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Clinical neuroscience and mental health: filling the gap

Orestis Giotakos

(MD. MSc, PhD), Founder of Obrela

Abstract

Recent developments in neuroscience can help inform clinicians' understanding of cognition, emotion, behavior, and social interactions—all critical aspects of people's lives that are dramatically affected in psychiatric disorders. Psychiatry is informed by a broad range of basic biological and social sciences and has at its disposal many tools, like brain imaging, genetics, neuropsychopharmacology, neurophysiology, epidemiological models, and neuropsychology, for developing new assessment and treatment approaches, grounded in understanding of etiology and pathophysiology. However, psychiatry as clinical neuroscience must strengthen its partnerships with the disciplines of public health, community and behavioral health science, and health economics. The WPA Action Plan 2017-2020 supports psychiatrists to promote mental health and improve care capacity, like service development, awareness raising and advocacy, education, research and publications. Establishing new approaches in publishing innovative research findings, I suggest that the creation of the new journal, *Dialogues in Clinical Neuroscience & Mental Health,* will contribute to guiding this interdisciplinary field in new directions.

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Human brain is probably the most complex object on Earth. In fact, the brain has an estimated 10^{11} neurons, each with an average 10^4 connections with other neurons. Zooming in and out of this dense jumble of cells and cables reveals non-random structural patterns at different levels. Better understanding of brain functioning and brain plasticity has allowed neuroscientists to transfer findings from research to education, therapy and rehabilitation programs. However, advancements in some areas which have dominated the literature, like "reward" and "fear" circuits, have gradually paved the way for a more nuanced conceptualization of valuation in the brain and the mesocorticolimbic system can no longer be categorized as a "reward" or a "dopaminergic" circuit, nor can the amygdala be deemed the "fear center." Based on this concept, Haye (2015) **[1]** suggested that "our goal is to help generate new hypotheses about how to better apprehend affective circuits". Recently, Boyle et al (2017) **[2]** found that disease risk is driven mostly by genes with no direct relevance to disease, but which act as modifiers of more fundamental biologic processes, perhaps related to individual genetic backgrounds and environmental experience. Based on these findings, Weinberger (2017) **[3]** suggested: "This proposal echoes the question of whether psychiatric disorders are really "diseases" rather than varying states of brain development that have a particular way of expressing difficulties in particular environmental contexts, based on genomic background, development and experience".

Understanding the brain represents one of the most profound and pressing scientific challenges of the 21st century. Recent developments in neuroscience can help inform clinicians' understanding of cognition, emotion, behavior, and social interactions—all critical aspects of people's lives that are dramatically affected in psychiatric disorders. Some areas of neuroscience, like the emotion regulation, are of particular relevance to clinicians because they help further the understanding of patients and can lead to the development of novel therapeutics. For many decades, scientists have thought of cognition and emotion as two largely separate systems in the brain, but even as researchers began to find evidence of the interdependence of the two, this interaction was often seen in the light of emotions interfering with the higher level of cognitive processes. Despite the *affective neuroscience* developments, scientists get still confused about what is meant by 'emotion', since there are distinctions between the functional emotion state ('the emotion state'), its conscious experience ('the experience of the emotion'), our ability to attribute emotions to others ('emotion perception'), our ability to think and talk about emotion ('conceptualizing emotion'), and the behaviors caused by an emotion state ('the expression of emotions', 'emotional reactions') **[4].** Although much still remains to be discovered, current findings in affective neuroscience have already influenced our understanding of drug use and abuse, psychological disorders such as panic disorder, and complex human emotions such as desire and enjoyment, grief and love.

During the last decade Neuroscience research has made also a step forward in virtual reality and big data analysis. The advent of plug-and-play technologies has simplified the connection between *Skinner boxes* and computers. At the present time, sophisticated software can provide user-friendly and intuitive panels for facilitating the creation of user-defined protocols, as well as direct access to analysis reports, providing straight-to-the-point integrated data, statistics and graphs. We know now that understanding how the brain works, needs to have three types of maps: first, **'**cell type map', i.e. to identify the diverse types of cells and their distributions in all brain regions, and the molecular expression pattern in each cell type. Using molecules specifically expressed in different cell types as markers, we can then draw the second type of map—**'**connectivity map', the so-called 'connectome'**,** which is the wiring diagram of nerve connections among all neurons in the brain. Mapping the 'connectome' is often compared to mapping the 'genome', the complete sequence of all nucleotides and genes they encode along the entire DNA of an organism. The third type, 'activity map'**,** refers to mapping of the firing or spiking pattern of all neurons in the brain associated with a particular state of the brain **[5].**

