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Neuroplastic effect induced by Brain Skills ® was observed in human brain cell culture

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Abstract

Cognitive function plays an important role in brain performance and it seems that brain functioning can be influenced by nutrition and dietary components. Nutrients regulate brain development during life, being essential for migration, growth, synapse, myelination and antioxidant/neuroprotective effect. Conversely, the young brain is remarkably plastic and therefore more amenable to repair after nutrient repletion. In this perspective, Brain Skills ®, which consists of an association of amino acids, vitamins, minerals, precursors of neurotransmitters, energy generators and antioxidants, was tested in human neuron cultures. Cultured human brain tissue was maintained in culture medium in the presence or absence of ®. Brain Skills ® promoted a neuroplastic effect after 72 hours of treatment, showing more developed neurons, larger cell bodies, greater dendritic and axonal plasticity, in addition to presenting a

greater amount of synaptic contact. The results demonstrated that the product Brain Skills ® had a significant effect on the neuroplastic development of human neurons.

Introduction

Dietary supplements or, more commonly, "supplements," are products taken by mouth that contain a "dietary ingredient." Dietary ingredients include vitamins, minerals, amino acids, botanicals (including herbs) and enzymes, as well as other substances alone or in combination that can be used to supplement one's diet. Supplements come in many formulations, including pills, capsules, tablets, powders, food bars and liquids (Dauncey & Bicknell, 199).

Products with the purpose of improving memory and focus, aiming at better cognitive performance and energy, are widely available in supplement stores and the internet. Therefore, studies established in the literature have shown that nutrition affects the structure and functioning of the brain throughout life (Dauncey and Bicknell, 1999; Dauncey, 2009; Dauncey, 2012).

Epidemiological studies that have focused on fruit and vegetable intake and cognitive function have also largely found that adequate consumption can prevent cognitive decline, while low intake is associated with increased cognitive decline (Miller *et al*., 2016). Specifically, increased intake of blueberries and strawberries, as well as increased intakes of anthocyanidins and total flavonoids, were associated with slowing the rate of cognitive decline by up to 2.5 years (Devore *et al*., 2012).

These studies highlight the importance of studying metabolic factors and nutrients in the early-life stress-induced effects on the brain. In the near future, it will be key to further understand the exact mechanisms mediating the effects of nutrients and metabolic factors and the windows of opportunity for interventions on brain function, as this will open entirely new avenues for targeted nutrition for vulnerable populations. However, while the early life period is a window of particular vulnerability to the programming effects of diet and other environmental influences, diet at other phases of life is also important in dictating mood and cognition.

The challenge is to integrate the various previously discussed perspectives on brain neuroplasticity to fulfill the anti-aging neuronal extensive benefits.

Methods

The biological samples were obtained from the brain tissue of patients traumatized with TBI, attended at the Regional Hospital Tarcísio de Vasconcelos Maia (HRTM), in Mossoró-RN, who required emergency neurosurgery, in some very specific situations.

Legal Requirements

The collection of the material was only carried out with the authorization of the legal representative of the

patient through the signing of the Term of Free and Clarified Assent (TALE). The collection was carried out in accordance with the specific legislation (Resolution 441/2011 and ordinance of the Ministry of Health 2201/11). This research project was approved by the local Research Ethics Committee (CAAE 65640517.0.0000.5294).

Criteria for Inclusion

Age between 18 and 70 years; signature of TALE by the legal guardian; patients who require emergency neurosurgery due to TBI in very specific situations:

in cases of patients with cranial dandruff with laceration of the dura-mater and cerebral cortex, with spontaneous (caused by trauma) brain tissue, which obviously need neurosurgery ; and in patients with traumatic intracranial hematomas with subcortical component and expansive effect, requiring surgical removal through a small opening in the cerebral cortex, known as corticectomy, for drainage of the clot.

Pre-Operative Preparation

After the indication of the need for surgical intervention, the patients were referred to the operating room of the surgical center in the usual manner, being routinely received by the nursing and anesthesiology team for anesthesia and subsequent surgery, as is the protocol for all patients who are operated. There was no change in the routine of the pre-surgery visits. Meanwhile, in another room, a member of the research team presented TALE with the legal guardian for the patient to read and authorize or not to collect the material.

