leaf)

Biometric indices	Ararat Valley		Foothills	NKR (Khanabad)
	Soil	hydroponic		
Plant height (cm)	110,0-115,0	118,0-133,0	104,0-108,0	98,0-120,0
Plant diameter (cm)	50,0-53,0	58,0-60,0	52,0-54,0	50,0-56,0

Root	Length (cm)	22,8-24,0	14,1-19,2	24,2-25,1	22,5-25,6
	Volume (cm 3)	54,0-56,5	52,7-74,8	51,7-55,7	55,6-60,7
	Root diameter (mm)	15,0-17,0	18,0-23,0	16.0-19.0	17,0-19,0
	Rootstock, the number of buds	7-11	19-23	4-7	9-16
Plant stem	I-class stem number	19-26	29-38	19-22	17-24
	Length (cm)	85,0-92,0	104,0-120,0	80,0-87,0	100,0-106,0
	II –class bush number	10-14	40-44	12-15	12-17
	Length (cm)	50,0-59,0	62,0-65,0	43,0-52,0	52,0-55,0
Leaf	Length (cm)	3,8-5,6	4,0-7,5	3,5-4,9	3,9-6,2
	Width (cm)	1,0-3,2	2,2-4,5	2,0-3,3	2,0-3,2
	Surface (dm2)	98,0	183,0	107,0	127,0
	Number of leaves on a bush	370-470	700-850	360-480	420-540
Yield (t/ha)		2,0-2,1	4,5-5,0	1,8-2,0	2,2-2,5

Table 2 presents biometric measurements of Stevia. According to the results obtained, the plant's biometric indicators are slightly different from those related to climatic conditions, except for the hydroponic version. Stevia is a short-day plant and the rate of its generative development depends on the distance from the region to the equator. This fact creates an opportunity to gather abundant, mature seeds in our area in autumn.

An average data of biochemical and chemical investigations of the last 5 years, which were done with cooperation with other institutes are presented in table 3.Toxicological analyses were also done, which proved that the medicinal raw material corresponds to the available standards. Plants grown hydroponically have predictable content, can be raised without soil, form their own nutrients based on composition of the nutrient solution. The facts tell us that regulation of chlorophyll, carotenoids and vitamin C content can be controlled from the outside by optimizing the composition of the nutritional solution.

Table 3. Biochemical, chemical and technical composition of Stevia leaves

Indices	Ararat valley		Foothills	Nagorno Karabagh Republic (NKR) (Khanabad Village)	Literature data
	Soil	Hydroponics			
Extractive agents , %	45,2-54.0	46,0-63,8	47,9-52,9	46,1-52,0	32,5-40,9
Stevioside , %	8,1-8,4	8,9-9,2	8,5-8,6	8,0-8,5	4,6-8,2
Nitrogen, %	3,5-4,4	3,7-4,7	3,4-3,8	3,5-4,3	-
Proteins, %	22,4-26,3	23,1-28,9	21,2-24,5	21,8-26,9	-
Carotene, mg %	64,3	66,1	68.4	65,4	-
Chlorophyll a+b, mg%	119,11	143,1	131,2	124,4	-
Vitamine "C" mg%	62,3	74,9	72,1	68,9	-
Tanning agent, %	13,9	10,2	12,5	12,9	-
Flavonoids, %	5,1	4,4	4,8	5,2	3,5
Essential oil, %	0,1	0,2	0,2	0,1	-
Endemic microelements, mg/100g					
,,_,, J	0,8	8,7	0,6	0,5	
,,_,, Zn	0,9	1,3	0,7	0,6	no detected
,,_,, Ge	0,00012	no detected	0,0001	0,00016	no detected

Diterpene glycosides from different samples of stevia leaves

Glycosides, % (76, 81, 69, 83 final product content)

Content of monosaccharides (final product content), %, (0,07; 0,09; 0,12; 0,05)

Stevia leaf samples

1. Hydroponics
2. Soil (Ararat plain)
3. Soil Ukraine (Crimea)
4. Soil Nagorno-Karabakh (Askeran district)

Stevia (Stevia rebaudiana Bertoni), has been studied according to biological, agro-technical, biochemical, radiation, toxicological, food safety and other indices. It has been introduced into RA and NKR, passed the production testing phase, provided above average yield according to international standards (in soil conditions 2.0-2.5 t/ha, in hydroponics 4.0-5.0 t/ha), as well as high content of endemic microelements (iodine – 8.7 mg/100g, zinc-1.3 mg/100g, germanium-0.12 mg/100g) and stevioside (8.0-9.2%). It has been registered as a new technical crop by the Institute of National Standards, RA (Patent N2779A). Ultramicroelement germanium (Ge), which is described as an anti-cancer element and can be a separate field for mineral nutrition and physiological studies of plant, has been discovered in Stevia. According to physiological studies Stevia has thermal regulation, anti-stress, antioxidant, anti-paradontal properties; it reduces and stabilizes the content of glucose in the blood plasma and promotes brain and spinal cord neuronal activity.

- We have shown that under impaired glucose metabolism conditions stevia adapts brain network by stabilizing of depression / or strengthening of depression mediated by activation of excitatory neurotransmitters [31]. Data on the short-term activation and long-term depressor effects were in favor in the functioning of Stevia as an allosteric modulator, which is consistent with the concept of molecular and cellular factors controlling signal transduction via transferred allosteric modulator proteins, a key one being G-protein-coupled receptor for natural substrates. At the systemic level, allosteric modulation of Stevia provides for neuroprotection by long-term changes of allosteric ligands, which in turn leads to an increase in neurotrophic factors. This intriguing possibility requires further investigation.
- The superoxide and consequently NADPH oxidase (Nox) are relevant targets involved in biological effects of Stevia. The presence of NADPH-containing superoxide-producing lipoprotein (suprol) in Stevia leaves has not yet been tested. The mechanism of producing superoxide radicals (O_2^-) by suprol was determined in vitro, which is associated with the electron transfer from NADPH in the composition of suprol by traces of transition metal ions (Fe^{3+} or Cu^{2+}) to molecular oxygen, turning it into O_2^-. It is expected that the therapeutic efficacy of Stevia leaves is caused by specific activity of superoxide--producing lipoprotein fraction [32].

Thus, biodiversity, biochemical, toxicological and complex studies on food security conducted in Armenia and Nagorno-Karabakh under the vegetation and industrial conditions confirm that Stevia is imported and raw materialcan be exported for sale on the international market as holder of the monopoly. Herbal product contains physiological active substances, endemic elements, and especially a microelement germanium [33]. In animal model, results of physiological studies have shown that Stevia has beneficial effects in cardiovascular diseases, blood glucose regulation [34] and functional changes in thermoregulation [35]. The effect of Stevia under stress conditions was also investigated [36].

A new electrophysiological direction has also been included [31]. After a single injection of a therapeutic dose of stevioside (the main pharmacologically active component of Stevia leaves) in vivo extracellular recording of neuronal activity in the hippocampus and amygdala revealed activation of excitatory responses, suggesting that Stevia adapts neural networks of the brain by imposing and / or strengthening depression mediated by activation of the excitatory neurotransmission system. Fundamental data of relationship between modulation of neuronal synaptic activity and NADPH--oxidase activity in the brain structures involved in learning and memory – a new theoretical basis for effectiveness of the Armenian Stevia to prevent the adverse effects of high fructose consumption, which dictates the feasibility of introducing the Armenian Stevia as a dietary food product with a view to improving the quality of life of the population [31; 37].

Various research data revealed that plants may worked as healing and regeneration of the tissue by multiple mechanisms [38]. Traditional uses and earlier reports have revealed, enhanced healing with less scarring of cuts, wounds, burns, acne, seborrhea, dermatitis, and psoriasis after topical application of aqueous Stevia extracts [39]. Stevia preparations in the traditional system of medicine to promote wound healing. This effect may be explained by several mechanisms such as coating the wound. Further the Stevia leaf powder did not produce any adverse effect and because of this it is possible to recommend its use in the treatment of wounds. We have shown the wound healing activity of hydroponic Stevia leaf powder and found to be effective in the functional recovery of the wound healing [40]. We have shown membrane stabilizing effects of Stevia rebaudiana Bertoni in fructose-induced type II diabetic rats associated with protection against the damaging effects of superoxide and hydroxyl radicals – active stimulators of tissue lipid peroxidation and oxidative stress [41]. We have recorded the activity of neurons in the hippocampus and the amygdala after a single injection of stevioside (the main pharmacologically active component in leaves of the stevia plant) in rats after prolonged consumption of dietary fructose. Predominant activation of ex-

citatory neurotransmission was revealed during single injection of therapeutic dose of stevioside [42]. I/m injection of stevioside after sciatic nerve crush-injury in condition of diabetes causes a rapid recovery of the sensory function of the damaged limb on 12 day post crush-injury and a restoration of the motor function after 7 days. Predominant inhibition of the background spike activity of spinal cord motoneurons after injection of therapeutic dose of stevioside was revealed. Stevia rebaudiana suppress the production of NADPH-dependent $O2^{-}$-producing activity by fructose-induced oxidative stress in spinal cord tissue through membrane-stabilizing effect. Generally, in conditions of sciatic nerve crush-injury at fructose-rich diet stevioside from Armenian Stevia rebaudiana is able to increase the positive adaptive changes of central nervous system in rats, improving the functional recovery [43].

Sumary

Thus, Stevia rebaudiana is gaining popularity in various developed and developing countries as an important crop for the production of nonnutritive, nontoxic, high-potency sweeteners. Stevia rebaudiana Bertoni has been imported to the RA and Artsakh, and passed through the phase of production test. Complex researches have been carried out concerning to biological, biochemical and physiological influence of Stevia rebaudiana Bertoni and food safety. As a result, it was provided, at least, 2,0-2,5 t/ha harvest in dry leaves soil culture and 4,5-5,0 t/ha harvest of preferable quality with the use of hydroponic phytotechnology. Stevia rebaudiana Bertoni was proposed by NAS Institute of Hydroponics Problems of RA and was registered in RA National institute of standards, as a new technical cultured plant in Armenia. The cultivation of stevia might represent a formidable opportunity for the growers, in order to diversify the cropping systems and to meet the increasing market demand for high-quality and traceable raw material. In addition, several legislative initiatives, such as the steviol glycosides approval as food additive in several countries, represent favorable factors for the development of a stevia-based agro-industry.

References

1. Kinghorn,A. D. and Soejarto,D. D. 1985. Current status of stevioside as a sweetening agent forhuman use. In H. Wagner, H. Hikino, and N. R. Farnsworth, eds. Economic and medical plant research. Academic Press, London, UK.

2. Megeji,N. W., Kumar,J. K.,Virendra,S.,Kaul,V. K. and Ahuja,P. S. 2005. Introducing Stevia rebaudiana, a natural zero-calorie sweetener. Current Sci. 88: 801804.

3. Lester,T. 1999. Stevia rebaudiana (sweet honey leaf). Aust. New Crops News Lett. 11. Nat. Prod. Radiance 2: 120.

4. Frederico,A. P., Ruas, P. M.,Marin-Morales,M. A., Fuas, C. F. and Nakajima,J. N. 1996. Chro-

mosome studies in some Stevia Cav. (Compositae) species from Southern Brazil. Braz. J. Genet. 19: 605609.

5. Dwivedi,R. S. 1999. Unnurtured and untapped super sweet nonsacchariferous plant species in India. Current Sci. (Bangalore) 76: 14541461.

6. Taiariol,D. R. 2004. Characterization of the Stevia rebaudiana Bert. [Online] Available: http://www.monografias.com/trabajos 13/stevia/stevia html.

7. Singh,S. D. and Rao,G. P. 2005. Stevia: The herbal sugar of the 21st century. Sugar Technol. 7: 1724.

8. Kinghorn,A. D. 1987. Biologically active compounds from plants with reputed medical and sweetening properties. J. Nat. Prod. 50: 10091024.

9. Sekaran,T.,Giridhar,P. and Ravishankar,G. A. 2007. Production of steviosides in ex vitro and in vitro grown Stevia rebaudiana Bertoni. J. Sci. Food Agric. 87: 420424.

10. DuBois,G. E. 2000. Sweeteners: non-nutritive. Pages 2245 2265 in F. J. Francis, ed. Encyclopedia of food science and technology Vol.4. 2nd ed. John Wiley and Sons, Inc., New York, NY.

11. Oddone,B. 1999. How to grow Stevia. Guarani Botanicals, Inc., Pawcatuck, Connecticut. pp. 130.

12. Kennely,E. J. 2002. Sweet and non-sweet constituents of Stevia rebaudiana (Bertoni) Bertoni. Pages 6885 in A. D. Kinghorn, ed. Stevia, the genus Stevia: Medical and plant industrial profiles. Vol.19. Taylor and Francis, London, UK.

13. Cioni,P. L.,Morelli,L.,Andolfi,L.,Macchia,M. and Ceccarini,L. 2006. Qualitative and quantitative analysis of essential oils of five lines Stevia rebaudiana Bert. genotypes cultivated in Pisa (Italy). J. Essential Oil Res. 18: 7679.

14. Totte´,N.,Caron,L., Rohmer,M.,Compernolle,F.,Baboeuf,I. and Geuns,J. M. C. 2000. Biosynthesis of the diterpenoid steviol, an ent-kaurene derivative from Stevia rebaudiana Bertoni, via the methylerythritol phosphate pathway. Tetahedron Lett. 41: 64076410.

15. Eisenreich,W., Rohdich, F. and Bacher,A. 2001. Deoxyxylulose phosphate pathway to terpenoids. Trends Plant Sci. 6: 678684.

16. Brandle,J. E.,Richman,A., Swanson,A. K. and Chapman, B. P. 2002. Leaf ESTs from Stevia rebaudiana: A resource for gene discovery in diterpene synthesis. Plant Mol. Biol. 50: 613622.

17. McGarvey,D. J. and Croteau, R. 1995. Terpenoid metabolism. Plant Cell 7: 10151026.

18. Shibata,H.,Sawa,Y.,Oka,T.,Sonoke,S.,Kim,K. K. and Yoshioka,M. 1995. Steviol and steviol-glucoside: glucosyltransferase activities in Stevia rebaudiana Bertoni: purification and partial characterization. Arch. Biochem. Biophys. 321: 390396.

19. Bondarev,N. I.,Sukhamova,M. A.,Reshetnyak, O. V. and Nosov,A. M. 2003. Steviol glycoside content in different organs of Stevia rebaudiana and its dynamics through ontogeny. Biol. Plant. 47: 261264.

20. Kang,K. H. and Lee,E. W. 1981. Physio-ecological studies on stevia (Stevia rebaudiana Bertoni). Korean J. Crops Sci. 26: 6989.

21. Anufrieva,E. N., Volodin,V. V., Nosov,A. M., Garcia,M. and Lafont,R. 1998. The content and composition of ecdysteroids in plants and tissue cultures of Serratula caranata. Russ. J. Plant Physiol. 45: 326332.

22. Yamazaki, T.,Flores, H. E.,Shimomura, K. and Yoshihira,K. 1991. Examination of steviol glucosides production by hairy root and shoot cultures of Stevia-rebaudiana. J. Nat. Products (Lloydia) 54: 986992.

23. Goettemoeller,J. and Ching,A. 1999. Seed germination in Stevia rebaudiana. Pages 510511 in J. Janick, ed. Perspectives on new crops and new uses. ASHS Press, Alexandria, VA.

24. Ermakov,E. I. and Kotechetov,A. A. 1996. Specific features in growth and development of Stevia plants under various light regimes in regulated conditions. Doklady Rossiiskoi Akademii Sel'skokhozyaistvennykh Nauk 10: 89.

25. Shock,C. C. 1982. Rebaudi's stevia: natural non-caloric sweeteners. California Agric. 36: 45.

26. Nakamura,S. and Tamura,Y. 1985. Variation in the main glycosides of stevia. Jpn. J. Trop. Agric 29: 109115.

27. Tateo,F.,Mariotti,M.,Bononi,M.,Lubian,E.,Martello,S. and Cornara,L. 1998. Stevioside content and morphological variability in a population of Stevia Rebaudiana (Bertoni) Bertoni from Paraguay. Italian J. Food Sci. 10: 261267.

28. Isoyan A.S., Hovhannisyan L.E., Babakhanyan M.A., Avetisyan L.G., Nebogova K. A., Nahapetyan Kh.H. Assessment of toxicity of hydroponic Stevia rebaudiana Bertoni: Biochemical approaches. Toxicon, Volume 159, Supplement 1, 5 March 2019, Page S15.

29. Ghochikyan V. T., Manukyan L. S., Tsaturyan A. O., Sahyan A. S., Kochikyan A. T., Hovhannisyan L. E., Hovsepyan H. Y., Babakhanyan M. A., Development of an effective method for the isolation of sweet glycosides from stevia leaves (Stevia rebaudina Bertoni), grown in Armenia and Nagorno-Karabakh. Biolog.Journ. Armenia, 2016, 1 (68), p. 70-74.

30. Abelyan V. A., Balayan A. M., Ghochikyan V. T., Markosyan A. A. Transglycosylation of Stevioside by Cyclodextrin Glucanotransferases of Various Groups of Microorganisms. Applied Biochemistry and Microbiology (2004) 40: 129.

31. Chavushyan V. A., Simonyan K. V., Simonyan R. M., Isoyan A. S., Simonyan G. M., Babakhanyan M. A., Hovhannisyian L. E., Nahapetyan Kh. H., Avetisyan L. G., Simonyan M. A., Effects of stevia on synaptic plasticity and NADPH oxidase level of CNS in conditions of metabolic disorders caused by fructose. BMC Complementary and Alternative Medicine, 2017, 17:540.