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Psychiatry is grounded in clinical neuroscience. The components of psychiatry and the components of neurology are often arbitrary and historical rather than rational. Whereas neurology has traditionally focused on discrete anatomical lesions, psychiatry or modern clinical neuroscience addresses dysfunction in anatomical circuits and connectivity. Psychiatry, like neurology, rests on a foundation of clinical neuroscience. It also encompasses and is informed by a broad range of basic biological and social sciences and has at its disposal many tools, like brain imaging, genetics, neuropsychopharmacology, neurophysiology, epidemiological models, and neuropsychology, for developing new assessment and treatment approaches, grounded in understanding of etiology and pathophysiology. Brain-imaging methods such as CT, MRI and PET are now serving useful diagnostic functions, but further advance in the use of MRI requires more fundamental understanding of the meaning of MRI signals and how they relate to the structure and activity of neural circuits. Psychiatry as clinical neuroscience must strengthen its partnerships with the disciplines of public health, community and behavioral health science, and health economics. Psychiatry needs to pay attention to the inequalities in the delivery of mental health services to vulnerable populations, as well as to the integration of mental health services into other areas of medicine, from pediatrics to geriatrics. Also, to the unmet mental health needs of medical students and physicians generally, whose rates of suicide are two to three times greater than in the general population. Moreover, several diagnostic concepts in psychiatry have changed to some extent through the years and that some of them have disappeared along this way. Several diagnostic categories have been split or lumped in a way that is questionable. The project launched in the early 1980s to validate DSM-III categories by elucidating their "specific" etiopathogenetic underpinnings seems to have failed **[6].** A dialogue should be kept between the neurosciences and other anthropological, psychological and social sciences. Psychiatry can improve both assessment and treatment strategies via deeper understanding of genetics, pathophysiology, functional neuroanatomy, and neuropsychopharmacology, allowing for the development of more personalized interventions.

On the other hand, mental health issues are found across the world and in every population. According to the World Health Organization, around a third of the adult population worldwide suffers from a mental disorder such as depression, anxiety and schizophrenia. However, treatments for depression and methods for preventing suicide, for example, are not evenly spread. There is clearly a gap between neuroscience research and mental health services. So it is important to find treatments for mental health disorders that can be delivered in culturally diverse low and middle-income countries, where there are challenges of poverty, stigma and a lack of clinicians with specialist training in mental health. Among all the conditions in the world of health, mental health occupies a unique and paradoxical place. There is an over-treatment and over-medicalization of mental health issues, often fueled by a pharmaceutical industry interested in the broadening of the boundaries of "illness" and in the creation of more and wider diagnostic categories and thus markets for "selling sickness." On the other hand, exists profound under-recognition of the suffering and breadth of mental health issues affecting millions of people across geographies, which is a global problem. The *WPA Action Plan 2017-2020* sets out a strategy for expanding the contribution of psychiatry to improved mental health for people across the globe **[7].** Three characteristics frame the strategic intent of the Action Plan. First, strengthening the contribution of psychiatrists to reducing distress, illness and suicidal behaviour among vulnerable populations, like women and girls, people under extreme stress, including those affected by conflict and emergencies, and people living with longstanding mental illnesses and their caregivers. Second, supporting psychiatrists to promote mental health and improve care capacity, like service development, awareness raising and advocacy, education, publications and research. Third, expanding the reach and effectiveness of partnerships and collaboration with service providers, service beneficiaries and policy makers. In addition, WPA proposes working with journals and other publications in low- and middle-income countries, re-establishing a task force on peer support for editors of psychiatric journals in low and middle-income countries.

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Finally, it should be noted that many talented young scientists, especially in biological sciences, are under the "curse" of 'high-impact' journals**.** In most cases these scientists need to conform to not only rigorous standards of data collection, analysis and interpretation, but also to the generally accepted peers' paradigm and thinking, as well as to the financial burden. Innovative science may needs to break the existing paradigm of the field. We can try to establish new approaches to publishing innovative research findings that are not necessarily acceptable by high-impact journals and new review criteria that stress the innovative aspects of the research. In parallel, we can strengthen the dialog, in order to fill the gap between the neuroscience knowledge and the mental health needs. I suggest that the creation of the new journal *Dialogues in Clinical Neuroscience & Mental Health,* will contribute to guiding this interdisciplinary field in new directions. I hope that in the future, our journal will become more than a repository of articles in an interesting area of science. I hope that the Journal *Dialogues in Clinical Neuroscience & Mental Health* will take an active role in shaping the field, by helping create a new community of researchers with common interests in this very different disciplinary background. I am deeply grateful to the founding members of the editorial board for their support, and to our publisher, *obrela*, for providing essential resources to this endeavor. I am confident that together we will make important contributions to the future of the emerging field of clinical neuroscience and mental health.