Surgical Procedure

Surgical interventions followed the internationally agreed steps for the treatment required for the patients in question, according to the following sequence: surgical table positioning, asepsis and antisepsis, placement of sterile surgical fields, surgical incision in the skin and subcutaneous tissue, rotation of the cutaneous or myocutaneous flap, craniotomy (opening of the skull) and opening of the dura mater. In this stage of the surgical procedure, after drainage of acute subdural hematoma, a small area of cortical laceration of the right temporal lobe was visualized in patient 1, and the biological sample of brain tissue was collected and the surgery followed its normal steps with the hemostasis, closure of the wraps (dura mater, skull, muscle, subcutaneous tissue, skin) and dressing. I reiterate that the neurosurgical technique was in no way modified (except at the time of material collection), in order to guarantee the best available treatment for the patient and to minimize the risks involved.

Signing the Sample for Culture

After collection, this material was immediately stored in Leibovitz-15

preservative medium (L-15: GIBCO Invitrogen Corporation) and shipped for treatment and plating on the addition of D-10 culture medium, being maintained in CO2 greenhouse. The technique of human nerve cell culture was adapted from the work of Lucena *et al*. (Lucena *et al*., 2014) – performed with rodents -with some modifications and described in detail in item 2.7 below.

Culture Technique

Inside the laminar flow, the brain tissue was processed and submitted to the technique of cleaning and obtaining tissue ex-plants for posterior plating. New 15 ml Falcon conical tubes with 4 ml of Dulbecco`s modified Eagle medium medium supplemented with 10% fetal bovine serum and ceftriaxone, a medium designated D-10, all obtained from Cultilab®, were prepared and received the nerve tissue to perform the preparation. 35 mm culture plates (P35) for plating were prepared with 1 ml of fetal bovine serum, which was removed and discarded after 30 minutes, and then 3.0 ml of D-10 was added to the P35, where, The plating of the explants was performed, and the plates were kept in a humid oven at 37°C with 5% CO2 and 95% air. In contrast light microscopy with phase contrast was used for the observation of the explants and the cell migration at the bottom of the plates. When the explant cells reached 70-90% confluence at the bottom of the plate, the basic medium was removed and 2 ml of trypsin / EDTA (0.25% trypsin containing 1 mM EDTA--Cutilab/Brazil®).

The cell suspension was then placed in Falcon-type conical tube with the same volume of D-10 medium for 10 minutes, in order to inactivate trypsin. The suspension was centrifuged at 1500 rpm for ten minutes; after this procedure, the supernatant was discarded and the cells resuspended in 1 ml of medium. The cells were deposited in P60 and observed in 72 hours' time periods and, in this way, it was possible to evaluate the adherence, proliferation and trophism of brain tissue cells at different times. For cellular observation, a CKX41 (Olympus®) phase contrast inverted microscope with a Moticam 3.0 digital camera (Motic®) coupled was used, and photomicrographs of the groups were made at 72 hours, taking the morphological development of the cells.

Results

Images A and B illustrate neurons from cultured human brains, in A being neurons maintained in culture medium without treatment and in B neurons maintained in culture medium treated with Brain Skills ®. Arrows point neuronal body and arrowhead neuronal arborization. Figure B demonstrates enhanced neurological development and transmission when treated with Brain Skills ®.