32. Isoyan A.S., Simonyan K.V., Simonyan R.M., Babayan M. A., Simonyan G.M., Chavushyan V.A., Simonyan M.A. Superoxide-producing lipoprotein fraction from Stevia leaves: definition of specific activity. BMC Complementary and Alternative Medicine. 2019; 19:88.

33. Avetisyan L. G., Chavushyan V. A., Simonyan K. V., Ghochikyan V. T., Hovhannisyan L. E., Babakhanyan M. A. The effect of stevioside on the functional recovery of damaged sciatic nerve in rats receiving fructose-rich food. Medical Science of Armenia 2017, vol. LVII, N1, p. 67-79.

34. Madan S, Ahmad S, Singh GN, Kohli K, Kumar Y, Singh R, Garg M. Stevia rebaudiana (Bert.) Bertoni- A Review. Indian J Nat Prod Resour. 2010. 1(3):267-286.

35. Harutjunyan R.A., Nahapetyan Kh.H., Simonyan K.V., Babakhanyan M.A., Hovsepyan H.Y., Hovhannisyan L.E. (2014). Separate and combined action of cicorium and stevia on thermoregulatory mechanisms in rats. Medical Science of Armenia, № 4, pp. 71-76.

36. Adamyan Ts. I., Gevorgyan E. S., Karapetyan H. M., Hovhannisyan L. E. The combined effect of Stevia rebaudina Bertoni and immobilization stress on the morphofunctional indices of leukopoiesis and the activity of proline enzyme biosynthesis. Biological Journal of Armenia,

vol. LXVII, N1, 2015, p. 64-69.

37. Babakhanyan M.A., Hovhannisyan L.E., Simonyan K.V., Isoyan A.S., Zakaryan Sh.S., Kochikyan V.T., Ghukasyan A.G. The production test, physiological influence and introduction of strategic cultured plant Stevia rebaudiana Bertoni in Armenia and Artsakh. Electronic Journal of Natural Sciences. 2019; 32(1):22-26.

38. Dewangan H, Bais M, Jaiswal V, Verma VK (2012) Potential wound healing activity of the ethanolic extract of Solanum xanthocarpum schrad and wendl leaves. Pak J Pharm Sci 25: 189-194.

39. Stephen YG, Emelia K, Francis A, Kofi A, Eric W (2010) Wound healing properties and kill kinetics of Clerodendron splendens G Don, A Ghanaian wound healing plant. Pharmacognosy Res 2: 63-68.

40. Babakhanyan MA, Nahapetyan Kh. H, Hovhannisyan LE, Simonyan KV, Avetisyan LG, Avetisyan RA, Chavushyan VA Wound healing potential of hydroponic stevia rebaudiana in rats. Physical Medicine and Rehabilitation Research 2017; 2(3):1-4.

41. Simonyan R.M., Chavushyan V.A., Isoyan A.S., Simonyan G.M., Simonyan K.V., Avetisyan L.G., Nahapetyan Kh.H., Babakhanyan M.A., Simonyan M.A. The membrane stabilizing effects of Stevia rebaudiana Bertoni on a fructose-induced rat model of type 2 diabetes. Medical Science of Armenia, 2015; № 3, pp. 51-60.

42. Isoyan A.S. Stevia rebaudiana as a potential modulator of neuronal activity in insulin resistant model, Issues in theoretical and clinical medicine, 2016; 6(110):35-39.

43. L.G. Avetisyan, V.A. Chavushyan, K.V. Simonyan, V.T. Ghochikyan, L.E. Hovhannisyan, M.A. Babakhanyan. Impact of stevioside on indices of the functional recovery of rat's injured sciatic nerve in conditions of fructose-rich diet. Medical Science of Armenia, 2017; 57(1):67-79.

16 - Neuronutrição no distúrbio do espectro autista
Neuronutrition on autism spectrum disorder

Nathália de Freitas Penaforte[1]

Resumo

O distúrbio do espectro autista (DEA) pode se identificar pela presença da: 1) deficiência qualitativa da interação social recíproca; 2) habilidades de comunicação deficiente; 3) comportamentos restritos, repetitivos e estereotípicos; 4) síndromes comportamentais neurodesenvolvidas do comprometimento verbal e comunicação não verbal. Trata-se de uma perturbação global do funcionamento cerebral, afetando numerosos sistemas e funções, eventualmente com múltiplas causas, apresentando alterações principalmente no corpo caloso, amígdala e cerebelo. Dentre as comorbidades associadas estão as alterações na autoimunidade, retardo mental e consumo alimentar com uma limitada variedade de alimentos coligada aos comportamentos característicos e a gravidade da sintomatologia, além de interferir na função da barreira intestinal que reflete na capacidade de ingestão e absorção dos alimentos e nutrientes. A criança com DEA desenvolve uma recusa a experimentar novas atividades, novos comportamentos e consequentemente novos alimentos, que combinado às alterações na capacidade sensorial faz com que a criança tenha um perfil alimentar diferenciado e individualizado, que segundo estudos, essas se apresentam em maior risco de ter sobrepeso e obesidade quando comparadas com crianças com desenvolvimento típico. A suplementação vitamínica e mineral é considerada um tratamento comum no DEA, por terem mais insuficiência, os quais apresentam uma elevação do estresse oxidativo, principalmente devido à diminuição sérica dos níveis de glutationa, anormalidades metabólicas e nutricionais, incluindo problemas de sulfatação, metilação e disfunção mitocondrial. Investigando quais micronutrientes estão relacionados a questões comportamentais típicos do DEA, e seus níveis. O desenvolvimento precoce do cérebro é considerado um dos fenômenos de grande interesse ainda estudado, ressaltando que o período gestacional pode haver índice de probabilidade quando há uma nutrição indesejada, principalmente em período crítico para o desenvolvimento cerebral. Também foi constatado que mais de 80% dos pais e profissionais da saúde analisados,

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utilizavam como tratamento a intervenção dietética, incluindo a dieta sem glúten e sem caseína, uma vez que essa exclusão seria eficiente, devido à teoria dos peptídeos opióides de origem exógena. Porém, diante da pluralidade de evidências sugestivas de diferentes respostas desse uso, foi proposto que só deva introduzir somente após diagnóstico de qualquer intolerância ou alergia a alérgenos alimentares a serem eliminados da dieta, ou outras condições de prováveis benefícios, solicitando novos estudos para consolidação. Além disso, evidências relatam uma coligação significativa entre o DEA e os distúrbios gastrointestinais, acreditando-se que tenha um papel imprescindível na homeostase intestinal e sistêmica, assim como na modulação comportamental e desequilíbrios microbianos recorrentes na microbiota gastrointestinal. Apesar das evidências ressaltarem a premissa terapêutica do uso de probióticos, prebióticos e simbióticos diante de alterações na microbiota gastrointestinal no DEA como uma estratégia eficaz, são necessários mais estudos.

Palavras-chave: autismo, eixo intestino-cérebro, micronutrientes, estado nutricional, dieta sem glúten e sem caseína.

Abstract

Autistic spectrum disorder (ASD) identified by the presence of: 1) qualitative deficiency of reciprocal social interaction; 2) poor communication skills; 3) restricted, repetitive and stereotyped behaviors; 4) neurodeveloped behavioral syndromes of verbal impairment and non-verbal communication. It is a global disturb of brain functioning, affecting numerous systems and functions, possibly with multiple causes, presenting changes mainly in the corpus callosum, amygdala and cerebellum. Among the associated comorbidities are alterations in autoimmunity, mental retardation and food consumption with a limited variety of foods related to the characteristic behaviors and severity of the symptomatology, besides interfering in the function of the intestinal barrier that reflects in the capacity of food intake and absorption of the nutrients. The child with ASD develops a refusal to experiment with new activities, new behaviors and consequently new foods, which, combined with changes in sensory capacity, causes the child to have a differentiated and individualized food profile, which, according to studies, these have a higher risk of overweight and obesity when compared to children with typical development. Vitamin and mineral supplementation considered a common treatment in ASD due to deficiencies, which present an elevation of oxidative stress, mainly due to serum levels of glutathione, metabolic and nutritional abnormalities, including sulfation problems, methylation and mitochondrial dysfunction. Investigating which micronutrients related to behavioral issues typical of the ASD, and their levels. The early development of the brain considered one of the phenomena of great interest

still studied, emphasizing that the gestational period may have probability index when there is undesired nutrition, especially in a critical period of brain development. It was also found that over 80% of parents and health professionals, used as treatment dietary intervention, including diet without gluten and casein, since such exclusion would be effective because of the theory of opioid peptides of exogenous origin. However, in view of the diversity of evidence suggestive of different responses to this use, it proposed that it should only introduced after diagnosis of any intolerance or allergy to food allergens to eliminated from the diet, or other conditions of probable benefits, requesting further studies for consolidation. In addition, evidence reports a significant association between ASD and gastrointestinal disorders and believed to have an essential role in intestinal and systemic homeostasis as well as behavioral modulation and recurrent microbial imbalances in the gastrointestinal microbiota. Although the evidence emphasizes the therapeutic premise of the use of probiotics, prebiotics and symbiotics in view of changes in the gastrointestinal microbiota in the ASD as an effective strategy, further studies are needed.

Key words: autism, intestine-brain axis, micronutrients, nutritional status, gluten--free diet and casein-free.

Introdução

O distúrbio do espectro autista (DEA) se identifica pela presença da deficiência qualitativa da interação social recíproca, habilidades de comunicação deficiente e por interesses e comportamentos restritos, repetitivos e estereotípicos, sendo cada vez mais prevalentes síndromes comportamentais neurodesenvolvidas do comprometimento verbal e comunicação não verbal[1,2] ,

No mundo, estima-se que o DEA afeta 1% da população, sendo quatro vezes mais prevalente entre pessoas do sexo masculino do que entre o sexo feminino[3]. Estimativas apresentadas pelo Centers for Disease Control and Prevention (CDC), revelou, com base em dados obtidos em 2014, nos Estados Unidos, uma prevalência de 16,8 por 1000, ou seja, uma em cada 59 crianças com oito anos de idade tem DEA no país[4].

No contexto brasileiro, a prevalência varia de 4 a 13 casos a cada 10.000 crianças, ocupando o 3º lugar entre os distúrbios do desenvolvimento infantil[5,6]. Diante de um estudo epidemiológico no Sul do Brasil, verificou-se uma prevalência de 3,85/10.000 habitantes, sendo o maior número de casos relatados na faixa etária de 5 a 9 anos de idade, observando que houve uma maior proporção de casos no sexo feminino em Santa Catarina comparado aos outros estados avaliados[7].

Foram realizadas diversas pesquisas acerca das causas do autismo, porém seu mecanismo ainda é idiopático, sendo associada a múltiplos fatores, seja genético, ambien-

tal, fatores imunológicos que desempenham papéis não-negligenciáveis na etiologia[8,9]. Trata-se de uma perturbação global do funcionamento cerebral, afetando numerosos sistemas e funções, eventualmente com múltiplas causas. Foi sugerido também que a idade dos pais possa ser um fator de risco claro à etiologia do DEA[9,10].

Outras pesquisas diagnósticas têm concentrado suas investigações nas causas cerebrais e genéticas, além do papel potencial de fatores bioquímicos e sistêmicos, que podem ser extrínsecos ao sistema nervoso, mas ter efeitos secundários sobre o cérebro[8]. Estudo comparou gêmeos monozigóticos (MZ) e dizigóticos, concluindo que a taxa de concordância para gêmeos MZ é mais elevada, ressaltando as taxas de concordância variam de acordo com o diagnóstico e com o subtipo de autismo considerado. Além disso, também demonstraram que a taxa de concordância do DEA em gêmeos MZ é incompleta [11].

O cérebro de uma pessoa com DEA apresenta falha de comunicação entre os neurônios, dificultando o processamento de informações. Apresentando alterações principalmente no corpo caloso, que é responsável por facilitar a comunicação entre os dois hemisférios do cérebro, a amígdala, responsável pelo comportamento social e emocional e o cerebelo, que está envolvido com as atividades motoras, como o equilíbrio e a coordenação[12].

Dentre as comorbidades comumente associadas incluem alterações na função da barreira intestinal, autoimunidade e retardo mental, além de interferir no consumo alimentar[8,13,14].Dentre esses sintomas podem-se classificar três categorias de comportamentos que vão afetar o perfil alimentar do autista, a seletividade alimentar, a recusa de alimentos, pelo fato de haver uma resistência a prova de novos alimentos e a indisciplina durante as refeições, fator que é bem característico na infância e no próprio transtorno. São esses fatores que fazem com que a criança com DEA possua uma dieta monótona e de variedade limitada, acarretando prováveis carências nutricionais[15].

Desconforto gastrointestinal, inflamação intestinal, diarréia, constipação e refluxo ácido, são algumas outras alterações características nessas crianças que afeta seu estado nutricional, refletindo na capacidade de ingestão e absorção dos alimentos e nutrientes, respectivamente[16,17]. Estudos consideram crianças com DEA como um grupo de risco no desenvolver de possíveis carências nutricionais, principalmente de micronutrientes[18,19].

Evidências relatam uma coligação significativa entre o DEA e os distúrbios gastrointestinais (DG), a partir do eixo intestino-cérebro por seu envolvimento na manutenção da barreira intestinal, expressão de neurotransmissores e seus receptores, sendo uma comunicação bidirecional envolvendo o sistema nervoso entérico, o nervo vago, o sistema nervoso simpático e parassimpático e o sistema endócrino e imune, o que tem despertado um interesse em pesquisas diante de sua[20-24].

Acredita-se que tenha um papel imprescindível na homeostase intestinal e sistêmica, assim como na modulação comportamental e desequilíbrios microbianos recor-

rentes na microbiota gastrointestinal (MG), como já foi evidenciado em pesquisas de modelo animal[24-26] e humano[27-32] além de estudos já terem relatado esses sintomas com a gravidade do DEA[13, 33-35].

Perfil Alimentar

A alimentação inadequada coligada a falta de equilíbrio energético torna-se de fato uma preocupação em crianças com DEA, uma vez que a ingestão insuficiente de macro e micronutrientes está estreitamente ligada ao estado nutricional, a limitada variedade de alimentos e a gravidade da sintomatologia associada ao transtorno, o que pode ser impactante na qualidade de vida desses pacientes[36].

Embora, mesmo que essa possua uma dieta variada e adequada sob a óptica nutricional, ela precisa ser capaz de executar algumas funções básicas, que infelizmente não se adequa, como: 1) digerir e quebrar adequadamente o alimento de forma absorvível; 2) absorver os nutrientes por meio do trato gastrointestinal saudável; 3) e converter os nutrientes a serem utilizados a nível celular[37].

Sabe-se que os alimentos ultraprocessados apresentam alta densidade energética, excesso de gorduras totais e saturadas, baixo teor de fibras e maior concentração de açúcar e sódio (Na), além de apresentarem em sua composição uma quantidade deficiente de vitaminas e minerais, essenciais para o controle das deficiências nutricionais apresentadas pelos autistas, mas que vem sendo associado ao excesso de peso nesse público, segundo estudos[38-40].

Estudo avaliou o consumo alimentar de macronutrientes de 62 crianças com DEA e constatou que apresentaram elevada ingestão de proteínas e adequado consumo de carboidratos e lipídeos[41], o que foi controverso em outro estudo em que detectaram um consumo reduzido de proteínas, abaixo do recomendado[42]. Sendo, portanto, evidenciado em estudo produzido na China, que crianças com DEA consumiam menos macronutrientes em comparação com as de desenvolvimento típico (DT)[43].

Já em outra pesquisa o teor de carboidrato e proteínas se apresentou dentro da recomendação para a faixa etária[44], porém o de lipídeos não alcançou a recomendação, ressalvando que dietas com essa insuficiência pode levar a redução da absorção de alguns micronutrientes, como as vitaminas lipossolúveis[45].

Estado Nutricional

Pesquisa desenvolvida na Espanha avaliou medidas antropométricas de 105 crianças com DEA e 495 crianças com DT de 6 a 9 anos de idade, concluindo que as diagnosticadas com DEA apresentaram maior risco para baixo peso, mencionando que existiam diferenças na ingestão total de energia, e que o índice de alimentação saudável e diferenças no escore de variedade alimentar não foram significantes[46]. O que foi controver-

so em estudo transversal no Maranhão do Brasil, o qual avaliou o estado nutricional de 29 crianças com DEA pelos indicadores de índice de massa corporal/idade e estatura/idade, verificando que 55,2% classificou-se com excesso de peso, ressaltando que o consumo de alimentos ultraprocessados foram responsáveis por 28% da contribuição calórica[40].

Evidências ressaltam que crianças e adolescentes com DEA e transtorno do déficit de atenção/hiperatividade estão em maior risco de ter sobrepeso e obesidade quando comparadas com crianças com DT, sendo imprescindível o rastreamento oportuno[47,48], o que foi conferido uma prevalência de 42,4% para sobrepeso e 21,4% para obesidade no DEA em outro estudo[49]. Já uma esquisa de cunho transversal no Sul do Brasil não encontrou diferença significativa do estado nutricional entre crianças de idade pré-ecolar com DEA leve e moderado[50].

Micronutrientes e sua relação com o comportamento

Evidências relatam que crianças com DEA apresentam uma elevação do estresse oxidativo (EO), principalmente devido à diminuição sérica dos níveis de glutationa, anormalidades metabólicas e nutricionais, incluindo problemas de sulfatação, metilação e disfunção mitocondrial[51,52]. Uma baixa concentração de enzimas antioxidantes séricas, nutrientes antioxidantes e níveis de glutationa assim como um aumento da concentração de pró-oxidantes que foram encontrados em estudos com esse público[53].