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Psychobiology of feeding behaviour

George Konstantakopoulos

Abstract

Adequate nutrition is essential for survival and therefore is ensured by a complex brain system regulating the levels of various nutrients in the blood and in the body stores. This system includes two distinct mechanisms of control on food intake, i.e. the homeostatic and the hedonic control. Over the last two decades our knowledge of neural circuits and molecules involved in these mechanisms has improved substantially, in large part due to the findings of research in experimental animals and functional neuroimaging in humans. These findings also provide insight into the mechanisms underlying obesity and abnormal feeding behaviour in neuropsychiatric disorders. The hypothalamic network that regulates food intake and energy balance consists of interconnected neurons located in the arcuate (infundibular in humans) nucleus, ventromedial nucleus, paraventricular nucleus, dorsomedial nucleus, and lateral hypothalamic area. Central regulation is mediated by α- and β- melanocyte-stimulating hormone, neuropeptide Y, Agouti-related protein, γ-aminobutyric acid, brain-derived neurotrophic factor, and melanin-concentrating hormone. Peripheral signals that stimulate (orexigenic) or inhibit (anorexigenic) food intake are received by neurons in the medial zones of the hypothalamus, including signals from circulating nutrients (glucose, fatty acids), hormones (insulin, leptin, ghrelin), and gastrointestinal peptides (cholecystokinin and peptide YY3-36). The pleasure of palatable food is associated with activation of the brain reward system, including the ventral tegmental area, dopaminergic system, nucleus accumbens, ventral pallidum, and amygdala. Dopamine release in the nucleus accumbens mediates the motivational aspects of food intake, especially the drive to eat food that is hedonically desirable ("wanting"). Orexin, cocaine- and amphetamine- regulated transcript, and galanin play significant role in hedonic regulation of feeding. The hedonic reaction per se to the pleasure of food reward ("liking") is regulated by endogenous opioids and endocannabinoids. There are homeostatic – hedonic control interactions via functional connections of nucleus accumbens with the prefrontal cortex, amygdala, and lateral hypothalamus, as well as between hypothalamic, cortical, and mesolimbic circuits. There is also a "top-down" control of human feeding behavior: interactions between cognitive and emotional processes could lead to different responses to food cues and changes in food intake.

Keywords: nutrition, homeostatic control, hedonic eating, hypothalamus, reward system

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1. Introduction

Feeding provides the energy that is essential for survival and therefore is subject to intense regulation by human brain. Adequate nutrition is ensured by a complex brain system regulating the levels of various nutrients in the blood and in the body stores. The hypothalamus is the centre of the network of control on food intake and metabolism in response to peripheral signals that reflect the feeding state and energy reserve, i.e. homeostatic control.

Hunger is associated with discomfort providing a strong drive for feeding and satiety is accompanied with satisfaction preventing further consumption of food. However, the rewarding nature of food goes beyond the feelings of hunger and satiety. Modern humans often eat in the complete absence of hunger and nowadays obesity is a serious public health problem. Hedonic eating, i.e. eating based on pleasure rather than energy needs, is controlled by complex neural mechanisms associated with reward. The insular cortex, orbitofrontal cortex, nucleus accumbens, amygdala, and ventral tegmental area have a key role in control of feeding behaviour in response to the hedonic aspects of food.

Over the last two decades our knowledge of neural circuits and molecules involved in homeostatic and hedonic control of food intake, has improved substantially, in large part due to the findings of research in experimental animals and functional neuroimaging in humans. These findings also provide insight into the mechanisms underlying obesity and abnormal feeding behaviour in neuropsychiatric disorders. Only the main aspects of the current knowledge on mechanisms controlling feeding behaviour can be emphasized here.