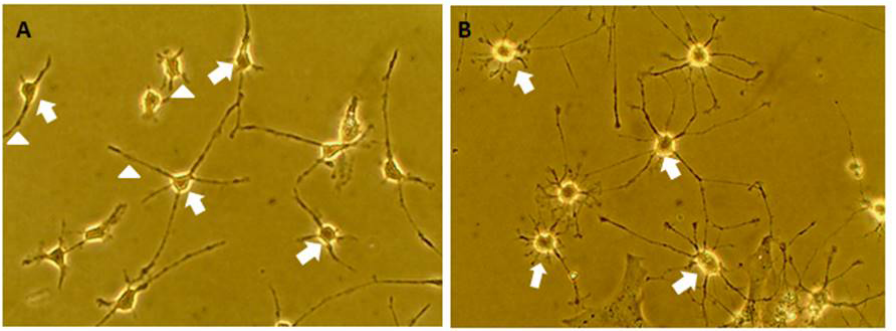
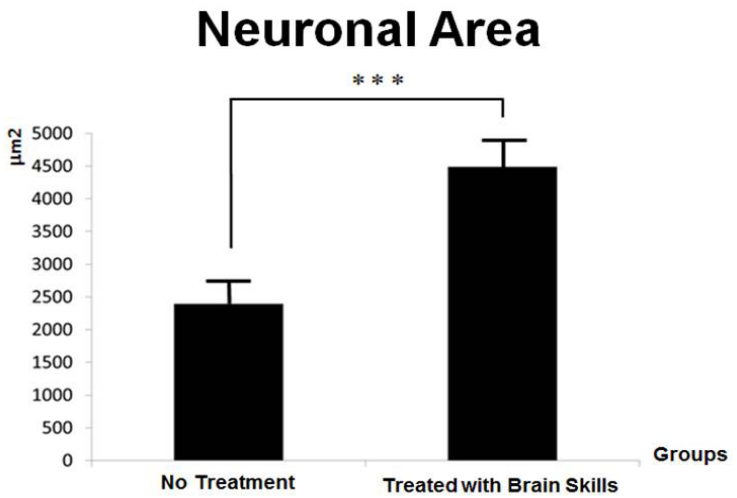


Figure 1: Images A and B illustrate neurons from cultured human brains, in A being neurons maintained in culture medium without treatment and in B neurons maintained in culture medium treated with Brain Skills®. Arrows point neuronal body and arrowhead neuronal arborization. Figure B demonstrates superior development when compared to untreated neurons. p<0.00001 Mann Whitney U-test.

Demonstrates quantitative analysis of the area of untreated brain neurons treated with Brain Skills ®. Neurons treated with Brain Skills ® demonstrate



Graph 1: Demonstrates quantitative analysis of the area of brain neurons without treatment and treated with Brain Skills®.

Neurons treated with ® demonstrate superior development when compared to untreated neurons. p<0.00001 Mann Whitney U-test.

Discussion

Cognition includes the mental processes involved in acquiring knowledge and the integration of these processes into numerous responses such as learning, decision-making, concentration and memory. The ageing process occurs throughout life, and ageing in later life can be associated with severe difficulties associated with cognitive decline and dementia. Cognitive decline involves an inability to reason, understand and interpret, and often also leads to dysfunctional behaviors. Dementia involves loss of intellectual abilities of sufficient severity to interfere with social or occupational functioning. The dysfunction is multifaceted and involves memory, behavior, personality, judgment, attention, language and other executive functions.

Tyrosine supplementation prevent declines in various aspects of cognitive performance and mood associated with stress encountered in some military settings (Lieberman, 2003). These treatments can produce changes in rain function; for example, in humans, administration of oral tryptophan can modify sleep and mood via its actions to stimu-late neuronal serotonin production and release (Borbély & Youmbi-Balderer, 1987; Young & Gauthier, 1981).

Guarana seed comes from plants found in the Amazon, and it contains theophylline, theobromine, and caffeine. Theobromine is a methylxanthine that is an adenosine receptor antagonist (as caffeine) and might improve cognitive function. Two studies (Haskell *et al*., 2007; Kennedy *et al*., 2004) have investigated the effects of guarana on cognitive performance and found that memory, mood, and speed during an attention task improved. The administration of a vitamin and mineral complex with guarana has been shown to attenuate mental fatigue and improve performance during cognitively-demanding tasks (Haskell *et al*., 2007; Kennedy *et al*., 2004).