As reações neuroimunes desempenham um papel patogênico em parte dos indivíduos com DEA, sendo provável que as anormalidades metabólicas de inflamação e EO estejam relacionadas às alterações de estrutura e função nestes pacientes, contribuindo para a diversidade de fenótipos, sendo necessária a compreensão do papel da neuroinflamação[54,55]. Sendo relatado que o cérebro de um paciente com DEA, há uma expressão alterada de genes associados à integridade da barreira hematoencefálica relacionada a um aumento desse processo inflamatório[56].

A suplementação vitamínica ou mineral é considerada o tratamento mais comumente usado para o DEA. Considerações específicas nesse público, como a característica e o comportamento do DEA, podem ser aumentadas ou, pelo menos, reduzir em maior risco devido ao estado nutricional sub-ótimo[52]. Além disso, crianças com DEA apresentaram mais insuficiências vitamínicas e minerais do que as crianças com DT em pesquisa, e seus níveis estavam relacionados aos sintomas característicos do transtorno[9].

As crianças necessitam relativamente mais zinco (Zn) em comparação aos adultos, tanto para o crescimento como para o desenvolvimento neurológico e cognitivo, ressaltando seu papel crucial no sistema imune, atuando como anti-inflamatório, bem como no sistema de defesa antioxidante[45,57]. Estudos têm demonstrado que um número considerável de crianças com DEA apresentaram pouca a grave deficiência desse mineral[58]. O que foi sugerido por pesquisadores uma possível alteração no funcionamento do

sistema de expressão da metalotioneína neuroprotetora[59].

Em indivíduos com DEA já foi evidenciado o diagnóstico de hipovitaminose A relacionada ao consumo alimentar inadequado[44,60]. O que se faz importante uma adequada ingestão de Zn uma vez que, uma inadequada ingestão de vitamina A (VA) afeta a função de Zn mesmo que este mineral seja consumido em quantidades suficientes, sendo válido pontuar que essa deficiência pode resultar em falha no crescimento e dano ocular[45].

Estudo na China examinou também por meio de avaliações dietéticas e questionários sobre comportamentos alimentares em 154 crianças com DEA e 73 crianças com DT, verificando que o grupo com DEA apresentou baixo nível sérico de VA, podendo ser um fator de risco para o agravamento dos sintomas típicos do distúrbio[43]. O que foi sugerido por pesquisadores que essa vitamina em alta concentração pode ser um fator de proteção para crianças com autismo[9].

Já com relação as concentrações plasmáticas do selênio (Se) nos indivíduos com DEA os resultados são heterogêneos, tendo sido encontrados em valores normais, diminuídos e elevados[61-63].

Estudo realizado na China, que avaliou 53 crianças com DEA e 53 crianças com DT, na faixa etária de 4 a 6 anos de idade, mostrou que os níveis séricos de cálcio (Ca), VA e folato estavam mais baixos nos indivíduos com DEA do que nos controles[64], que corroborou com os resultados encontrados no estudo realizado na Espanha que apontaram 5 vezes mais risco de crianças com DEA, comparado com as crianças controle de não atingirem a meta recomendada de Ca e ferro (Fe) comparado ao grupo controle e 3 vezes mais risco no alcance de vitamina C (VC)[65].

Pesquisadores compararam o consumo alimentar por meio do inquérito Recordatório 24 horas (R24H) e grupos alimentares por faixa etária, encontrando um maior consumo de vitamina B6 (VB6) e vitamina E (VE) nos indivíduos com DEA em comparação aos de DT, e um menor consumo de Ca no grupo de crianças com DEA[66]. Outro estudo usando o mesmo método de avaliação identificou que crianças com DEA, com faixa etária de 6 a 9 anos, não alcançaram as recomendações dietéticas para VC e VE em comparação com os de DT[67]. Outro estudo avaliou o consumo alimentar nesse público e averiguou um menor consumo de Ca, fósforo (P), Se, tiamina, riboflavina e vitamina B12 (VB12) e um maior consumo de VE[42].

Diante do consumo alimentar de fibras por crianças com DEA estudo relatou que 38% apresentou adequação, porém 27% de forma inadequada e 35% nociva. Nesse mesmo estudo também verificou possível redução de 50% e 69,2% no consumo de Ca e Na, respectivamente, e o magnésio (Mg) apresentando-se com consumo nocivo além do recomendado em 58% das crianças avaliadas[44]. Em Virtude do maior envolvimento do Mg nas funções neurológicas, seus níveis elevados no plasma podem causar efeitos adversos, tornando-se muito grave[45].

A redução do consumo de Ca está diretamente ligada a diversas funções orgânicas

como modulação de sinais de transdução e metabolismo de produção de energia e proliferação celular, sendo alguns sintomas resultantes de sua deficiência: 1) ansiedade; 2) hiperatividade; 3) agitação; 4) alucinações; 5) irritabilidade; 6) nervosismo; 7) agressão; 8) estresse crônico; 9) e dificuldade de aprendizagem[45,68]. O que foi evidenciado essa deficiência em crianças com DEA, podendo refletir no seu comportamento[44,64-66]. Dando ênfase no seu envolvimento da atividade de transportar o aspartato/glutamato e no EO em cérebros autistas[69].

Já em outro estudo as concentrações de Ca, Mg, Fe, Zn e Folato foram positivamente correlacionadas com comportamento adaptativo, motor grosso, motor fino, linguagem e comportamento pessoal-social no DEA[9]. Disfunção mitocondrial, inflamação, desregulação imune, e oxidativa indicam a base biológica para os problemas comportamentais relatados em indivíduos com DEA, o que visa avaliar o tratamento de intervenção vitamínica e de minerais[70-72].

O folato e o VB12 são indispensáveis para o desenvolvimento neuronal normal, função e deficiências graves destas vitaminas pode afetar diretamente a função cerebral[45,73], o que foi relatado menores valores em dieta e soro em crianças com DEA quando comparado com crianças de DT[9,74].

Nutrientes como a VB6 também são de extrema importância para a metilação, transulfatação e sulfatação, que representam um conjunto de atividades bioquímicas que não funcionam adequadamente nos portadores com DEA. Quando da limitação dessas transformações metabólicas, os neurotransmissores não são adequadamente ativados, ocasionando sintomas de ansiedade, depressão, déficit de atenção e transtorno do sono[9].

Período gestacional: índice de probabilidade

O desenvolvimento precoce do cérebro é um dos fenômenos de maior interesse em pesquisas sobre autismo, a partir disso, estudo identificou o período crítico para o desenvolvimento cerebral atípico em indivíduos com DEA durante o período intrauterino, usando parâmetros pré-natais e pós-natais, após a 22ª semana de amenorréia, o qual é crucial para a laminação cortical e ativação glial[75].

Dentre os fatores de risco modificáveis associados, a deficiência de vitamina D gestacional está vinculada com o desenvolvimento cerebral alterado e traços relacionados ao DEA, o que foi determinado em uma grande amostra populacional de mães e seus respectivos filhos (n = 4229), em que a 25-hidroxivitamina D (25OHD) foi avaliada a partir de soros maternos de meia gestação e de soros neonatais, colhidos de sangue de cordão umbilical[76].

Enfatizando que o ácido fólico é vital para o neurodesenvolvimento inicial e por seu efeito protetor contra defeitos do tubo neural[45], foi sugerido, em estudo de modelo animal, que a sua suplementação possa facilitar a reversão ou compensação dos efeitos

epigenéticos de outras exposições pré-natais precoces que atrapalham o neurodesenvolvimento[77]. Já em estudo norueguês relatou que o risco de traços autistas em crianças expostas a drogas antiepilépticas no útero pode ser atenuado pela suplementação periconcepcional de ácido fólico e pelo status de folato[78].

Diante disso, o excesso da suplementação de ácido fólico já foi correlacionado com a incidência de 8,2 vezes no DEA, porém, tal estudo não pôde provar relacionamento direto, por haver limitação de informações acerca da quantificação suplementada[79]. Outros achados mostraram baixos níveis de S-adenosilmetionina em S-adenosil-homocisteína, sugerindo comprometimento nos processos de metilação e hipótese de que o metabolismo do folato é afetado em crianças com DEA, ressaltando que isso não significa necessariamente que tenha sido resultado de contribuições dietéticas maternas[51].

Os potenciais efeitos do excesso de suplementação de ácido fólico na gestação ainda são desconhecidos[80],uma vez que no período periconcepcional pode reduzir o risco de desenvolver DEA em indivíduos com deficiências no metabolismo do folato, mas o alerta se dá em não exceder a dose diária recomendada[80-82].

O etanol é conhecido por perturbar a memória através de perturbações induzidas no hipocampo, mais notavelmente durante o desenvolvimento cerebral[83,84]. Estudo avaliou a associação entre o uso materno de etanol e DEA, conferindo que no primeiro trimestre, 21,2% das mães avaliadas relataram uso de álcool em comparação com 18,1% e 18,2% das mães de crianças com DEA ou DT, respectivamente, entretanto, não foi capaz de concluir os dados[85].

Além disso, evidências apontam que gestantes que sofrem de doenças infecciosas, distúrbios inflamatórios ou condições auto-imunes estão com risco consideravelmente elevado de ter filhos com DEA[86-90]. Destacando que a ativação imune materna desregulada pode impedir a maturação normal do cérebro e promover o desenvolvimento de fenótipos relacionados ao DEA[91].

Glúten e caseína: dos mecanismos inflamatórios a alcance

Constatou-se que mais de 80% dos pais e profissionais da saúde analisados, utilizavam como tratamento a intervenção dietética, incluindo a dieta sem glúten e sem caseína (DSGSC)[92]. Uma vez que a exclusão do glúten e da caseína seria eficiente, devido à teoria dos peptídeos opióides de origem exógena[93,94].

Baseando-se que o glúten, constitui a principal proteína estrutural do trigo, que pode ser encontrado em outros cereais a exemplo do centeio, da cevada e da aveia, em que quando não é totalmente digerido pelo organismo, produz as exorfinas que são peptídeos estimulantes dos receptores opióides no cérebro[95-97].

Estudo recente relatou que em um subgrupo de crianças com DEA exibe reatividade imune aumentada ao glúten, cujo mecanismo parece ser distinto da doença celíaca,

cujo o aumento da resposta de anticorpos anti-gliadina e sua associação com sintomas gastrointestinais apontam para uma coligação de anormalidades de permeabilidade imunológica e/ou intestinal[98]. Ressaltando que foi sugerido por pesquisadores que o sistema imunológico disfuncional no DEA pode impactar profundamente o neurodesenvolvimento, função cognitiva e o comportamental[99].

Já os peptídios derivados das proteínas do leite são inicialmente liberados pela pepsina no estômago sob condições de pH ácido. Em seguida, são ainda hidrolisados por enzimas pancreáticas, a exemplo da tripsina, quimotripsina e peptidases de membrana, resultando em peptídios de diferentes comprimentos[96,100]. As hipóteses giram em torno da ocorrência de respostas imunes a proteínas alimentares e a presença de uma permeabilidade intestinal anormal que possivelmente resultaria na absorção de peptídeos incompletamente quebrados, seguindo de uma atuação opióde no Sistema Nervoso Central através da barreira hematoencefálica[101].

Estudo duplo-cego avaliou a introdução da DSGSC por 4 a 6 semanas em crianças com DEA com faixa etária de 3 a 5 anos, e estudo de desafio placebo controlado por 12 semanas, continuando a dieta, com um seguimento de 12 semanas, dentre os resultados, não houve estatísticas significativas nas medidas de funcionamento fisiológico, problemas de comportamento e/ou sintomas característico do distúrbio, sendo justificado pelo número relativamente pequeno da amostra[102].

Pesquisa com 80 crianças diagnosticadas com DEA foram divididas em dois grupos, avaliando a intervenção de dieta sem glúten (DSG) por 6 semanas, verificando que no grupo da DSG a prevalência de sintomas gastrointestinais reduziu significativamente (40,57% vs 17,10%), assim como em distúrbios comportamentais (80,03 ± 14,07 vs 75,82 ± 15,37), o que foi sugerido sua eficácia no controle desses sintomas[103].

Já uma recente investigação foi incapaz de demonstrar uma diferença de grupo estatisticamente significativa com relação a permeabilidade do intestino delgado em um coorte de crianças com DEA relativamente grande (n = 103) e um grupo controle pareado por idade e quociente de inteligência com necessidades educativas especiais (n = 30)[104].

Pesquisa realizada no Reino Unido investigou pais e especialistas a respeito de suas experiências diante do tratamento com DSGSC no DEA, verificando que 83% das crianças receberam uma variedade de manipulações dietéticas, sendo DSGSC em 29%, o que foi relatado pelos pais uma melhoria significante em 54% de sintomas gastrointestinais, 42% na concentração e atenção, 29% na comunicação e 25% na interação social[92].

Com intuito de determinar o efeito da suplementação de glúten e caseína em comportamento mal adaptativo, gravidade do sintoma gastrointestinal e excreção de proteína de ligação de ácidos graxos intestinais em 74 crianças com DEA por uma semana, verificou que não houve alteração, necessitando de mais estudos por tempo

prolongado ou outros mecanismos de dano enterocitário a ser explorado[105].

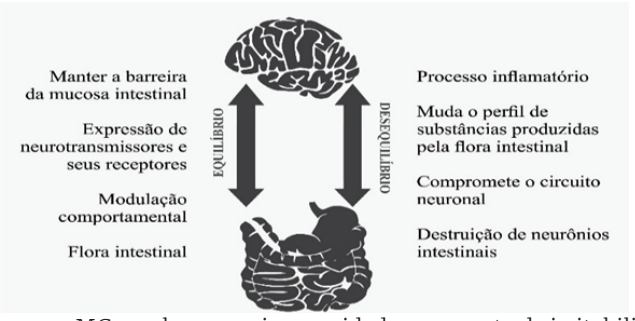
Diante da pluralidade de evidências sugestivas de diferentes respostas pelo uso de uma DSGSC, foi sugerido a combinação com algum progresso na determinação dos correlatos genéticos e biológicos subjacentes potencialmente relacionados a esses elementos dietéticos[65,107]. Assim como a solicitação de novas abordagens em nutrigenômica e nutrigenética, explorando a hipótese de que a resposta comportamental com DSGSC seja determinada por um endofenótipo relacionado específico[107]. Sendo proposto que só deva ser introduzida somente após diagnosticar qualquer intolerância ou alergia a alérgenos alimentares a serem eliminados da dieta, ou outras condições de prováveis benefícios[9].

Eixo intestino cérebro

Nos últimos anos, a MG tem sido implicada como uma via potencial que afeta a manifestação dos sintomas em distúrbios cognitivos e do neurodesenvolvimento, assim como na resposta imunológica, possivelmente associada a mudanças metagenômicas que compromete a função da barreira intestinal[32,108]. Há também a possibilidade de alterações induzidas por dieta podendo influenciar em alterações comportamentais e na flexibilidade cognitiva[25,26].

Além disso, estudos sugerem que há associação de atraso no desenvolvimento comportamental por desordens gastrointestinais, como a diminuída produção de enzimas digestivas, inflamações da parede intestinal e a permeabilidade intestinal alterada, nessas crianças[109-111]. Na Figura 1 é possível verificar o ponto de homeostase e que alterações podem ser desencadeadas diante de desequilíbrios no eixo intestino-cérebro no DEA.

Figura 1: Eixo intestino-cérebro e sua interação no DEA



Os problemas na MG revelaram maior gravidade no aspecto de irritabilidade, ansiedade, distúrbios do sono, transtornos de humor, intolerância alimentar e abstinência social, além de ter demonstrado esse grupo como menos propenso a responder o tratamento com tendência a desenvolver comportamentos agressivos[30,112,113]. Foi evidenciado que indivíduos com DEA que apresentam DG podem produzir mais citocinas pró-inflamatórias relacionadas à inflamação da mucosa e menos citocinas regulatórias, quando

comparado a indivíduos com DEA que não apresentam essas alterações intestinais[32].

As causalidades desses DG ainda não foram totalmente esclarecidas em autistas, porém, estudos tem associado ao uso excessivo de antibióticos orais, os quais devido ao sistema imunológico debilitado podem alterar a flora intestinal ao resultar no crescimento excessivo da flora patogênica[34,114-116].

Diante disso, se faz necessário classificar os microrganismos da MG como benéficas ao sistema ou patogênicas[35], o que vem sendo amplamente explorado se é a elevação desses ou o déficit dos microrganismos benéficos existentes no DEA como o principal provocador dessas alterações. As causalidades desses DG ainda não foram totalmente esclarecidas em autistas, porém, estudos tem associado ao uso excessivo de antibióticos orais, os quais devido ao sistema imunológico debilitado podem alterar a flora intestinal ao resultar no crescimento excessivo da flora patogênica[35,114-117].

O Clostridium, considerado um microrganismo produtor de toxinas, tem se revelado em maior abundância em indivíduos com DEA, principalmente em especial aqueles que tem demonstrado DG em comparação às que não apresenta esses sintomas[31,118]. Já a Bifidobactéria é classificada como um micróbio benéfico por ser capaz de melhorar a função da barreira epitelial, reduzir sintomas alérgicos, prevenir infecções por patógenos e produzir metabólitos bioativos, como ácidos graxos voláteis, vitaminas ou ácidos graxos poliinsaturados, que contribuem para a modulação da função intestinal e imunológica, porém se apresenta em menor abundância em crianças com DEA[108,116,118-120].

Ressalta-se que a Enterobacteriaceae já foi proposta como marcador de disbiose de MG e disfunção epitelial[121], e vale destacar que o Lactobacillus já foi relatado estar presente em altas concentrações em amostras fecais de crianças com DEA[33,116]. Prevotella, Coprococcus e Veillonellaceae são outras três espécies que se apresenta em menor abundância nesses indivíduos, sendo importantes degradadores de carboidratos e/ou de fermentadores e consequentemente necessárias para a homeostase intestinal[118].