2. Homeostatic control of food intake

The hypothalamic network that regulates food intake and energy balance consists of interconnected neurons located in the arcuate (infundibular in humans) nucleus (ARC), ventromedial nucleus (VMH), paraventricular nucleus (PVN), dorsomedial nucleus (DMH), and lateral hypothalamic area (LHA). Peripheral signals that stimulate (orexigenic) or inhibit (anorexigenic) food intake are received by neurons in the medial zones of the hypothalamus, including signals from circulating nutrients (glucose, fatty acids), hormones (insulin, leptin, ghrelin), and gastrointestinal peptides (cholecystokinin and peptide YY_{3-36}) **[1]**. The dorsomedial and lateral hypothalamic neurons receive circadian influences and interact with neural circuits for thermoregulation and arousal **[2]**. The integration between orexigenic and anorexigenic signals proceeds via complex interactions between the hypothalamic nuclei mediated by a variety of neurotransmitters **[3]**. The hypothalamic network exerts control on food intake and peripheral metabolism acting via projections to sympathetic and parasympathetic nuclei (nucleus of the solitary tract, area postrema, dorsal motor nucleus of the vagus, and locus coeruleus) on the endocrine glands and the gastrointestinal system **[4]**. Cognitive and emotional aspects of food intake relay on reciprocal connections of hypothalamus with cortical and mesolimbic circuits, and hippocampus **[5].**

In the following we present the main peripheral and central signals and hypothalamic pathways related to feeding behaviour, which are also briefly displayed in *Table 1*.

2.1. Central regulation of feeding and energy balance

The ARC is a key regulator of food intake and energy balance containing a group of neurons that synthesizes *α- and β- melanocyte-stimulating hormone (MSH)*, neuropeptides derived from *pro-opiomelanocortin (POMC)*, and another group of neurons synthesizing *neuropeptide Y (NPY), Agouti-related protein (AgRP),* and *γ-aminobutyric acid (GABA).* A- and β- MSH decrease food intake and increase energy expenditure acting on melanocortin 4 receptors (MC4R) in the PVN and VMH **[6].** By contrast, NPY via Y1, Y2, Y5 receptors and AgRP acting as an inverse agonist of MC4R in the PVN increase food intake and reduce energy expenditure **[3].** Moreover, the same group of neurons can inhibit POMC neurons in the ARC via GABA and NPY projections **[7].** Thus, the ARC mediates both orexigenic and anorexigenic signals from periphery and regulates feeding and energy metabolism integrating these mutually opposing influences.

Neurons in the VMH that synthesize *brain-derived neurotrophic factor (BDNF)* receive signals from POMC neurons of the ARC and they also respond to glucose and leptin re-

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ducing food intake and increasing energy metabolism **[8]**. Groups of neurons in the PVN receiving signals from the ARC synthesize hormones with anorexigenic effects – corticotrophin releasing hormone (CRH), thyrotrophin releasing hormone (TRH), and oxytocin **[7].**

The LHA has also a key role in regulation of feeding and metabolism, integrating signals from the periphery (i.e., glucose, leptin, ghrelin) and interacting with other hypothalamic areas and the mesolimbic system **[4] [9] [10]**. A group of neurons in the LHA synthesizing *orexin* (or *hypocretin*) plays a significant role in the short-term regulation of energy balance. Orexin neurons are inhibited by glucose and stimulated during fasting and they promote food intake acting on specific receptors (OX1 and OX2) in the PVN **[11]** up of LHA neurons synthesizes *melanin-concentrating hormone (MCH)* and they act on specific receptors (MCH1 and MCH2) increas-