A growing body of literature has investigated the effects of caffeine on cognitive performance, particularly during/after sleep deprivation. Caffeine works by blocking adenosine receptors within the brain and has demonstrated positive changes (at varying doses) on the alerting, orienting and executive control attention networks within the brain, specifically enhancing alertness, vigilance and reaction time. It has not demonstrated improvements in memory performance or other executive functions, such as decision-making (McLellan, Caldwell & Lieberman, 2016)

Choline and lecithin have also been studied as potential memory enhancers, on the basis of the notion that acetylcholine neurons in the hippocampus play an important role in memory and that enhancing transmitter production might improve memory. Patients with Alzheimer disease are the group most studied; in general, the outcome has been that neither choline nor phos- phatidylcholine offers much improvement in memory (Rathman & Conner, 1984; Thal *et al*., 1981; Etienne *et al*., 1981; Spring, 1986).

BDNF is a neurotrophin conside-

red generally beneficial for maintaining neuronal function and for promoting recovery after neurologic insult. BDNF is profuse in the hippocampus and cerebral cortex, and is found in lesser amounts in the hypothalamus and spinal cord. In addition to regulating the survival, growth, and differentiation of neurons during development, BDNF stimulates synaptic and cognitive plasticity in the adult brain (Zuccato & Cattaneo, 2009). Dietary supplementation with nutrients such as omega-3 fatty acids and curcumin have been shown to elevate levels of brain-derived neurotrophic factor (BDNF) and can exert their influences on repair and maintenance of neural circuits, important for learning, memory and locomotion (Gomez-Pinilla & Gomez, 2011).

The folate or folic acid, which is found in spinach, meat liver, beans, broccoli, etc., during three years of supplementation, can reduce or prevent the brain-aging decline and dementia (Fioravanti, 1997; Durga, 2007). Alpha lipoic acid is an important mitochondrial energy maintenance coenzyme; present in kidney, liver, heart, broccoli, spinach and potatoes (Liu, 2008). Alpha lipoic acid can reduce the cognitive decline in Alzheimer's disease (Holmquist, 2007). Vitamin E repairs the cellular synaptic membrane against oxidation and affects neuroplasticity (Wu, 2004). Vitamin D is a CNS active neurosteroid with the capability to cross the cell membranes and link to their vitamin D nuclear receptors, switching on more than 1000 genes and modulating a gigantic network of neurological mechanisms such as cellular proliferation, differentiation and calcium homeostasis (Eyles, Burne & McGrath, 2011). Further antioxidants may be also part of a functional neuroplastic diet, such as choline, combination of vitamins [C, E, carotene], calcium, selenium, zinc and creatine, which delay brain decline, promote longevity and cellular energetic nerve tissues storage and repair etc (McCann, Hudes & Ames, 2006; Schram, 2007; Gao, 2007; Ortega, 1997; Blaylock, 2012).

Cognitive aging does not occur simultaneously across cognitive domains, with various domains peaking in early adulthood before reaching a plateau or declining. Therefore, interventions early in life may yield health benefits that are only measureable in later life.

As humans age, their ability to defend against the effects of oxidative stress and inflammation weakens, putting elderly people at increased risk for neuronal disease and degradation. Neuroprotective foods, such as berries and other dark-colored fruits, represent one way to protect aging brains against this damage by reducing inflammation and oxidative stress in the brain, thereby protecting against cognitive declines in aged populations.

Nutrients may influence brain architecture, chemistry, and function. Given this organ's vulnerability and the potential for lifelong deficits in brain function, more work is needed to identify which nutrients and micronutrients, when deficient in the diet, put the brain at risk. Several nutrients affect brain chemistry and function in the fully formed (adult) brain.

Conclusion

Brain Skills ® promoted a neuroplastic effect after 72 hours of treatment, showing more developed neurons, larger cell bodies, greater dendritic and axonal plasticity, in addition to presenting a greater amount of synaptic contact. The results demonstrated that the product Brain Skills ® had a significant effect on the neuroplastic development of human neurons. The functional brain has the ability to repair itself trough neuroplastic mechanisms that depends on anti-inflammatory/antioxidant type diet, caloric restriction, moderate exercises, new experiences, meditation, hormonal balance and regenerative cell neurotrophic factors.

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