Diante do aumento da permeabilidade intestinal que impacta o desenvolvimento neurológico, é sugerido modificar a MG com a administração de probióticos e prebióticos, definidos como ingredientes alimentares não digeríveis, que afetam beneficamente o hospedeiro constituindo uma estratégica terapêutica nesses pacientes, aumentando a integridade da mucosa intestinal assim como nos sintomas gastrointestinais, podendo ainda reduzir a presença de microrganismos nocivos e seus metabólitos[118,119].

Estudo com 160 crianças com DEA foi descoberto que 59% tinham DG, como diarreia ou fezes não formadas, constipação, inchaço e/ou refluxo gastroesofágico, também foram identificadas associações entre gêneros microbianos específicos[30]. Outros estudos corroboraram com esses sintomas relatados, sendo a constipação e a diarreia crônica moderada ou grave como os mais prevalentes nesse público estudado[27-29,112,122]. Diferentes graus desses sintomas em crianças com DEA foram identificados em um estudo pros-

pectivo, detectando 79,5% apresentando disbiose, sendo 61,29% dessas em grau III[117].

O comportamento alimentar seletivo (CAS) é comumente observado em crianças com DEA, sendo comportamentos repetitivos, sensibilidade sensorial e inflexibilidade à alimentação alguns dos sintomas para esse diagnóstico, associado à dificuldade motora e sensorial de forma persistente[29,123,124]. De certa forma foram observadas diferenças na ingestão de nutrientes e perfil da MG, visto que o grupo que apresentou características de CAS demonstraram maior abundância de Actinobacteria, Coriobacteriaceae, Clostridiales, Collinsella, Lactobacillus e Acidaminococcus, mas menor abundância de Bacteroidetes, Bifidubactéria, Cyanobacteria, Eggerthella, Bacteroides, Dialister e Anaerotuncs em sua MGI. Foi elucidada uma associação muito forte da quantidade de Desulfovibrio ssp. com a gravidade do autismo no escore do CAS[33,108].

Outros achados sugeriram a restrição alimentar para ter maior impacto no crescimento de bactérias do tipo Bifidobactérias e Veillonellaceae nesse tipo de transtorno, ressaltando uma abordagem dietética combinada de uma dieta prebiótica, o que resultou em uma melhora significativa no comportamento antissocial e dos DG, uma vez que já foi estimado que 50% das alterações da MG possam ser atribuídas à dieta. Pesquisadores que usaram essa terapia no DEA relataram como resultado ter tido uma melhora na barreira da mucosa e da disbiose do intestino, assim como elevações em vários metabólitos associados, abolindo perturbações comportamentais característicos desse distúrbio[118,125,126].

A terapia probiótica pode ser uma alternativa de intervenção eficaz diante de dietas restritivas, como é o caso de muitas destinadas à indivíduos com DEA. Justificando essa terapia por ter potencial de reconstituir ou estabilizar a barreira intestinal por meio da produção de mucina, sintetizar antioxidantes para proteção contra microrganismos nocivos, produzir enzimas digestivas e modulação de respostas imunes suprimindo as funções desreguladas[22,127,128].

A introdução probiótica por 4 meses em crianças com DEA de faixa etária de 2 a 9 anos de idade que apresentavam DGI, reduziu não só esses sintomas como também a taxa de inflamação[33]. Corroborando com os resultados de um estudo mais recente que fez essa introdução de três cepas probióticas composta por Lactobacillus acidophilus, Lactobacillus rhamnosus e Bifidobacteria longum, por 3 meses em crianças com DEA que tinham 5 a 9 anos de idade, o que melhorou significativamente a gravidade do autismo e os sintomas do DGI, além da elevação da contagem dessas colônias constituídas pelas cepas[129].

Outra evidência mostrou que a administração diária de cinco cepas probióticas e imunomodulador em crianças com DEA de 3 a 16 anos de idade, melhorou 48% da gravidade da diarreia e 52% da constipação, reduzindo também sintomas associados ao DEA como linguagem, sociabilidade de comunicação, consciência sensorial e cognitiva, saúde física e comportamental em um período de 3 semanas[130].

Em outra linha de pesquisa com crianças com DEA com 3 a 16 anos de idade, fez a suplementação de Lactobacillus por 12 semanas o que resultou na redução da abundância fecal de Clostridium de forma significativa[131], em outro estudo reduziu os produtos metabólicos dos microrganismos patogênicos de leveduras Candida, as quais se apresentam caracteristicamente elevadas em indivíduos com DEA[132].

Foi sugerido um padrão alimentar com maior consumo de alimentos saudáveis associado a um melhor perfil bacteriano em crianças com DEA, o que poderia estar relacionado à uma redução dos DG a partir de uma menor abundância de Enterobacteriaceae, Lactococcus, Roseburia, Leuconostoc e Ruminococcus em um estudo comparativo. O que foi evidenciado em outro estudo que a ingestão de uma dieta ocidental contribui beneficamente para mudanças cognitivas associada à alteração da MG[26,108]. A terapia probiótica teve alta evidência por ter resultado em uma redução de 80% dos DGI, incluindo dor abdominal, diarreia, constipação e indigestão, além de melhorar os sintomas do DEA[133].

Conclusão

Partindo do que vem sendo evidenciado em crianças portadoras do DEA, e levando em consideração a nutrição necessária para essa fase da vida, entende-se que essa desenvolve uma recusa a experimentar novas atividades, comportamentos e consequentemente novos alimentos, que combinado às alterações na capacidade sensorial faz com que a criança tenha um perfil alimentar diferenciado e individualizado, principalmente no que diz a respeito de macro e micronutrientes na dieta sendo capaz de subestimar possíveis impactos dessas alterações, como resultado de longo prazo da dieta habitual.

Ressaltando que o suporte clínico adequado e dietético deve ser imprescindível nessas crianças, assim como estudos que avaliem seu consumo alimentar, possibilitando a aquisição de medidas preventivas contra deficiências e/ou excessos nutricionais, devendo concentrar estudos com foco nos micronutrientes, objetivando seus mecanismos no DEA e seu impacto no aspecto comportamental, o que ainda permanece limitado. Enfatizando esse suporte também no período gestacional.

Apesar da popularidade da DSGSC na terapia do DEA ser disseminado, sua base científica sobre sua eficácia ainda se encontra escassa e questionável, uma vez que se há um grande número de estudos deficientes e outros metodologicamente aceitáveis, mas que não foram capazes de firmar suas conclusões sobre os possíveis benefícios desse método intervencional. O que se recomenda a implementação de estudos bem conduzidos sob ensaios controlados de longa duração, permitindo determinar seus efeitos e resultados comportamentais.

Embora as evidências ressaltem a premissa terapêutica dos probióticos, prebióticos

e/ou simbióticos diante de alterações na MG no DEA como uma estratégia eficaz, ainda são necessários mais investigações a fim de que se avaliem os padrões de comportamento dessas crianças na pós-suplementação e seu potencial benefício, visto que ainda se encontra falhas metodológicas na literatura disponível. Sendo válido enfatizar que existe limitações para esse tipo de estudo devido ao alto custo, heterogeneidade das cepas, espécies, dosagens necessárias, tempo de intervenção e variáveis de confusão, necessitando alinhar a faixa etária específica.

Referências

1. Alanazi AS. The role of nutraceuticals in the management of autism. Saudi Pharmaceutical Journal, 2013, 21.3: 233-243.

2. Lombardi VC, Meirleir KLD, Subramanian K, Nourani SM, Dagda RK, Delaney SL, et al. Nutritional Modulation of the Intestinal Microbiota; Future Opportunities for the Prevention and Treatment of Neuroimmune and Neuroinflammatory Disease. The Journal of nutritional biochemistry, 2018. disorders (ASDs). Microbiome, 2018, 6.1: 133.

3. Oliveira KG, Sertié AL. Autism spectrum disorders: an updated guide for genetic counseling. Einstein. São Paulo. 2017, v. 15, n.2 p. 233-238.

4. Baio J, Wiggins L, Christensen DL, et al. Prevalence of autism spectrum disorder among children aged 8 years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, MMWR Surveillance Summaries, 2014, 67(6):1-23.

5. Gadia CA, Tuchman R, Rotta NT. Autism and pervasive developmental disorders. Jornal de pediatria, 2004, 80.2: 83-94.

6. Muhle R, Trentacoste SV, Rapin I. The genetics of autism. PEDIATRICS-SPRINGFIELD-, 2004,113(5; ISSU 1), 1389-1389.

7. Beck RG. Estimativa do número de casos de transtorno do espectro autista no sul do Brasil. Pós-Graduação em Ciência da Saúde, 2017.

8. Lázaro CP. Construção de escala para avaliar o comportamento alimentar de indivíduos com transtorno do espectro do autismo (TEA). Tese (Doutorado) - Escola Baiana de Medicina e Saúde Pública, Programa de Pós-Graduação em Medicina e Saúde Humana. Salvador, 2016.

9. Guo M, Li L, Zhang Q, Chen L, Dai Y, Liu L, et al. Vitamin and mineral status of children with autism spectrum disorder in Hainan Province of China: associations with symptoms. Nutritional neuroscience, 2018, 1-8.

10. Bonnet-Brilhault F, Rajerison TA, Paillet C, Guimard-Brunault M, Saby A, Ponson L, et al. Autism is a prenatal disorder: Evidence from late gestation brain overgrowth. Autism Research, 2018. doi:10.1002/aur.2036

11. Tordjman S, Somogyi E, Coulon N, Kermarrec S, Cohen D, Bronsard G, et al. Gene× Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. Frontiers in psychiatry, 2014, 5, 53.

12. Siqueira CC, Ferreira EO, Bittencourt RG, Cavalheiro FR, de Abreu Silveira JA, Santos MFR. 221 O CÉREBRO AUTISTA: A BIOLOGIA DA MENTE E SUA IMPLICAÇÃO NO COMPROMETIMENTO

SOCIAL. Revista Transformar, 2016, 8(8), 221-237.

13. Buie T, Campbell DB, Fuchs GJ, Furuta GT, Levy J, Vandewater J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. Pediatrics 125 (Suppl 1), 2010, S1–S18.

14. Leal M, Nagata M, Cunha NDM, Pavanello U, Ferreira NVR. Terapia nutricional em crianças com transtorno do espectro autista. Cadernos da Escola de Saúde, 2017, 1(13).

15. Silva NID. (2011). Relação entre hábito alimentar e síndrome do espectro autista (Doctoral dissertation, Universidade de São Paulo).

16. Zimmer MH, Hart LC, Manning-Courtney P, Murray DS, Bing NM, Summer S. Food variety as a predictor of nutritional status among children with autism. Journal of autism and developmental disorders, 2012, 42(4), 549-556.

17. Meguid N, Anwar M, Zaki S, Kandeel W, Ahmed N, Tewfik I. Dietary patterns of children with autism spectrum disorder: a study based in Egypt. Open access Macedonian journal of medical sciences, 2015, 3(2), 262.

18. Pedraza DF, de Queiroz D. Micronutrientes no crescimento e desenvolvimento infantil. Journal of Human Growth and Development, 2011, 21(1), 156-171.

19. Curtin C, Anderson SE, Must A, Bandini L. The prevalence of obesity in children with autism: a secondary data analysis using nationally representative data from the National Survey of Children's Health. BMC pediatrics, 2010, 10(1), 11.

20. Bercik P, Collins SM, Verdu EF. Microbes and the gut-brain axis. Neurogastroenterology and Motility, 2012, 24(5), 405–413.

21. Louis P. Does the human gut microbiota contribute to the etiology of autism spectrum disorders? Digestive Diseases and Sciences, 2012, 1-3.

22. Mangiola F, Ianiro G, Franceschi F, Fagiuoli S, Gasbarrini G, Gasbarrini A. Gut microbiota in autism and mood disorders. World Journal of Gastroenterology, 2016, 22, 361-368.

23. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous system and the gut microbiome. Cell, 2016, 167, 915-932.

24. El-ansary A, Bacha AB, Bjorklund G, Nora AO, Ramesa SB, Nadine M, et al. Probiotic treatment reduces the autistic-like excitation/inhibition imbalance in juvenile hamsters induced by orally administered propionic acid and clindamycin. Metabolic brain disease, 2018, 1-10.

25. Pyndt Jørgensen B, Hansen JT, Krych L, Larsen C, Klein AB, Nielsen DS, et al. . (2014). A possible link between food and mood: dietary impact on gut microbiota and behavior in BALB/c mice. PLoS ONE 9:e103398. 10.1371/journal.pone.0103398

26. Magnusson KR, Hauck L, Jeffrey BM, Elias V, Humphrey A, Nath R, et al. Relationships between diet-related changes in the gut microbiome and cognitive flexibility. Neuroscience, 2015, 300, 128–140.

27. Molloy CA, Manning-Courtney P. Prevalence of chronic gastrointestinal symptoms in children with autism and autistic spectrum disorders. Autism. 2003, 7 (2): 165-171.

28. Adams JB, Holloway CE, George F, Quig D: Analyses of Toxic Metals and Essential Minerals in the Hair of Arizona Children with Autism and their mothers. Biol Tr El Res. 2006, 110: 193-209.

29. Valicenti-McDermott M, McVicar K, Rapin I, Wershil BK, Cohen H, Shinnar S. Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease. J Dev Behav Pediatr. 2006, 27 (2 Suppl): S128-36.

30. Ming X, Brimacombe M, Chaaban J, Zimmerman-Bier B, Wagner GC. Autism spectrum disorders: concurrent clinical disorders. Journal of child neurology, 2008, 23(1), 6-13.

31. Finegold SM, Summanen PH, Downes J, Corbett K, Komoriya T. Detection of Clostridium perfringens toxin genes in the gut microbiota of autistic children. Anaerobe, 2017, 45, 133-137.

32. Rose DR, Yang H, Serena G, Sturgeon C, Ma B, Careaga M, et al. Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. Brain, behavior, and immunity, 2018, 70, 354-368.

33. Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K, et al. Gastrointestinal microbiota in children with autism in Slovakia. Physiology & behavior, 2015, 138, 179-187.

34. Adams JB, Romdalvik J, Ramanujam VMS, Legator MS. Mercury, Lead, and Zinc in Baby Teeth of Children with Autism vs. Controls. J Toxicol Environ Health. 2007, 70 (12): 1046-51.

35. Campion D, Ponzo P, Alessandria C, Saracco GM, Balzola F. Role of microbiota in the autism spectrum disorders. Minerva gastroenterologica e dietologica, 2018.

36. Zuchetto AT, Cavalcante TM, Pimenta RA, Zanon PA, Nasser JP. Avaliação da composição corporal de crianças e jovens com deficiência. Revista Portuguesa de Ciências do Desporto, 2014.

37. Reichow B. Overview of meta-analyses on early intensive behavioral intervention for young children with autism spectrum disorders. Journal of autism and developmental disorders, 2012, 42(4), 512-520.

38. Bielemann RM, Motta JVS, Minten GC, Horta BL, Gigante DP. Consumption of ultra-processed foods and their impact on the diet of young adults. Revista de saude publica, 2015, 49, 28.

39. Louzada MLDC, Martins APB, Canella DS, Baraldi LG, Levy RB, Claro RM, et al. Impact of ultra-processed foods on micronutrient content in the Brazilian diet. Revista de saude publica, 2015, 49, 45.

40. De Araújo Almeida AK, de Ameida Fonseca PC, Oliveira LA, Santos WRCC, Zagmignan A, de Oliveira BR, et al. Consumo de ultraprocessados e estado nutricional de crianças com transtorno do espectro do autismo. Revista Brasileira em Promoção da Saúde, 2018, 31(3).

41. Levy SE, Souders MC, Ittenbach RF, Giarelli E, Mulberg AE, Pinto-Martin JA. Relationship of dietary intake to gastrointestinal symptoms in children with autistic spectrum disorders. Biological psychiatry, 2007, 61(4), 492-497.

42. Esteban-Figuerola P, Canals J, Fernández-Cao JC, Arija Val V. Differences in food consumption and nutritional intake between children with autism spectrum disorders and typically developing children: A meta-analysis. Autism, 2018, 1362361318794179.

43. Liu X, Liu J, Xiong X, Yang T, Hou N, Liang X, et al. Correlation between nutrition and symptoms: nutritional survey of children with autism spectrum disorder in Chongqing, China. Nutrients, 2016, 8(5), 294.

44. Caetano MV, Gurgel DC. Perfil nutricional de crianças portadoras do transtorno do espectro autista. Revista Brasileira em Promoção da Saúde, 2018, 31(1).

45. Cozzolino SMF. Biodisponibilidade de nutrientes. São Paulo: Manole, 2005.

46. Marí-Bauset S, Llopis-González A, Zazpe I, Marí-Sanchis A, Morales Suárez-Varela M. Comparison of nutritional status between children with autism spectrum disorder and typically developing children in the Mediterranean Region (Valencia, Spain). Autism, 2017, 21(3), 310-322.

47. Ranjan S, Nasser JA. Nutritional status of individuals with autism spectrum disorders: do we know enough?. Advances in Nutrition, 2015, 6(4), 397-407.

48. Kummer A, Barbosa IG, Rodrigues DH, Rocha NP, da Silva Rafael M, Pfeilsticker L, Teixeira AL. Frequência de sobrepeso e obesidade em crianças e adolescentes com autismo e transtorno do déficit de atenção/hiperatividade. Revista Paulista de Pediatria, 2016, 34(1), 71-77.

49. Criado KK, Sharp WG, McCracken CE, De Vinck-Baroody O, Dong L, Aman MG, Leventhal JM. Overweight and obese status in children with autism spectrum disorder and disruptive behavior. Autism, 2018, 22(4), 450-459.