Table 1: Main signals and mechanisms for homeostatic control of food intake

Signal	Source	Target (receptors)	Effect	Mechanisms of action
Peripheral (hormones)				
Insulin	Pancreas	Hypothalamus (Insulin Receptors, IR)	Food intake	Activation of POMC neurons in ARC Activation of BDNF neurons in VMH Inhibition of LHA neurons
Cholecystokinin Peptide YY_{3-36}	Gut	Hypothalamus via vagus nerve (CCK-1, Y2)	Food intake	Stimulation of vagus nerve – signals via NTS and PBN projections to POMC neurons in ARC
Leptin	Adipose tissue	Hypothalamus (Leptin Receptors, OB-R)	Food intake Metabolism	Activation of POMC neurons in ARC Activation of BDNF neurons in VMH Inhibition of LHA neurons
Ghrelin	Stomach	Hypothalamus (GHR1)	Food intake	Activation of Neuropeptide Y, Agouti-related peptide, and GABA neurons in ARC
Central				
α- and β-MSH	ARC	Hypothalamus (MC4R)	Food intake	Agonists of MC4R in PVN and VMH
Agouti-related peptide	ARC	Hypothalamus (MC4R)	Food intake Metabolism	Inverse agonist of MC4R in PVN
Neuropeptide Y	ARC	Hypothalamus (Y1, Y2, Y5)	Food intake Metabolism	Direct activation of PVN Inhibition of POMC neurons in ARC
BDNF	VMH	Hypothalamus (Tropomyosin receptor kinase B, TrkB)	Food intake	Agonist of TrkB and MC4R in PVN and VMH
Melanin-concentrating hormone	LHA	Hypothalamus, VTA (MCH1 and MCH2)	Food intake Metabolism	Agonist of MCH receptors in hypothalamus and VTA
Orexin/hypocretin	LHA	Hypothalamus (OX1 and OX2)	Food intake	Agonist of OX1 and OX2 in PVN (short-term regulation of energy balance)
Endocannabinoids	LHA	Hypothalamus (cannabinoid-1 receptors, CB1)	Food intake Metabolism	Inhibition of anorexigenic signals via CB1

Abbreviations: ARC, arcuate (infundibular in humans) nucleus; BDNF, brain-derived neurotrophic factor; GABA, γ-aminobutyric acid; LHA, lateral hypothalamic area; MC4R, Melanocortin 4 receptor; MSH, melanocyte-stimulating hormone; NTS, nucleus of the solitary tract; PBN, parabrachial nucleus; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus; VMH, ventromedial nucleus; VTA, ventral tegmental area.

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ing food intake and decreasing energy metabolism **[12].** The function of LHA on food intake is related to sleep-wake cycle: the MCH neurons are active during slow-wave sleep while the orexin neurons are activated in wakefulness **[7].**

2.2. Peripheral factors regulating food intake and metabolism

Gut peptides (cholecystokinin, peptide YY$_{3-36}$) are released after a meal and suppress food intake and meal size activating via vagal afferents the nucleus of the solitary tract, which signals fullness to the hypothalamus and other brain regions, initiating satiety and resulting in meal termination **[13], [9].** Another gut peptide under investigation with similar effects on food intake and a significant role in the control of glucose and energy homeostasis is *glucagon-like peptide-1* **[14].** On the other hand, *ghrelin* is the hormone that is released from the stomach during fasting and provokes hunger and meal initiation. Ghrelin, acting on the growth hormone secretagogue receptor (GHSR) in the ARC, stimulates NPY, AgRP and GABA neurons **[15].** *Leptin* is a hormone synthesized in the adipose tissue that circulates at levels proportional to the amount of fat. Leptin, acting on specific receptors in the ARC, stimulates POMC neurons and inhibits the release of NPY and AgRP, thus contributing in long-term weight and glucose homeostasis **[16], [14]**. It also produces anorexigenic effect stimulating BDNF neurons in VMH while inhibiting LHA neurons **[16].** *Insulin*, the hormone released by beta-cells in pancreas and regulating glucose homeostasis, has also anorexigenic effects possibly through similar mechanisms of action as those of leptin **[17], [14].**

3. Hedonic control of feeding behaviour

Many aspects of human behaviour, like seeking for pleasant food, cooking, or obesity, indicate that feeding is not controlled solely by homeostatic mechanisms but is also influenced by the rewarding nature of food.

3.1. Gustatory regulation of feeding

Food reward is associated with palatability qualities, particularly taste and smell. Animals consume sweet and salty food beyond their homeostatic needs and avoid sour or bitter food even if they are hungry. In human brain, taste information passes via the nucleus of the solitary tract and parabrachial nucleus to the thalamus, the lateral frontal cerebral cortex, the central nucleus of amygdala, and several hypothalamic areas, including LHA. Although gustatory thalamus is critical for hedonic aspects of taste, other subcortical areas also mediate the motivational qualities of palatable food cues **[2].**

3.2. Reward system for feeding

The pleasure of palatable food is associated with activation of many areas of the brain reward system, including the ventral tegmental area (VTA) dopaminergic system, nucleus accumbens (NAc), ventral pallidum, and amygdala **[10], [18].** Dopamine release in the NAc mediates the motivational aspects of food intake, especially the drive to eat food that is hedonically desirable ("wanting") **[19]**. As yet, the mechanisms by which food stimulates dopamine release are not well understood. It has been found that food can stimulate dopamine signalling independent of the processing of taste information **[20].**