50. Ferreira NVR. Estado nutricional em crianças com transtorno do espectro autista. 2016.

51. James SJ, Cutler P, Melnyk S, Jerniga S, Janak L, Gaylor DW, Neubrander JA. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. The American journal of clinical nutrition, 2004, 80(6), 1611-1617.

52. Bjørklund G, Waly MI, Al-Farsi Y, Saad K, Dadar M, Rahman MM, et al. The Role of Vitamins in Autism Spectrum Disorder: What Do We Know?. Journal of Molecular Neuroscience, 2019, 1-15.

53. McGinnis WR. Oxidative stress in autism. Alternative Therapies in Health and Medicine, 2004,10(6), 22-36.

54. Herbert MR. Large brains in autism: the challenge of pervasive abnormality. The Neuroscientist, 2005, 11(5), 417-440.

55. Pardo CA, Vargas DL, Zimmerman AW. Immunity, neuroglia and neuroinflammation in autism. International review of psychiatry, 2005, 17(6), 485-495.

56. Fiorentino M, Sapone A, Senger S, Camhi SS, Kadzielski SM, Buie TM, Fasano A. Blood–brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. Molecular autism, 2016, 7(1), 49.

57. Fisberg M, Barros MJL, Castillo-Duran C, Martinez FE, Schwartzman JS, Braga JAP, Tumas R. (2008). O papel dos nutrientes no crescimento e desenvolvimento infantil.

58. Yasuda H, Yoshida K, Yasuda Y, Tsutsui T. Infantile zinc deficiency: association with autism spectrum disorders. Scientific reports, 2011, 1, 129.

59. Crăciun EC, Bjørklund G, Tinkov AA, Urbina MA, Skalny AV, Rad F, Dronca E. Evaluation of whole blood zinc and copper levels in children with autism spectrum disorder. Metabolic brain disease, 2016, 31(4), 887-890.

60. Cruz AAV, Attié-Castro FA, Fernandes SL, Cortes JFF, Paulo de Tarso Filho PP, Rocha EM, Marchini JS. Adult blindness secondary to vitamin A deficiency associated with an eating disorder. Nutrition, 2005, 21(5), 630-633.

61. Jory J, McGinnis WR. Red-cell trace minerals in children with autism. American Journal of Biochemistry and Biotechnology, 2008, 4(2), 101-104.

62. Priya MDL, Geetha A. Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. Biological trace

element research, 2011, 142(2), 148-158.

63. De Palma G, Catalani S, Franco A, Brighenti M, Apostoli P. Lack of correlation between metallic elements analyzed in hair by ICP-MS and autism. Journal of autism and developmental disorders, 2012, 42(3), 342-353.

64. Sun C, Xia W, Zhao Y, Li N, Zhao D, Wu L. Nutritional status survey of children with autism and typically developing children aged 4–6 years in Heilongjiang Province, China. Journal of nutritional science, 2013, 2.

65. Marí-Bauset S, Llopis-González A, Zazpe-García I, Marí-Sanchis A, Morales-Suárez-Varela M. Nutritional status of children with autism spectrum disorders (ASDs): a case–control study. Journal of autism and developmental disorders, 2015, 45(1), 203-212.

66. Herndon AC, DiGuiseppi C, Johnson SL, Leiferman J, Reynolds A. Does nutritional intake differ between children with autism spectrum disorders and children with typical development?. Journal of autism and developmental disorders, 2009, 39(2), 212.

67. Marí-Bauset S, Llopis-González A, Zazpe I, Marí-Sanchis A, Morales Suárez-Varela M. Comparison of nutritional status between children with autism spectrum disorder and typically developing children in the Mediterranean Region (Valencia, Spain). Autism, 2017, 21(3), 310-322.

68. Vitolo MR. (2014). Nutrição–da gestação ao envelhecimento. Editora Rubio.

69. Palmieri L, Papaleo V, Porcelli V, Scarcia P, Gaita L, Sacco R, Militerni R. Altered calcium homeostasis in autism-spectrum disorders: evidence from biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier AGC1. Molecular psychiatry, 2010, 15(1), 38.

70. Goh S, Dong Z, Zhang Y, DiMauro S, Peterson BS. Mitochondrial dysfunction as a neurobiological subtype of autism spectrum disorder: evidence from brain imaging. JAMA psychiatry, 2014, 71(6), 665-671.

71. Rossignol DA, Frye RE. Evidence linking oxidative stress, mitochondrial dysfunction, and inflammation in the brain of individuals with autism. Frontiers in physiology, 2014, 5, 150.

72. Naviaux JC, Wang L, Li K, Bright AT, Alaynick WA, Williams KR, Naviaux RK. Antipurinergic therapy corrects the autism-like features in the Fragile X (Fmr1 knockout) mouse model. Molecular autism, 2015, 6(1), 1.

73. Mitchell ES, Conus N, Kaput J. B vitamin polymorphisms and behavior: Evidence of associations with neurodevelopment, depression, schizophrenia, bipolar disorder and cognitive decline. Neuroscience & Biobehavioral Reviews, 2014, 47, 307-320.

74. Al-Farsi YM, Waly MI, Deth RC, Al-Sharbati MM, Al-Shafaee M, Al-Farsi O, Al-Adawi S. Low folate and vitamin B12 nourishment is common in Omani children with newly diagnosed autism. Nutrition, 2013, 29(3), 537-541.

75. Bonnet-Brilhault F, Rajerison TA, Paillet C, Guimard-Brunault M, Saby A, Ponson L, Roux S. Autism is a prenatal disorder: Evidence from late gestation brain overgrowth. Autism Research. 2018. doi:10.1002/aur.2036

76. Vinkhuyzen AA, Eyles DW, Burne TH, Blanken LM, Kruithof CJ, Verhulst F, McGrath JJ. Gestational vitamin D deficiency and autism-related traits: the Generation R Study. Molecular psychiatry, 2018, 23(2), 240.

77. Roth C, Magnus P, Schjolberg S, Stoltenberg C, Suren P, McKeague IW, Davey SG, Reich-

born-Kjennerud T, Susser E (2011). Folic acid supplements in pregnancy and severe language delay in children. Journal of the American Medical Association 306, 1566–1573.

78. BJØRK M, et al. Association of folic acid supplementation during pregnancy with the risk of autistic traits in children exposed to antiepileptic drugs in utero. JAMA neurology, 2018, 75.2: 160-168.

79. Beard CM, Panser LA, Katusic SK. Is excess folic acid supplementation a risk factor for autism? Med Hypotheses. 2011;77:15–17

80. Murray LK, Smith MJ, Jadavji NM. Maternal oversupplementation with folic acid and its impact on neurodevelopment of offspring. Nutrition Reviews, 2018, 76(9), 708–721. doi:10.1093/nutrit/nuy025

81. Schmidt RJ, Tancredi DJ, Ozonoff S, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. Am J Clin Nutr. 2012;96:80–89.

82. O'Neill RJ, Vrana PB, Rosenfeld CS. Maternal methyl supplemented diets and effects on offspring health. Front Genet. 2014;5:289. doi:10.3389/fgene.2014. 00289

83. White AM, Swartzwelder HS. Hippocampal function during adolescence: a unique target of ethanol effects. Annals of the New York Academy of Sciences, 2004, 1021, 206220.

84. Alfonso-Loeches S, Guerri C. Molecular and behavioral aspects of the actions of alcohol on the adult and developing brain. Critical Reviews in Clinical Laboratory Sciences, 2011, 48(1), 1947.

85. Singer AB, Aylsworth AS, Cordero C, Croen LA, DiGuiseppi C, Fallin MD, Windham GC. Prenatal alcohol exposure in relation to autism spectrum disorder: Findings from the study to explore early development (SEED). Paediatric and perinatal epidemiology, 2017, 31(6), 573-582.

86. Atladottir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, et al. Association of family history of autoimmune diseases and autism spectrum disorders. Pediatrics, 2009, 124, 687–694.

87. Braunschweig D, Van de Water J. Maternal autoantibodies in autism. Arch Neurol, 2012, 69, 693–699.

88. Krakowiak P, Walker CK, Bremer AA, Baker AS, Ozonoff S, Hansen RL, et al. Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics, 2012, 129, e1121–e1128.

89. Brimberg L, Sadiq A, Gregersen PK, Diamond B. Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. Mol Psychiatry, 2013, 18, 1171–1177.

90. Bilbo SD, Block CL, Bolton JL, Hanamsagar R, Tran PK. Beyond infection—maternal immune activation by environmental factors, microglial development, and relevance for autism spectrum disorders. Exp Neurol, 2018, 299, 241–251

91. Paysour MJ, Bolte AC, Lukens JR. Crosstalk Between the Microbiome and Gestational Immunity in Autism-Related Disorders. DNA and Cell Biology, 2019. doi:10.1089/dna.2019.4653

92. Winburn E, Charlton J, McConachie H, et al. Parents' and child health professionals' attitudes towards dietary interventions for children with autism spectrum disorders. J Autism Dev Disord 2014; 44:747–757.

93. Nandhu MS, Naijil G, Smijin S, Jayanarayanan S, Paulose CS. Opioid system functional reg-

ulation in neurological disease management. Journal of neuroscience research, 2010, 88(15), 3215-3221.

94. Reichelt KL, Tveiten Bioengineer D, Knivsberg AM, Brønstad G. Peptides' role in autism with emphasis on exorphins. Microbial ecology in health and disease, 2012, 23(1), 18958.

95. Nalini Sehgal M, Howard Smith M, Laxmaiah Manchikanti M. Peripherally acting opioids and clinical implications for pain control. Pain Physician, 2011, 14, 249-258.

96. Wada Y, Loennerdal B. Bioactive peptides derived from human milk proteins—mechanisms of action. The Journal of nutritional biochemistry, 2014, 25(5), 503-514.

97. Lange KW, Hauser J, Reissmann A. Gluten-free and casein-free diets in the therapy of autism. Current Opinion in Clinical Nutrition and Metabolic Care, 2015, 18(6), 572–575.doi:10.1097/mco.0000000000000228

98. Lau NM, Green PH, Taylor AK, et al. Markers of celiac disease and gluten sensitivity in children with autism. PLoS One 2013; 8:e66155.

99. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. Brain, behavior, and immunity, 2012, 26(3), 383-392.

100. Muro Urista C, Álvarez Fernández R, Riera Rodriguez F, Arana Cuenca A, Tellez Jurado A. Production and functionality of active peptides from milk. Food Science and Technology International, 2011, 17(4), 293-317.

101. Galiatsatos P, Gologan A, Lamoureux E. Autistic enterocolitis: fact or fiction? Canadian Journal of Gastroenterology and Hepatology, 2009, 23(2), 95-98.

102. Hyman SL, Stewart PA, Foley J, Peck R, Morris DD, Wang H, Smith T. The gluten-free/casein-free diet: a double-blind challenge trial in children with autism. Journal of autism and developmental disorders, 2016, 46(1), 205-220.

103. Ghalichi F, Ghaemmaghami J, Malek A, Ostadrahimi A. Effect of gluten free diet on gastrointestinal and behavioral indices for children with autism spectrum disorders: a randomized clinical trial. World Journal of Pediatrics, 2016, 12(4), 436-442.

104. Dalton N, Chandler S, Turner C, et al. Gut permeability in autism spectrum disorder. Autism Res 2014; 7:305–313.

105. Pusponegoro HD, Ismael S, Firmansyah A, Sastroasmor S, Vandenplas Y. Gluten and casein supplementation does not increase symptoms in children with autism spectrum disorder. Acta Paediatrica, 2015, 104(11), e500-e505.

106. Whiteley P. Nutritional management of (some) autism: a case for gluten- and casein-free diets? Proceedings of the Nutrition Society, 2014, 74(03), 202–207.doi:10.1017/s0029665114001475

107. Whiteley P, Shattock P, Knivsberg AM, Seim A, Reichelt KL, Todd L, et al. Gluten- and casein-free dietary intervention for autism spectrum conditions. Frontiers in Human Neuroscience, 2013, 6, 344.

108. Berding K, Donovan SM. Diet Can Impact Microbiota Composition in Children With Autism Spectrum Disorder. Frontiers in neuroscience, 2018, 12.

109. Ibrahim SH, et al. Incidence of Gastrointestinal Symptoms in Children: APopulation-Based Study. NationalInstituteof Health. EUA: Agosto, p. 680 – 686, 2009.

110. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism

spectrum disorder: a meta-analysis. Pediatrics, 2014, 133(5), 872-883.

111. Frye RE, Rose S, Slattery J, MacFabe DF. Gastrointestinal dysfunction in autism spectrum disorder: the role of the mitochondria and the enteric microbiome. Microbial ecology in health and disease, 2015, 26(1), 27458.

112. Nikolov RN, Bearss KE, Lettinga J, Erickson C, Rodowski M, Aman MG, et al. Gastrointestinal symptoms in a sample of children with pervasive developmental disorders. Journal of autism and developmental disorders, 2009, 39(3), 405-413.

113. Mazurek MO, Vasa RA, Kalb LG, Kanne SM, Rosenberg D, Keefer A, et al. Anxiety, sensory over-responsivity, and gastrointestinal problems in children with autism spectrum disorders. Journal of Abnormal Child Psychology, 2013, 41, 165-176.

114. Niehus R, Lord C. Early medical history of children with autism spectrum disorders. Journal of Developmental & Behavioral Pediatrics, 2006, 27(2), S120-S127.

115. Adams JB, Romdalvik J, Levine KE, Hu L-W: Mercury in First-Cut Baby Hair of Children with Autism vs. Typically-Developing Children. Toxicological and Environmental Chemistry. 2008, 90 (4): 739-753.

116. Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism–comparisons to typical children and correlation with autism severity. BMC gastroenterology, 2011, 11.1: 22.

117. Moreno X, Santamaria G, Sánchez R, De La Torre B, Garcés F, Hernández C, et al. Microbiota gastrointestinal aeróbica en niños con trastorno del espectro autista. Estudio preliminar. Revista GEN, 2015, 69(2), 36-44.

118. Grimaldi R, Cela D, Swann JR, Vulevic J, Gibson GR, Tzortzis G, et al. In vitro fermentation of B-GOS: impact on faecal bacterial populations and metabolic activity in autistic and non-autistic children. FEMS microbiology ecology, 2017, 93.2.

119. Bottacini F, Ventura M, Van Sinderen D, Motherway MOC. Diversity, ecology and intestinal function of bifidobacteria. Microb. Cell Fact. 2014, 13:S4.

120. Hidalgo-Cantabrana C, Delgado S, Ruiz L, Ruas-Madiedo P, Sánchez B, Margolles A. Bifidobacteria and their health-promoting effects. Microbiol. Spectr. 2017, 5, BAD-0010-2016.

121. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015, 33, 496–503.

122. Son JS, Zheng LJ, Rowehl LM, Tian X, Zhang, Y, Zhu W, et al. Comparison of fecal microbiota in children with autism spectrum disorders and neurotypical siblings in the simons simplex collection. PloS one, 2015, 10(10), e0137725.

123. Provost B, Crowe TK, Osbourn PL, McClain C, Skipper BJ. Mealtime behaviors of preschool children: comparison of children with autism spectrum disorder and children with typical development. Phys. Occup. Ther. Pediatr. 2010, 30, 220–233.

124. Diolordi L, del Balzo V, Bernabei P, Vitiello V, Donini LM. Eating habits and dietary patterns in children with autism. Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity. 2014, 19, 295–301.

125. Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, et al. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. ISME J. 2010, 4:232.

126. Bonder MJ, Tigchelaar EF, X Cai, Trynka G, CM Cenit, Hrdlickova B, et al. A influência de uma dieta sem glúten a curto prazo sobre o ser humano microbioma intestinal. Genoma Med. 2016; 8: 1-11.

127. Campbell DB, Warren D, Sutcliffe JS, Lee EB, Levitt P. Association of MET with social and communication phenotypes in individuals with autism spectrum disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 2010, 153(2), 438-446.

128. Critchfield JW, Van Hemert S, Ash M, Mulder L, Ashwood P. The potential role of probiotics in the management of childhood autism spectrum disorders. Gastroenterology research and practice, 2011.

129. Shaaban SY, El Gendy YG, Mehanna NS, El-Senousy WM, El-Feki HS, Saad K, et al. The role of probiotics in children with autism spectrum disorder: A prospective, open-label study. Nutritional Neuroscience, 2017, 1-6.

130. West R, Roberts E, Sichel LS, Sichel J. Improvements in gastrointestinal symptoms among children with autism spectrum disorder receiving the Delpro® Probiotic and immunomodulator formulation. Journal of Probiotics and Health, 2013, 1, 2.

131. Parracho HM, Gibson GR, Knott F, Bosscher D, Kleerebezem M, McCartney AL. A double-blind, placebo-controlled, crossover-designed probiotic feeding study in children diagnosed with autistic spectrum disorders. International Journal of Probiotics & Prebiotics, 2010, 5, 69-74.

132. Kałużna-Czaplińska J, Błaszczyk S. The level of arabinitol in autistic children after probiotic therapy. Nutrition, 2012, 28, 124-126.

133. Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome, 2017, 5, 10.

17 - Levels cortisol and female sex hormone in elderly female capuchin monkeys (Sapajus libidinosus)

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Abstract

The aim of this study was to measure estradiol, progesterone and cortisol levels in two elderly female tufted capuchin monkeys from the Primate Center-UNB. During ten weeks, three times per week, fecal samples were collected for hormonal extraction and dosage. Hormonal extraction was performed by means of two distinct processes: hydrolysis and solvolysis, respectively, while the dosage was made by the technique ELISA. The results indicate irregular menstrual cycles and low hormonal levels, not corresponding to the normal menstrual cycle profile of this species. The highest values recorded were 200 ng/g feces (estradiol) and 20 µg/g feces (progesterone). There was synchrony between estradiol and cortisol values. It is possible that these females are in reproductive senescence, because, as in women and other primates, they will also show a decrease in levels of sex hormones, probably due to morphological and structural changes, characteristic of the physiological aging process.