Release of *orexin* during feeding directly stimulates dopamine neurons in the VTA increasing dopamine release in the NAc **[21]**. Other hypothalamic neuropeptides may also play a role in hedonic regulation of feeding influencing dopamine release. The *cocaine- and amphetamine- regulated transcript (CART)* which is found in several hypothalamic areas decreases food intake possibly inhibiting dopaminergic neurons in VTA. However, the anorexigenic effect of CART is associated with its multiple actions in hedonic and homeostatic regulating systems, which are not clear yet **[22].** By contrast, *galanin* stimulates food intake, in particular the intake of fat, possibly acting on specific receptors in the PVN. However, it still remains unknown which of the multiple central and peripheral effects of galanin might be related with this effect **[23].**

The hedonic reaction *per se* to the pleasure of food reward

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("liking") is regulated by *endogenous opioids* and *endocannabinoids* acting via μ-type opioid receptors and CB1 receptors respectively, within the shell of the NAc and possibly within the ventral pallidum **[10].** Although 'liking' and 'wanting' are needed together for complete food reward, are mediated by interacting but partially independent neural substrates.

3.3 Interactions of homeostatic and hedonic regulatory mechanisms

The stability of body weight over adult life in spite of the availability of highly palatable and energy dense food, as well as the discrepancies from normal eating, e.g. overweight, obesity and eating disorders, indicate an interface between the metabolic and hedonic drives of eating. Therefore, the possible neural circuits and mechanisms that underlie interactions between homeostatic and hedonic regulation of feeding have been a focus of research during the last two decades.

The NAc plays a key role in the integration of homeostatic, hedonic, and cognitive aspects of food intake via its connections with the prefrontal cortex, amygdala, and lateral hypothalamus **[10], [24]**. There are also multiple functional connections between hypothalamic, cortical, and mesolimbic circuits mediated by POMC, orexin and MCH that may play a role in homeostatic – hedonic control interactions **[18].** Hormones involved in homeostatic regulation of feeding, such as leptin, insulin, and ghrelin, also exert effects on motivation to obtain food through their influence on mesolimbic dopamine signalling, especially on the dopaminergic neurons in the VTA **[25].** Leptin decreases the firing rate of the VTA dopaminergic neurons. Insulin increases dopamine release and the firing rate of dopaminergic neurons but reduces dopamine levels in the VTA probably by upregulation

Table 2: Main signals and mechanisms for hedonic control of eating behaviour

Signal	Source	Target (receptors)	Effect	Mechanisms of action
Peripheral (hormones)				
Leptin	Adipose tissue	VTA (Leptin Receptors, OB-R)	Food intake Metabolism	Inhibition of dopaminergic neurons in VTA
Insulin	Pancreas	VTA (Insulin Receptors, IR)	Food intake	Reduction of dopamine levels in VTA probably by upregulation of DAT
Central				
Ghrelin	ARC	VTA (GHR1)	Food intake	Activation of dopaminergic neurons in VTA Increase of the activation of dopamine D1 and D2 receptors and dopamine levels in NAc
Orexin/hypocretin	LHA	VTA (OX1 and OX2)	Food intake	Activation of dopaminergic neurons in VTA
Endocannabinoids	Local	Nucleus accumbens (cannabinoid-1 receptors, CB1)	Food intake Metabolism	Enhancement of dopamine effect on nucleus accumbens
Endogenous opioids	Local	Nucleus accumbens (μ-opioid receptors)	Food intake	Increase of dopamine release in nucleus accumbens
CART	ARC, LHA	Hypothalamus, Mesolimbic system	Food intake	Unknown
Galanin	ARC	Hypothalamus, especially PVN (GALR)	Food intake	Unknown

Abbreviations: ARC, arcuate (infundibular in humans) nucleus; CART, cocaine- and amphetamine- regulated transcript; DAT, dopamine active transporter; LHA, lateral hypothalamic area; NAc, nucleus accumbens; PVN, paraventricular nucleus; VTA, ventral tegmental area.

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of the dopamine active transporter (DAT). Ghrelin enhances signalling from the VTA to the NAc increasing the activation of dopamine D_1 and D_2 receptors and dopamine levels.

Like ghrelin, other factors involved in meal-to-meal regulation of feeding may also affect food reward in a way that even highly palatable food may be unpleasant after satiation. There is evidence that the rewarding effects of food are potently modulated by indicators of satiety, such as peptide YY_{3-36} that was found to elicit a switch of activation from the hypothalamus to the orbitofrontal cortex and diminished orbitofrontal activation in response to the rewarding aspects of food **[26].** The main pathways related to hedonic control of feeding behaviour are briefly displayed in *Table 2*.

4. Cognitive and emotional control of feeding behaviour

Homeostatic and hedonic mechanisms controlling feeding behaviour described above only partially operate outside awareness. However, there is also a "top-down" control of human feeding behavior: interactions between cognitive and emotional processes could lead to different responses to food cues and changes in food intake **[27].** Thus, humans can voluntary inhibit their drive to eat or develop involuntary changes in their appetite and body weight related to emotional states.

Cognitive control of feeding behaviour involves integration of peripheral signals related to energy status of the body, food-related signals in the form of sensory and environmental cues, and memory of past feeding experiences **[7].** The insular, orbitofrontal, and anterior cingulate cortical areas have a key role in the processing of interoceptive and food-related information and participate in motivational aspects of feeding behaviour **[28], [29], [2].**

There is now evidence from preclinical studies that emotional factors influence both hedonic and homeostatic aspects of food intake, altering the activation of many mediators such as ghrelin, orexin and leptin. For example, chronic stress may influence feeding and body weight independent of palatability of food or energy status of the individual **[19].** This is more obvious in human behaviour, since changes in appetite and body weight are frequent symptoms and one of the core diagnostic features of major depressive disorder. Furthermore, the association rate between mood disorders and obesity is about 25% **[30].** Influences of mood on hedonic and homeostatic control of feeding may partially mediated by the effects of serotoninergic system, e.g. action of serotonin on POMC neurons in ARC via $5HT_{2C}$ receptors **[31].** Aside from depression, serotonin dysfunctions are also implicated in the pathophysiology of eating disorders, i.e. anorexia nervosa and bulimia nervosa **[32].**

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The intriguing role of the Gut Microbiome in the etiology and pathogenesis of Neuropsychiatric Disorders

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Abstract

Humans live in a symbiotic, evolutionarily endorsed, relationship with the plethora of microbes residing within them, but not until very recently we came to understand that our microbes are more than simply bystanders co-existing with us. They have established a communication, an inter-kingdom connection, with the host's cells, which is responsible for many physiological aspects concerning human health. The gut microbiome, by its very definition, represents the collective genome material of all microbes inhabiting our intestines, and holds tremendous capacities, for it is able to affect the host in terms of health and disease. It's intriguing the fact that the gut microbes are engaged not only in local events, but may influence remote tissues and organs as well. Via the gut-brain axis, a multichannel system of pathways connecting the two organs, microbes can affect mood, behavior and cognition and become implicated in the pathogenesis of many neuropsychological disorders. They are able to impact brain function in a variety of ways, however, their true potential lies in their ability to regulate the neural development, a delicate process whose defects can lead to long-term mental health outcomes later in life. In this article, we will review the contribution of the gut microbes in the process of neurodevelopment and attempt to shed light on the etiology of many neuropsychological disorders from the perspective of gut dysbiotic states. Unraveling the mystery behind the true meaning of symbiosis with microbes may provide novel therapeutic strategies against neuro-psychological disorders.

Key words: gut-brain axis, gut microbiome, Gut microbiota, mental health, neuro-psychological disorders

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Introduction

For millions of years the human body was inhabited by a group of little creatures, which afterwards were named microbes. Once we learned of their existence we started to observe and study them. We found them living and thriving in every possible environment, even within us. We related them with diseases and started fighting them to extinction. In time, we discovered a connection, a prosperous symbiotic relationship between some of them and us. We came to understand that the human being isn't a solely organism, but a living ecosystem with a ratio of indigenous cells to microbes 1:1 to 1:10[1,2]. We have yet to unravel the precise nature of this symbiotic relationship, for this is a topic of research that is still in its infancy.

The Gut Microbiome (Gut microbiota, formerly called *gut flora*), a vast ecosystem of bacteria, archaea, protozoa, parasites, fungi and viruses thrives within our intestines[1]. It carries an important role on the homeostatic regulation of the human body, on immune maturation and metabolic function [1,3]. The vast variety of its activities, the interaction and communication between the microbes and the human body cells, and the apparent importance of the microbiome on human health and development have led to its description as "a forgotten organ" [1,4].