Key words: Sexual Hormones; Nonsexual Steroid; Primates; Senescence; Menstrual Cycle

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Introduction

In many mammalian females, including humans, the aging process is characterized by several factors which are indicative of reproductive senescence. Among them is the decrease in hormone levels, fertility rate and sexual activity (1-2). There are several species which are suitable for the study of reproductive senescence, such Pan troglodytes. These species have morphological and physiological similarities with human females such as the presence of menstrual cycle and hormonal fluctuations (2). Thus, these characteristics are fundamental to choose the suitable animal model for studies on reproductive senescence. Among these models, the species Sapajus libidinosus is particularly interesting to be investigated because the reproductive physiology of females is also very similar to that of women with menstrual cycle accompanied by hormonal fluctuations (3). Furthermore, the peaks of sex hormones coincide with the period of cycle phases whose duration is around 23 (4).

Although various aspects of the reproductive physiology of female capuchin monkeys have already been described (4-5), reproductive aging still deserves prominence. The reason for that is that it is not known for sure until what age females of this species are capable of reproduction or may be considered reproductively active. It is worth mentioning that capuchin monkeys can live in captivity for more than forty years (4). Therefore, since this species is the only New World Primate that present menstrual cycle, there is a need for more detailed studies on the hormonal profile in elderly female capuchin monkeys. It has been shown that there is a reproductive senescence phase in the female Old World Primates. This is known as menopause (6) and is possibly a result of low levels of sex hormones leading to meaningful changes in reproductive success (2, 1).

Aware of the importance of better understanding the natural process of aging and the set of phenomena related to the reproductive decline, we aimed to analyze sexual (estradiol and progesterone) and non-sexual (cortisol) steroid levels in the menstrual cycles of two elderly female capuchin monkeys. The females in question are thirty-six years old and may be considered elderly taking into consideration that the life expectancy for these primates is around forty.

The data in this study allowed the verification of the levels of sexual hormones and cortisol in this age group. These are important data since the phenomenon of reproductive senescence in capuchin monkey has not been studied enough. With the exception of the studies by Rodrigues and colleagues (7) there are no other studies in the literature presenting outcomes about levels of sexual hormones in elderly female capuchin monkeys. Our hypothesis for such is that both sexual hormones and cortisol levels suffer changes due to advanced age.

Material and methods

Humane Care Guidelines

Before the beginning of this study, the research project was evaluated and approved by the Ethics Committee on Animal Use (CEUA) of the Institute of Biological Sciences at the University of Brasilia (Protocol number UnBDOC No. 32945/2009)

Subjects and Site

It was possible to use two females for purposes of this study, since there are difficulties associated with the high cost of maintenance of these animals in captivity, and about the ethics of use of primates in scientific studies. Furthermore our goal was to characterize the levels of sex steroids in elderly female capuchin monkey, so it is extremely rare animals, which is known to have reliable records of the age of the subjects. So they parciparam only two females in this study, since we have safely age the same, ie both is 36 years old. Theses monkeys – called M. Flor and M. Rosa – were housed and tested at the Primate Center of the University of Brasilia - Brazil, under natural light, temperature and humidity conditions. The Primate Research Center is located within the grounds of an ecological reserve, where home cages are surrounded by nearby native tropical semideciduos gallery forest.

Subjects were housed in a colony room consisting of two rows of six cages (4m length, 2.9m width, 2m height per cage). Each cage consists of two concrete walls, separating adjacent cages, and a wire mesh front, back and ceiling forming an outdoor/semi-indoor housing system. Each home cage contains a suspended wood nest-box, several wood perches at different heights, a food tray where a food bowl is placed, and a thick layer of natural dry leaves and twigs on the floor. Olfactory and acoustic, but not visual, contact is possible between the members of the colony. Food is provided in the home cage once per day at 07:30, where it remains until 17:30. The provisions include a variety of fresh fruits and vegetables. Dry banana pellets and fresh water are available ad libitum. Animals are weighed and clinically evaluated by a veterinarian once a month. Housing and maintenance conditions are in accordance with the laws and regulations of the Brazilian Institute of Environment and Renewable Natural Resources - IBAMA.

Procedure

Data were collected during ten weeks, between April and June 2009 (from 08:00 am and 12:00 pm). Subjects' fecal samples were collected three times a week, identified and stored in 5 ml Eppendorf bottles at -20ºC.

The extraction of hormones was performed at the Behavioral Endocrinology Laboratory, Federal University of Rio Grande do Norte (UFRN). After taken out of the freezer, the samples were placed at room temperature for a period of about 10 minutes to defrost. Then, with the aid of a spatula, each fecal sample was homogenized and, by means of a precision scale, an aliquot of 0.1 g feces was weighed and transferred to a 15

ml plastic tube which had been properly identified.

From the stool samples, progesterone, estradiol and cortisol were evaluated. In order to perform hormonal dosage procedures in stools, it is necessary to do the hormonal extraction, which is the separation of hormones in fecal residues. For this, two sequential hydrolysis and solvolysis processes were respectively used, which served to separate united hormones through single, double and triple bonds for subsequent quantification. The hydrolysis process aimed to separate steroids that were in free form and under sample conjugation from the remainder sample material, while the solvolysis aimed to separate the steroids that were under double and triple conjugation. Hormonal dosage was performed by an ELISA method.

The data from this study are only statistically descriptive because it is a case study of only two subjects. For the tabulation of hormone data outcomes, Excel® version 2010 for Windows® was used. The graphics were constructed through of use of the statistical software Graphpad Prism 5.

Results

In the female M. Rosa, there was synchrony between the values of cortisol and the sex hormones estradiol and progesterone, considering that cortisol peaks came before the increased levels of estradiol and progesterone. Between day 0 and day 18 very low values were recorded in all dosed hormones. The first hormonal peaks came after the 19th day. Regarding estradiol levels, there were recurring peaks between the twentieth and forty-fifth day, and progesterone peaks showed oscillations between the forty-fifth and sixtieth day. After the sixtieth day estradiol levels remained at basal levels, progesterone presented discreet increases, which were also observed for the cortisol hormone (Figure 1-A).

For the female M. Flor, cortisol levels were in some moments synchronized to the estradiol levels. Unlike the results found in M. Rosa, there wasn't any synchrony between values of progesterone and cortisol because, when progesterone values were high, cortisol was at low levels. This could be observed over the days, with the exception of the peak that occurred between the twentieth and fortieth day (Figure 1-B). Another distinct outcome between the two females regarded values of sex hormones between day 0 and day 20 because of the high levels of hormone presented by M. Flor (Figure 1-A and B).

In both females, the cortisol levels remained at basal levels from day 0 to 19, practically a complete menstrual cycle. However, from the twentieth day on, the cortisol levels of M. Rosa presented a small elevation of 4 µg/g of feces, which was corresponded then by M. Flor, but in superior value of 23 µg/g feces. The latter was the highest value recorded during the study. Other hormonal elevations had synchro-

nized peaks, but these values were low (Figure 2).

Estradiol levels also showed synchrony between the two females, but the rise of this hormone came first in M. Flor, unlike the cortisol levels (Figure 2 and Figure 3, respectively). Similar to the cortisol levels, an increase in estradiol levels was not registered in none of the females observed between days 0 and 20. However, the female M. Flor presented a small rise in estradiol levels from the fifteenth day on that came before the estradiol peak of M. Rosa. From the twentieth day the female M. Rosa began presenting a significant regular rise of estradiol levels, until around the sixtieth day. This pattern always came after the peaks of the other female. After the sixtieth day, there was a decrease in estradiol levels of M. Rosa that remained at basal levels, whereas M. Flor, throughout the days, showed recurrent peaks with the highest registered value of 200 ng/feces until practically the end of analyzed days (Figure 3).

Discussion

Most studies about non-human primates' reproductive senescence have been done with Old World Primates such as chimpanzees (1) rhesus (8) and baboons (9), where there is a clear senescence phase (10). One of the most marked features of this phase is the low levels of sex hormones (11). The present study is one of the few to investigate the topic using a New World Primate.

Our results demonstrated that, throughout all menstrual cycles studied, very low and irregular values of sex hormones were recorded. This points towards a possible reproductive senescence in the female capuchin monkeys studied. Regarding estradiol levels, the highest value recorded was of 200 ng/ml, and this value is well below what is commonly found for sexually active females (503 pg/ml) (3). In female capuchin monkeys, as well as in female humans, the follicular phase which precedes ovulation is characterized by continuous increase in estradiol levels. In the ovulation phase this hormone reaches the highest registered value throughout all menstrual cycle (5,3). In our study it was not possible to determine whether elderly females are still ovulating, because estradiol and progesterone peaks did not match the normal hormone profile which is typical of menstrual cycle (3). It is worth highlighting that estradiol is directly correlated to increased sexual behavior and when female tufted capuchins are closer to ovulation, they begin to present higher estradiol levels, and consequently an increase in the expression of sexual behavior (5).

In studies developed by Rodrigues (7), a lower expression of sexual behavior was registered in elderly tufted capuchin females, in such a way that several behavioral expressions named as sexual were not displayed by these elderly females. However, these same sexual expressions were displayed by younger females, as for ins-

tance, raising their eyebrows, facing, looking with a tilted head, massaging breasts and genitalia (5). It is worth highlighting that tufted Capuchin females emit various behavioral expressions in the proceptive phase of their menstrual cycle, and such behaviors are directed to alpha males as a sign of fertility (4). Furthermore in the study of Rodrigues (9) the progesterone levels were also dosed in both groups (adult and elderly). The results of that study showed irregularities in the elderly menstrual cycles, since they presented fluctuations that were different from the adult's, with extremely low values in almost all monitored menstrual cycles.

Aging can be defined as a natural progression of changes in the organism structure and functioning (12). It is part of the biological cycle of all living beings and brings with it a number of undesirable consequences for the animal organism, such as loss of vitality and changes in the functioning of biological systems, affecting the reproductive function both directly or indirectly (12,13,14). In humans, after they reach certain age, a reduction of levels of sex hormones begins (8). These stages are known as andropause in men and menopause in women, characterized by changes in the reproductive physiology and consequently a decline in the reproductive functions (14).

In women, the reproductive aging is characterized by a continuous process, which begins near birth and is extended up to the menopause (13). It occurs in average around the age of 51 (15) and this phase is characterized by a series of physiological, psychological and behavioral changes. Among these alterations, there are the structural and functional changes in the hypothalamic-pituitary-ovary axis (16), which affect other physiological aspects such as menstrual cycle, ovulation and libido (12). As a result, females gradually lose their capacity to reproduce. This is typically indicated by a disruption in normal menstrual cyclicality, which begins by eventual periods until complete menstruation cessation (16). The first signs of menopause are increased levels of Follicle-Stimulating Hormone (FSH) and reduction in estradiol levels, that lead to irregularities in the menstrual cycle or to amenorrhea (16).

Similarly to what happens in women, studies have shown that non-human primate females also suffer important hormonal and morphological changes with advancing age (17). Elderly females of rhesus monkeys have hormonal changes characteristic of climacteric and menopause (14). These changes include the reduction in estrogen levels, fertility reduction and increased circulating levels of FSH (8,11).

In non-human primates, besides the physiological changes, other aspects also change with age, for example the ovarian follicles reserve. Studies with elderly females of chimpanzees (1) and rhesus (8) have shown that, similarly to what happens in humans, these females also demonstrate morphological changes in the germinal epithelium. They also show reduction in the number of primordial or preantral follicles, in such a way that it was verified the complete exhaustion of ovarian follicles

with age (1).

In our study, the FSH levels and the number of ovarian follicles were not evaluated, which brings a few limitations of this work, since these two parameters are essential for the affirmation of female reproductive senescence. However, the advanced age (14) and low level of sex hormones (16) are also crucial factors for the characterization of a reproductive senescence phase. In relation to the count of ovarian follicles reserve we could not perform an ovariectomy because the females in question participate in other experiments in which it is fundamental that their ovaries remain intact. One of these studies evaluates the sexual behavior and hormonal aspects after chronic administration of testosterone (data not yet published). We will also analyze the FSH levels to corroborate with low levels of estradiol in these females.

Another goal of our study was to measure the cortisol levels. In non-human primates, this steroid is directly involved in the response to stress (17). Stress can be caused by either social or environmental factors, such as access to sexual partners, reproductive state, and food resources (12). Cortisol levels are also linked to reproductive dominance aspects (21) since dominant individuals have higher cortisol levels than subordinated individuals (22).

In women there is a relationship between cortisol levels and the menstrual cycle, because significant increase in levels of cortisol in the ovulatory phase has been registered (23). Just as in humans, females of non-human primates have synchrony between the levels of cortisol and the menstrual cycle phase. For these females, however, increased cortisol levels occur in the periovulatory phase, i.e., the one that precedes ovulation in which the highest estradiol levels are recorded. It is worth highlighting that the cortisol increase does not occur in the luteal phase of the menstrual cycle, i.e., increase of progesterone levels do not significantly changes cortisol levels, (24) just as noted in women (25). As with other primates, our study showed that elderly female tufted Capuchinh has a synchrony between estradiol levels and cortisol levels, even though the ovarian cycle of these females present an abnormal pattern. Probably the cortisol receptors are sensitive to any small variation in the estradiol levels and not only to the ovulation.

According to the literature, rats, non-human and human primates show changes of cortisol levels as a result of aging (25,26). Physiological aging may be responsible for the morphological and functional changes of the hypothalamic-pituitary-adrenal (HPA) (26,27). That would make the axis less sensitive to and that is expressed as a deficiency in the negative feedback (23,28). In this way, cortisol levels change with aging (25,26), such as decreased levels of cortisol (26) and adrenocorticotropic cortisol (ACTH) (29,30).

In short, we can conclude that these tufted capuchin females, as other primates, present reproductive senescence. It is worth highlighting that this work is just a case

study. Nevertheless, the females in question have showed very low levels of sexual hormones, similar to those found in senescent women and in Old World Primates. Probably the hormonal levels decrease has occurred due to morphological and structural changes of the aging process, which has, as one of its features, the changes on hormonal levels. This alterations compromise the reproductive functions because sex hormones are directly related to the activation of sexual behavior. Besides that, the low levels are related to a decline of reproductive activities (30) and reproductive aging (2).

Acknowledgements

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References

1. Jones KP, Walker LC, Anderson D, Lacreuse A, Robson SL, Hawkes K. Depletion of ovarian follicles with age in chimpanzees: similarities to humans. Biol Reprod 2007; 77: 247-51.

2. Neal-Perry G, Nejat E, Dicken C. The neuroendocrine physiology of female reproductive aging: An update. Maturitas 2010; 67: 34-38.

3. Nagle CA, Denari JH, Quiroga S, Riarte A, Merlo A, Germino NI, Gómez-Argaña F, Roster JM. The plasma pattern of ovarian steroids during the menstrual cycle in capuchin monkeys (Sapajus apella). Biol Reprod 1979; 21: 979-83.

4. Fragaszy DM, Visalberghi E, Fedigan LM. The complete capuchin. the biology of the genus Cebus. Cambridge: Cambridge University Press, 2004.

5. Carnegie SD, Fedigan LM, Ziegler TE. Behavioral indicators of ovarian phase in white-faced capuchins (Cebus capucinus). Am J Primatol 2005; 67: 51-68.

6. Lancaster JB, King BJ. An evolutionary perspective on menopause. In: In her prime: New views of middle-aged women. Kerns & Brown (eds). Champaigh: University of Illinois Press,1992: 7-15,.

7. Rodrigues RC, Carrijo C, Uribe CE, Gasbarri A, Tavares MCH. Sexual behavior and progesterone levels in elderly female capuchin monkeys (Cebus libidinosus). Neurobiologia 2011; 74:10-21.

8. Nichols SM, Bavister BD, Brenner CA, Didier PJ, Hrrison RM, Kubisch HM. Ovarian senescence in the rhesus monkey (Macaca mulatta). Hum Reprod 2005; 20: 79-83.

9. Altmann J, Gesquiere L, Galbany J, Onyango PO, Alberts SC: Life history context of reproductive aging in a wild primate model. Ann NY Acad Sci 2010; 12: 127-38.

10. Atsalis S, Videan E. Reproductive aging in captive and wild common chimpanzees: Factors influencing the rate of follicular depletion. Am J Primatol 2009; 71: 271-82.

11. Walker ML. Menopause in female rhesus monkeys. Am J Primatol 2005; 35: 59-71.

12. Soules MR, Sherman S, Parrott E, Rebar R, Santoro N, Utian W, Woods N. Executive summary:

stages of reproductive aging workshop (STRAW). Climacteric 2001; 4: 267-72.

13. Hansen KR, Knowlton NS, Thyer AC, Charleston JS, Soules MR, Klein NA. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod 2008; 23: 699-708.

14. Shideler SE, Gee NA, Chen J, Lasley BL. Estrogen and progesterone metabolites and follicle-stimulating hormone in the aged macaque female. Biol Reprod 2001; 65: 18-25.

15. Treloar AE. Menstrual cyclicity and the pre-menopause. Maturitas 1981; 3: 249-64.

16. Burger HG, Dudley EC, Robertson DM, Dennerstein L. Hormonal changes in the menopause transition. Recent Prog Horm Res 2002; 57: 257-76.

17. Petticrew M, Smith GD: The Monkey Puzzle. A Systematic Review of Studies of Stress, Social Hierarchies, and Heart Disease in Monkeys. PLOS ONE 2012; 7:e 27939.

18. Atsalis S, Margulis S: Primate reproductive aging. from lemurs to humans. Primate Reproductive Aging: Cross-Taxon Perspectives 2008; 36: 186-194.

19. Carnegie SD, Fedigan LM, Ziegler TE: Social and environmental factors affecting fecal glucocorticoids in wild, female white-faced capuchins (Cebus capucinus). Am J Primatol 2011; 73: 861-869.

20. Abbott DH, McNeilly AS, Lunn SF, Hulme MJ, Burden FJ: Inhibition of ovarian function in subordinate female marmoset monkeys (Callithrix jacchus jacchus). J Reprod Fertil 1981; 63: 335-345.

21. Albuquerque ACSR, Sousa MBC, Santos HM, Ziegler TE. Behavioral and hormonal analysis of social relationships between oldest females in a wild monogamous group of common marmosets (Callithrix jacchus). Int J Primatol 2001; 22: 631-645.

22. Wolfram M, Bellingrath S, Kudielka BM. The cortisol awakening response (CAR) across the female menstrual cycle. Psychoneuroendocrino 2011; 36:905-12.

23. Ziegler, T.E., G. Scheffler, and C.T. Snowdon. The relationship of cortisol levels to social environment and reproductive functioning in female cotton-top tamarins, Saguinus oedipus. Hormones and behavior, 1995; 29: 407-424.

24. Ferrari, E., et al. Age-related changes of the hypothalamic-pituitary-adrenal axis: pathophysiological correlates. European Journal of Endocrinology 2001; 144: 319-329.

25. Gust DA, Wilson ME, Stocker T, Conrad S, Plotsky PM, Gordon TP. Activity of the hypothalamic-pituitary-adrenal axis is altered by aging and exposure to social stress in female rhesus monkeys. Journal of Clinical Endocrinol Metab; 2000; 85: 2556-2563.

26. Hess G, Riegle G: Effects of chronic ACTH stimulation on adrenocortical function in young and aged rats. Am J Physiol--Legacy Content 1972; 222:1458-1461.

27. Garrido P, de Blas M, Del Arco A, Segovia G, Mora F. Aging increases basal but not stress-induced levels of corticosterone in the brain of the awake rat. Neurob Aging 2012; 33: 375-382.

28. Hauger R, Thrivikraman K, Plotsky P: Age-related alterations of hypothalamic-pituitary-adrenal axis function in male Fischer 344 rats. Endocrinology 1994; 134: 1528-1536.

29. Magri F, Locatelli M, Balza G, Molla G, Cuzzoni G, Fioravanti M, Solerte SB, Ferrari E, Cravello L, Muzzoni B, Casarotti D, Paltro M, Solerte SB, Fioravanti M, Cuzzoni G, Pontiggia B, Magri

F: Age-related changes of the hypothalamic-pituitary-adrenal axis: pathophysiological correlates. Eur J Endocrinol 2001; 144: 319-329.

30. Van Cauter E, Leproult R, Kupfer D. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocr Metab 1996; 81: 2468-2473.

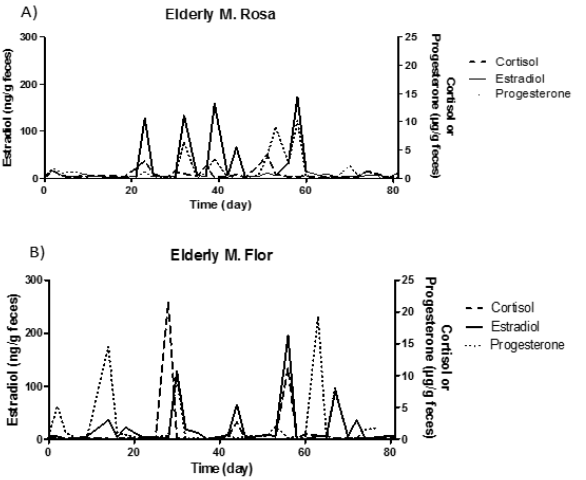

Figure 1 Estradiol , progesterone, and cortisol hormone Levels in fecal metabolites (ng/g feces) of elderly female Capuchin monkeys. A) M. Rosa and B) M. Flor, from the Primate Center at the University of Brasilia-UnB. The number 0 corresponds to the first day of biological material assessmen**t.**

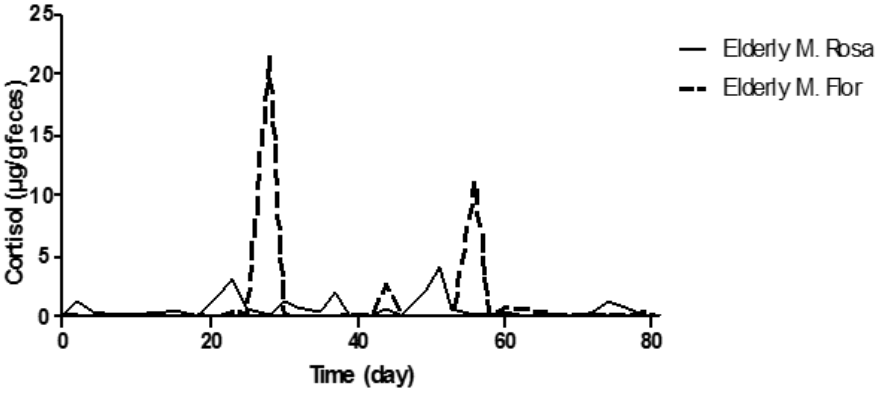

Figure 2 Cortisol levels in fecal metabolites (ng/g feces) of elderly female capuchin monkeys M. Rosa and M. Flor from the Primate Center at the University of Brasilia-UnB. The number 0 corresponds to the first day of biological material assessment.

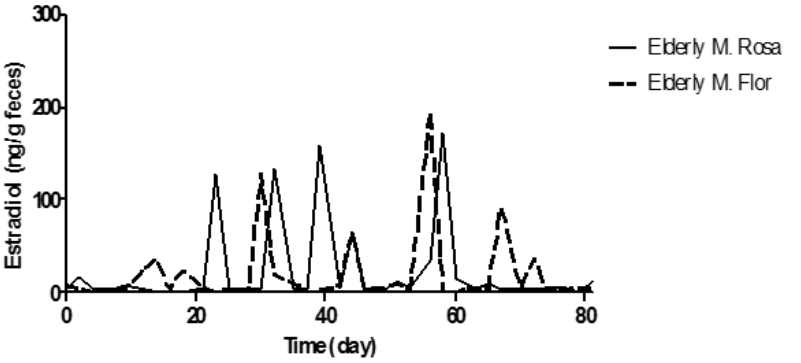

Figure 3 Estradiol levels in fecal metabolites (ng/g feces) of elderly female capuchin monkeys M. Rosa and M. Flor from the Primate Center at the University of Brasilia-UnB. The number 0 corresponds to the first day of biological material.

18 - The use of hallucinogenic plants in the history of *Homo sapiens*: prehistory and ancient peoples

Marcelo Moraes Valença, MD, PhD, FAHS[1], *Juliana Ramos de Andrade, PhD*[2], *Josiene Maria Falcão Fraga dos Santos, PhD*[3]

From time immemorial to prehistory, through the ancient egyptians to indigenous peoples, ***Homo sapiens*** uses hallucinogenic plants looking for ways to connect with the supernatural in order to communicate with divinities or as an escape from reality. Contact with the supernatural was intended to receive messages that help to understand and face possible threats from the forces of nature. Ancient peoples (and even current isolated populations) believed that hallucination was a way of contacting the gods (Figure 1).

A hallucinogen is any chemical that distorts the senses and produces perceptions or experiences that drift dramatically away from ordinary reality. One way that the human species found to reach such a mental stage was by consuming plants by inhaling, chewing or ingesting the plant itself (*e.g.*, lotus flower and poppy leaf) as well as in the form of beverages usually extracted from the fruits or in way of teas as the example of peyote (see list of plants in Table 1).

The most widespread form of consumption among primitive peoples of substances that exerted influence on their psychoactive capacity was beverages produced through fruit juice. The more frequent substance responsible for altering one's level of consciousness was alcohol. In this case, alcohol intoxication was the main explanation for behavioral changes that occurred hours after ingestion. The use of a combination of alcoholic drinks with hallucinogenic plants was a common practice during ritualistic ceremonies[18].

There are robust archaeological clues suggesting the use of alcohol by humans dating back 10,000 years ago. Recently a prehistorical brewery was discovered in 2018 in a

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burial site in a cave near Haifa, Israel. The residue of beer estimated as 13,000-year-old was found, believed to have been used for ritual feasts to honor the dead.

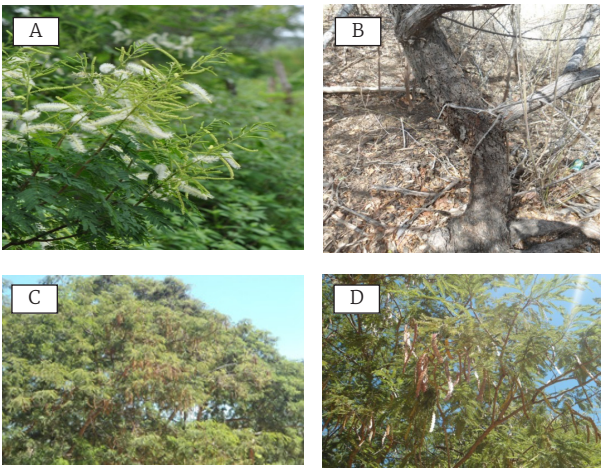

Figure 1. Images of hallucinogenic plants used by Amerindians in Brazil. A. Detail of the inflorescence of ***Mimosa tenuiflora*** "black jurema", a native species of the Caatinga used by indigenous peoples of the region; B. The detail of the "black jurema" bark; C and D. show part of the plant used to make tea ***Anadenanthera colubrina*** "angico" with fruit, the seeds of the species are scraped off and their powder is inhaled causing delusions and trance. (Photo courtesy of teacher PhD Diego Nathan Nascimento Sousa).

Early records of the use of hallucinogenic beverages by primitive peoples are linked to the well-known ayahuasca. From Quechua ayahuasca means "vine of the dead" or "vine of the spirit," also known as "hoasca', "daime", "iagê', 'saint-daime" and is produced from the combination of the vine ***Banisteriopsis caapi, Psychotria viridis*** and ***Diplopterys cabrerana,*** among other plant species. It was initially used by the Incas, with the first reports of its use by indigenous people in the West of North America in 1768, when it was used for divination, mystification and bewitching. However, it has been estimated that indigenous populations have used beverages with these plants for at least 3,500 years. The substances encountered in the tea act altering perception and cognition probably through activation of serotonin receptors in a way similar to LSD. During its action, the drink causes an increase in pupillary diameter and dramatically increases the neuroendocrine responses regarding prolactin, cortisol and growth hormone secretion. These are typical actions of dimetiltriptamina (DMT), one of the major psychoactive substance found in the ayahuasca tea.

The search for substance extracted from elements of nature, such as plants, which would bring "pleasurable sensations" is a type of behavior present not only in man,

but also in the animal world. As examples, we can mention the quest for marula by elephants, ostriches, warthogs and baboons which, after eating fermented marula fruit, may become behaviorally intoxicated, with typical inebriation.

Such human behavior in seeking different forms of altering his or her level of consciousness is very marked, even nowadays. It may be an intrinsic mechanism in the search for such foods by trial and error. It is clear that this is a learned behavior transmitted from one generation to the next.

In the Guarani tribe, the use of a pipe is a common custom, but the shaman uses it as a form to communication with the other world with additional help from drinks from special plants. During the sessions some members lose consciousness and are taken for dead. The Guarani believe that the dead are in the other world and the shaman, by using the smoke from his pipe, is able to bring them back to life.

There are countless examples of the use of hallucinogenic plants in primitive rituals by shamans in different parts of the world, employing a large variety of plants.

Table 1. List of hallucinogenic and/or entheogenic plants used by primitive and ancient peoples. Historical period of first recorded uses; Place and/or peoples who used the plant during the respective period; Plant species used for hallucinogenic stimulation; Popular name of the plant species; Part plant used for the extraction of psychoactive substances; Psychoactive substance with higher hallucinogen percentage in plant; and Substance extraction method and nervous system effect. (-) no record or information.

Historical period	Local/ People	Species used	Popular name	Part plant	Psychoactive substance	Substance extraction method	Nervous system effect
Prehistory[1-4]	Africa	*Tabernanthe iboga* Baill.	iboga, ibogaina	root	ibogain	chewing, tea	hallucinations, visions, feeling sick and unwell
10.000 BC[2,5]	New Zeland/ Maori	*Radula perrottetii* Gottsche ex Steph.	radula	aerial part	perrotinolene	-	catalepsy, hypolocomotion
9.000 BC[3,6]	Europe and Asia	*Artemisia absinthium* L.	wormwood, absinthe	aerial part	tuinona	infusion, decoction	-
5.000 BC[3,7,8]	China	*Cannabis* sp.	marijuana, haxixi	flower	cannabinoids	vaporization	stimulant and hyperlocomotion
4.000 BC[3,7]	Egypt	*Nelumbo nucifera* Gaertn.	lotus	flower	aporphine	tea, alcohol drink	clutching, hyperlocomotion
4.000 BC[2,3]	Incas and Mayas	*Erythroxylum coca* Lam.	coca	leaf	cocaine	vaporization	hyperlocomotion
3.400 BC[2,3,7,9]	Sumerians	*Papaver somniferum* L.	poppy	leaf	alkaloides	tea, steam inhalation (opium)	hypnotic, narcotic sedative
3.000 BC[2-4,6]	North America	*Lophophora williamsii* (Lem. ex Salm-Dyck) J.M.Coult	peyote	stalk	mescaline (3,4,5-trimethoxyphenylethylamine)	chewing, tea	stimulant and hyperlocomotion
2.700 BC[3,7]	Egypt	*Nymphaea caerulea* Savigny	blue lotus	flower	alkaloides	tea, soaked in wine	lucid dreams

Continuation table 1.

Historical period	Local/ People	Species used	Popular name	Part plant	Psychoactive substance	Substance extraction method	Nervous system effect
2.000 BC[2,3,4,6,10-14]	Incas	*Psychotria viridis* Ruiz & Pav.	-	leaf	N, N-dimethyltryptamine	tea (ayahuasca)	alteration of perception and cognition
2.000 BC[2,3,4,6,10-14]	Incas	*Psychotria carthagenensis* Jacq.	-	leaf	N, N-dimethyltryptamine	tea (ayahuasca)	alteration of perception and cognition
2.000 BC[2,3,4,6,10-14]	Incas	*Diplopterys cabrerana* (Cuatrec.) B. Gates	-	leaf	N, N-dimethyltryptamine	tea (ayahuasca)	alteration of perception and cognition
2.000 BC[2,3,4,6,10-14]	Incas	*Banisteriopsis caapi* Spruce ex Griseb.	vine, ayahuasca	bark	harmina, harmalina e tetrahidroharmina	tea (ayahuasca)	alteration of perception and cognition
1.500 BC[2,3,9,14]	Israel	*Mandragora officinarum* Bertol.	mandragora	root, leaf	alkaloides	infusion, maceration	mydriasis, drowsiness
1.500 BC[2,3,14]	Egypt	*Datura stramonium* L.	datura	root, leaf, stalk and flower	alkaloides	tea	delusions and loss of consciousness
AD 1.300 - 1.521[23]	Mexico/ Aztec	*Ipomoea tricolor* Cav.	morning glory	seed	ergine	chewing	lucid dreams
AD 1.300[2,3]	Ethiopia and Yemen	*Catha edulis* Forssk.	khat	leaf	catinone	chewing, tea	stimulant and hyperactivity
AD 1.300 - 1.521[2,3]	Mexico/ Aztec	*Ipomoea violacea* L.	black badoh	seed	ergine	chewing	lucid dreams
-	Europe[2,3]	*Hyoscyamus niger* L.	black hembane	seed	alkaloides	mixed into drink	deep sleep
-	Iran e India[2,3]	*Peganum harmala* L.	harmal	flower	alkaloides	inhalation of dust	spontaneous locomotor activity and hyperlocomotion
-	-	*Atropa belladonna* L.[2,3,9,14]	beladona	root, leaf and fruit	atropine	chewing	hallucinations
-	Mexico[2,3,16]	*Salvia divinorum* Epling & Játiva	salvia	leaf	salvinorin A	smoked dry leaf, chewing	loss of consciousness and hallucinations
-	Brazil[3,15]	*Mimosa tenuiflora* (Wild.) Poiret	jurema preta	stem bark or root	N, N-dimetiltriptamina	tea	alteration of perception and cognition
-	South of Africa/ Xhosa[2,3]	*Silene capensis* Otth.	xhosa	root	alkaloides	inflatable dust (yopo)	lucid dreams
-	Brazil[2,3,17]	*Dorstenia multiformis* Miquel	carapiá	root	-	tea and smoke	lucid dreams
-	India[2,3]	*Celastrus paniculatus* Willd.	intellected tree	seed	N, N-dimethyltryptamine	chewing	lucid dreams
-	China[2,3]	*Asparagus officinalis* L.	willd aspargus	root	-	extract (dust)	lucid dreams
-	South of America[2,3]	*Anadenanthera colubrina* (Vell.) Brenan	angico	seed	bufotenin	inflatable dust (yopo)	delusions and trance
-	South of America[2,3]	*Anadenanthera peregrina* (L.) Speg.	angico	seed	bufotenin	inflatable dust (yopo)	delusions and trance

In conclusion, the search for plants that would alter the level of conscience, particularly those provoking "pleasure" sensations, is an old practice of the humankind, still present nowadays.

References

1. Papadodima SA, Dona A, Evaggelakos CI, Goutas N, Athanaselis SA. (2013) Ibogaine related sudden death: a case report. Journal of Forensic and Legal Medicine 20(7):809-11. https://doi.org/10.1016/j.jflm.2013.06.032.

2. Ivison Ramos Blog (2015) As drogas da antiguidade. https://ivisonramos.wordpress.com/2012/10/11/as-drogas-da-antiguidade/. Accessed 02 Jul 2019

3. Entheology: preserving shamanism's ancient sacred knowledge (1990) Search. http://entheology.com/category/news-articles/. Accessed 25 Jun 2019

4. Hypescience (2011) 8 alucinógenos que alteram a mente humana. https://hypescience.com/8-alucinogenos-que-alteram-a-mente-humana/. Accessed 25 Jul 2019

5. Leman J. (2018) Liverwort plants contain a painkiller similar to the one in marijuana: The THC-like substance may have medical benefits minus the same kind of high. Science News 194(10):16.

6. Schutles RE (1982) Plantas Alucinógênicas. México, DF: Prensa Médica Mexicana 164p.

7. History Play (2019) https://br.historyplay.tv/noticias/como-drogas-eram-usadas-no-antigo-egito. Accessed 18 Jul 2019

8. Honório KM, Arroio A, Silva ABF. (2006) Aspectos terapêuticos de compostos da planta Cannabis sativa. Química Nova 29(2):318-325.

9. Martinez ST, Almeida MR, Pinto AC. (2009) Alucinógenos naturais: um voo da Europa Medieval ao Brasil. Química Nova 32(9):2501-2507.

10. McKenna DJ, Towers GHN, Abbott F. (1984) Monoamine oxidase inhibitors in South American hallucinogenic plants: Tryptamine and β-carboline constituents of Ayahuasca. Journal of Ethnopharmacology 10(2):195-223.

11. Costa MCM, Figueiredo MC, Cazenave SOS. (2005) Ayahuasca: uma abordagem toxicológica do uso ritualístico. Revista de psiquiatria clínica 32(6):310-318. http://dx.doi.org/10.1590/S0101-60832005000600001

12. Bois-Mariage F. (2002) Ayahuasca: une synthèse interdisciplinaire. Psychotropes 8:79-113. http://dx.doi.org/10.3917/psyt.081.0079.

13. Naranjo P. (1979) Hallucinogenic plant use and related indigenous belief systems in the Ecuadorian Amazon.Journal of Ethnopharmacology 1:121- 45.

14. Cultural Survival (1985) Hallucinogenic plants and their use traditional societies. https://www.culturalsurvival.org/publications/cultural-survival-quarterly/hallucinogenic-plants-and--their-use-traditional-societies. Accessed 02 Jun 2019

15. Grünewald RA. (2018) On the Trails of Jurema. Religião & Sociedade 38(1):110-135. http://dx.doi.org/10.1590/0100-85872018v38n1cap05

16. Calado VG. (2013) Novas Substâncias Psicoativas. O caso da salvia divinorum. Lisboa: Serviço de Intervenção nos Comportamentos Aditivos e nas Dependências.

17. Balestrin L, Dias JFG, Miguel OG, Dall'Stella DSG, Miguel MD. (2008) Contribuição ao estudo fitoquímico de Dorstenia multiformis Miquel (Moraceae) com abordagem em atividade antioxidante. Revista brasileira de farmacognósia 18(2): 230-235

18. Wikipedia (2019) History of alcoholic drinks. https://en.wikipedia.org/wiki/History_of_alcoholic_drinks. Accessed 29 Jul 2019

In memoriam

Dr. Peter Morgane (1927-2010)

By: Dr. David Mokler, University of New England, USA.

Peter J. Morgane, PhD, neuroscientist and neuropharmacologist, had a distinguished career spanning five decades. He was born in Atlanta, Georgia and spent numerous early years in New Orleans. He received his bachelor's degree from Tulane University in New Orleans. Dr. Morgane was a page in the United States Senate in 1943 and 1944. He served in the U.S. Army in 1947-1948 at Fort Hood and Camp Moxey, Texas.

He did his graduate studies at Northwestern University Medical School in Chicago, receiving both his master's and doctorate degrees in neurophysiology. His work at Northwestern focused on feeding behavior and he was particularly intrigued with the Klüver-Bucy Syndrome. His interest in feeding behavior and the effects of nutrition on brain development were key aspects of his research throughout his career.

He did post-graduate work at the University of Oregon in Portland, Oregon and the Brain Research Institute in Mexico City, working at the latter with the famous sleep researcher, Raúl Hernández Peón. He continued his interest in sleep throughout his career, publishing more than 40 papers in the field. His interest in sleep led to a deeper interest in serotonin and the limbic system which he worked on until his death.

He then moved to Miami where he joined John Lilly in investigating the whale brain. He published seminal work on the neuroanatomy of the whale brain, working with the famous neuropathologist Paul Yakovlev at the Department of Neuropathology, Harvard Medical School. He had many collaborators in this work including Willard McFarland at the NIH and Myron Jacobs. He continued to work with Ilya Glezer of the City University of New York and Patrick Hof at the Mount Sinai School of Medicine in New York City. Dr. Morgane's studies on whale brains were published in several monographs and some 40 scientific papers from 1962 until 1998. These studies form the definitive works on whale brain: both large and small (dolphins, porpoises and whales). His colleague David Mokler at the University of New England maintains the archives of his research on cetacean brains. In addition, Patrick Hof has the histological collection of whale brains started by Dr. Morgane.

Dr. Morgane moved to Worcester MA in 1968 as head of the sleep research laboratories at the Worcester Foundation for Experimental Biology in Shrewsbury MA. There he was director of the Training Program for Neurobiology from 1970-1975. He also began work on a Program Project dealing with effects of prenatal protein malnutrition on the developing brain, which he worked on until the time of his passing. That program moved to Boston University Medical School in 1987 under the leadership of Janina Galler and was funded by the National Institute of Child Health and Human Development. The program moved to Judge Baker Children's Center at Harvard Medical School in 2007 with funding from the National Institute of Mental Health. He was Professor in the Department of Psychiatry at Boston University School of Medicine and Professor of Pharmacology at the University of New England College of Osteopathic Medicine in Biddeford, Maine. He had many colleagues and collaborators in his work on prenatal protein malnutrition. One colleague in particular was Leon Cintra, who did a sabbatical with him at the Worchester Foundation and continued their friendship and collaboration until Dr. Cintra's death in 2009.

In his scientific career Dr. Morgane published over 230 scientific papers and edited the four volume Handbook of the Hypothalamus. He was a member of 16 scientific societies. He served for 11 years on grant reviews at the National Science Foundation and at the National Institutes of Health for over 35 years. At the University of New England, Dr. Morgane worked with David Mokler on serotonin and the limbic system and they edited a volume of Neuroscience and Biobehavioral Reviews entitled "The Limbic Brain: Continuing Resolution". He became Professor Emeritus at UNE in June 2010.

Dr. Morgane gave considerable support to the University of New England. He funded the Cécile Morgane Research Laboratory, as well as the Peter and Cécile Morgane Hall. This created valuable research space for the College of Osteopathic Medicine, and offices and laboratories for the College of Arts and Sciences.

Dr. Morgane's principal endeavors outside of science included book collecting, antique gathering, champagne tasting and world-wide travel to over 45 countries, including Japan, India, Australia, East Africa, Israel and all of the countries of Europe. He and his wife, Cécile, who was born in France, particularly loved going to France to sample the great beauty of the country, as well as the wine and food. In recent years, he enjoyed the pleasure of good champagne and will be remembered for his champagne tasting parties. He loved animals, especially his cat Monet who was his constant companion since his wife's death. He contributed to the care of animals at the Animal Welfare Society in West Kennebunk. He died as a member of the Roman Catholic Church. He passes away on Monday, September 27, 2010 at Southern Maine Medical Center following a short illness. He was predeceased by his wife, Cecile Murette Morgane, who died in 2001.

He is greatly missed by his friends and colleagues.

Cintra's remembrance

Dr. Leon Cintra: A life full of passion - 10th Anniversary Remembrance

By Pilar Durán, PhD[1]

Leon Cintra's passing happened ten years ago. His life was taken prematurely and suddenly in a highway accident. Since then, his presence has been deeply missed and remembered not only for his brilliant mind, but also for his noble and gentle heart.

Leon died as he lived, his last act of kindness was towards his fellow men. That is something I will never forget. I suffered the great pain, but the fortune to share his final moments.

Leon was an empathic being, always willing to help others without receiving anything in return, a lover of the simplest pleasures, delicious speeches and endless themes. Those who knew him can attest to that.

As Scientist, his career was exceptional, full of passion and vocation. It was a pleasure to see him in the laboratory, conducting experiments and asking questions. He was recognized respected and admired by his own and strangers. He was also, an internationally recognized speaker, invited around the world to impart his knowledge.

To honor life and work of Leon Cintra, a special issue was published in the journal Nutritional Neuroscience (2011). There is a testament beautifully written by others about his scientific career. Since others have already written his achievements and much better than I could, I will do my best to summarize almost 40-year span in few lines.

His career began as undergraduate student at the Insitituto de investigaciones Biomédicas, Universidad Nacional Autónoma de México (IIB-UNAM) of México. Where he earned his bachelor, master and PhD. Initially, León Cintra focused his studies on the impact of malnutrition on electroencephalographic activity in an animal model, under Dr. Manuel Salas' mentorship.

His postdoctoral training at the Worcester Foundation marked the course of his

1 The National Autonomous University of Mexico/UNAM.

subsequent research lines, there, under the advice of Dr. Peter Morgane, directed his studies to the detrimental effects produced by pre and postnatal protein malnutrition in the developing central nervous system, using the sleep phenomenon as an index of functional integrity. Following his return to Mexico in the late 1980s and as an independent researcher at UNAM (IIB, Instituto de Neurobiología) Cintra continued his long-term collaboration until the end of his scientific career, with Dr. Morgane first at the Worcester Foundation and later at the University of Boston Center for Behavioral Development and Mental Retardation, where he strengthened academic ties with the group, then led by Dr. Janina Galler.

His scientific and inquisitive interest led him to deepen the study of the sleep-wake cycle and circadian rhythms in mammals. His research laid the foundations for the scientific study of prenatal and chronic protein malnutrition and sleep physiology. He demonstrated chronic protein malnutrition produces deleterious effects in several brain circuits and nuclei such as Raphe, Locus Coerulus, Suprachiasmatic and Hippocampal Formation, as well as the behavioral responses controlled by those circuits.

Dr. Cintra left an indelible mark through his contribution to the field of malnutrition and developmental neurophysiology. Nowadays, his contributions are a mandatory reference when we refer to sleep, malnutrition and brain development.

Obituary

Dr. Kenneth Charles Moore (1943 – 2018): O homem, o tempo e o vento

Dr. Kenneth Charles Moore (1943 – 2018): The man, the weather and the wind

Dr. Carlos Augusto Carvalho de Vasconcelos[1]

Kenneth Charles Moore (Ken) faleceu inesperadamente num final de domingo, dia 29 de abril de 2018, aos 74 anos de idade na cidade de Iowa, IA/USA, estava sofrendo com uma das doenças mais terríveis da humanidade atualmente, depressão, ele nasceu em 21 de maio de 1943 em Long Beach, Califórnia, filho de Forrest e Lillian Williams Moore. Em 2 de setembro de 1967, Ken casou-se com Sharon Sweetland (Sherry) em Garden Grove, Califórnia. Ele frequentou o Harbour Junior College, em seguida, recebeu seu bacharelado em Biologia pela California State University em Long Beach e seu mes-trado pela University of Iowa em 1971. Em associação com a Faculdade de Bioquímica do Iowa, Ken desenvolveu o Central Electron Microscopy Research Facility (CEMRF) em 1973. Em 1979, tornou-se um assistente na universidade sob os auspícios do Ga-binete do Vice-Presidente de Pesquisa. Em sua função como Diretor do Laboratório de Microscopia, ele e sua equipe colaboraram com o corpo docente em até 200 projetos de pesquisa por ano. Ele lecionou seis cursos por ano, colaborou em bolsas de pesquisa en-volvendo melanoma ocular e fibrose cística e foi autor ou co-autor de inúmeros artigos de pesquisa científica. Ken representou a Universidade de Iowa e Iowa City nos Estados Unidos e no Japão, China, Venezuela, Brasil, Canadá e Europa. Ele também atuou como palestrante internacional para organizações científicas profissionais, consultou recur-sos de microscopia em universidades dos EUA e estrangeiras e contribuiu para o desen-volvimento de instrumentação para empresas como DuPont, Emitech e Hitachi. A foto acima ilustra

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Dr. Ken em Olinda/PE, Catedral da Sé, lugar que adorou, posteriormente em outro momento com sua esposa em na Praia de Japaratinga em Alagoas (Foto 2).

Dr. Ken como era carinhosamente conhecido serviu dois mandatos como Presidente do Conselho de Pessoal da Universidade e foi membro fundador de comitês que estabeleceram o programa Mulheres na Ciência e Engenharia da Universidade de Iowa e o Conselho de Transferência de Tecnologia de Iowa. Ken recebeu o Prêmio de Excelência da Universidade de Iowa e o Prêmio de Reagentes por Contribuições Extraordinárias para a Universidade e o Estado de Iowa. Após 32 anos de serviço na Universidade de Iowa, Ken se aposentou em 2010. Ken era um defensor e um mentor para os jovens. Ao longo de sua longa carreira, Ken entusiasticamente apresentou alunos, do jardim de infância à pós-graduação, à maravilha da ciência. Em sua aposentadoria, Ken continuou a fazer amizade e encorajar os jovens em sua vida. Além de sua família amorosa e suas contribuições para a expansão do conhecimento científico, Dr. Kenneth Moore deixou um legado daqueles cujas vidas são mais ricas por tê-lo conhecido. Deixou sua esposa Sherry, com 51 anos, seu filho, Kenneth Charles, de Phoenix, Arizona/AZ; sua irmã, Sue Mack, da Folsom, Califórnia/CA; meio-irmão, Jody Tarango e meia-irmã, Diane Prim, ambos de Phoenix; e seus Yorkies (Yorkshires) Charlie e Sophie.

Eu particularmente o conheci através da Dra. Valéria Fazan de Ribeirão Preto/SP, grande amiga e colaboradora em Ribeirão Preto, durante minha longa estadia na Faculdade de Medicina de Ribeirão Preto, e posteriormente minha co-orientadora de Doutorado na UFPE, fui convidado por ela em setembro de 2008 a realizar visita e estágio de doutorado na The University of Iowa, em Iowa city, no Central Microscopy Research Facility, que se realizou ano seguinte, de março a abril de 2009, a foto mostra um momento bem agradável num restaurante na hora do Almoço (Foto 3), o que me levou a grande oportunidade de conhecer o serviço de perto e ter maior contato com todos, professores, técnicos, alunos e novos amigos, enriquecendo enormemente meus conhecimentos, lá nosso chefe era Dr. Kenneth Moore, lembro-me bem do primeiro encontro, fomos almoçar num pub inglês, ele me perguntou se eu estava gostando e onde eu tinha nascido no Brasil, daí retornei ao Brasil realizado e feliz pela brilhante oportunidade e experiências adquiridas. Para minha surpresa maior ainda foi saber repentinamente que ele queria vir ao Brasil para minha banca de tese de doutorado e conhecer a cidade do Recife e Olinda, bem como as praias mais tranquilas, gostou muito das histórias que contei para ele, das praias paradisíacas da nossa região, ataques de tubarões, fauna, flora, arte e cultura regional. Em fevereiro de 2010 veio ao Recife, o recebemos com toda honra e glória, uma imensa felicidade contagiou a todos, ainda era o grande chefe (Big Boss), Diretor competente e uma personalidade ímpar, um ser humano de extrema sensibilidade, além do seu tempo, a defesa de doutorado foi excelente e tudo se resolveu brilhantemente, ele participou como Membro Titular Externo

da Banca examinadora da minha defesa de tese na UFPE no Recife em fevereiro de 2010 (Foto 4). Dr. Ken também visitou e ficou encantado com o Laboratório de Imunopatologia Keizo Asami/LIKA - UFPE (Foto 5). Meses depois do mesmo ano ele se aposentaria (Foto 6), na foto ladeado por colegas, alunos e amigos, eu não pude comparecer, mais fui representado pelos outros. Soube também que Dr. David Mokler trabalhou com Dr. Ken no início de sua carreira universitária no doutorado. Quando soube de sua morte pelo Dr. Randy Alan Nessler, seu pupilo e atual Diretor do Central Microscopy Research Facility of The University of Iowa (Foto 7), fiquei muito triste, bem como todos os familiares e amigos. Ainda penso muito nele, deixou um legado precioso/irreparável. Recentemente Dr. Randy não só autorizou o necrológio, bem como agradeceu muito a homenagem e lembranças, bem como frequentemente pensa nele e em mim. Tudo isso é a amizade. Saudades eternas.

Foto 2 – Com a esposa Sherry na paradisíaca praia de Japaratinga, Alagoas, Brasil

Foto 3 – Restaurante na cidade de Iowa, Estados Unidos, no horário de almoço

Foto 4 – Felicitações pelo doutoramento na UFPE em 2010

Foto 5 – Visita ao LIKA/UFPE

Foto 6 – Comemoração pela de aposentadoria no CMRF, Iowa city, entre colegas, amigos e alunos.

Foto 7 – Dr. Randy Nessler, atual Diretor do Central Microscopy Research Facility da Universidade de Iowa, Iowa city, USA.

Reference

1.https://www.legacy.com/obituaries/press-citizen/obituary.aspx-?n=kenneth-c-moore&pid=188894496&fhid=13623

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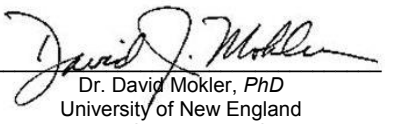

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