The last years, its role has been implicated in the pathogenesis of various diseases, gastrointestinal and systemic[1,2,5,6,7]. In this article, we will study its peculiar part on development and function of the CNS, focusing on the data which correlates the microbiome with the pathogenesis of neuropsychological disorders. It's unfamiliar the fact that many forms of neuro-immune and neuro-psychiatric diseases are now being related with gut dysbiotic states (disruption of a balanced composition of the gut microbiome), the microbes' detrimental activities and gut-derived metabolites.

How microbes talk to the brain: The Gut-Brain Axis

The Gut-Brain axis is a concept of connection between the two major systems, the gut and the brain. It consists of a range of multichannel pathways that integrate and transmit the brain signals to the intestines and vice versa[8]. The brain, as the prime neural organ, controls the gastrointestinal functions (e.g. motility, muscular tension, visceral sensitivity, local immune cell activity, hormonal production, nutrient absortion) and therefore shapes the luminal microenvironment affecting bacterial establishment and growth. Although it was believed for many years that the communication between the gut and the brain was one way (top-down, from brain to intestines), nowadays this axis is found to be bidirectional[1], mediating the fundamental functions of these two peculiar systems[9,10]. There have been described several paths that convey the intestinal signals centrally. Each and every of these paths comprise what we described before as "the Gut-Brain axis".

Neural Avenue

Vagus nerve, the widest distributing nerve in the body, consisting of afferent and efferent neurons, is a vital part of the MGB axis. It collects all the information from intestinal events and creates signals to be transmitted through four consecutive neurons cephalically[1,11]. Afferent projections are spread into higher brain centers such as brainstem nuclei, the thalamus, the basal ganglia and the cortex[1,11]. In this way, gut microbiota can induce changes on brain function across the vagus nerve. Compatible with this notion are the experiments done in vagotomized mice, in which probiotic treatment didn't manage to change behavioral traits, although it has been previously reported to be effective[12,13]. Moreover, the sickness behavior followed by pharmaceutical induced colitis in mice was substantially attenuated following vagotomy[14]. Spinal sympathetic neurons are also conducting information from the gut to the brain through the spinal cord[1,9]. A well documented route is the activation of GPR41 (a receptor found in sympathetic ganglia) by microbial products, which transmits the luminal signals centrally[15].

Neuroendocrine Route

Enteroendocrine cells (EECs), specialized cells of the gastrointestinal tract, secrete neurotransmitters and other signaling peptides (i.e. serotonin, CCK, GLP-1, PYY) in response to

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changes on luminal contents, acting as transducers for the endocrine-CNS route [16,17]. Receptors of these peptides are found locally, on the afferent endings of the vagus nerve, but also centrally, where they are involved in behavioral responses [17]. Bacteria may produce metabolites that act as paracrine signals that influence host's cells behavior and function. C1pB protein, for example, produced by commensal microbes, can stimulate the release of PYY and GLP-1 from the EECs [18].

Microbial fermentation products may act as signaling molecules with great significance on gut-brain interaction. Short Chain Fatty Acids (SCFAs), the most studied metabolic products, originate from fibers and undigested carbohydrates [16]. It has been shown to act as modulators on intestinal hormone production and as immune regulators, with many luminal and systemic interactions [19]. They can cross the BBB, regulate microglia homoeostasis [8] and have been implicated in brain development and pathogenesis of autism [8,20]. Locally, SCFAs regulate the production of gut peptides from enteroendocrine cells [8], and the synthesis of gut-derived serotonin from enterochromaffin cells, activating afferent nerve endings to signal to the CNS [8,21]. Experiments done in animal with neurodegenerative disorders have shown a profound increase in cognitive function related to the production of SCFAs[22].

Immune Pathways

The enterocytes, the immune cells and the neurons express in their surface PRRs (Pattern Recognition Receptors) which interact with bacteria molecules or products [1,23]. TLRs, a class of PRRs, are an integral part of the local innate immune system. Activation of these receptors triggers a pro-inflammatory cytokine release (Il-1, Il-6) which spreads locally and systemically via the bloodstream, reaching the brain, to activate the Hypothalamic–Pituitary–Adrenal (HPA) axis [16]. Cytokines may also signal to the brain indirectly, via the vagus nerve [24]. Local immune activation and cytokine production against microbial antigens or products leads to a stress response that stimulates the HPA axis which, in turn, induces hormonal changes in the blood [1]. An immune-endocrine activation affect cognition and behavior, mimicking the effects of bacterial infection [4,25]. LPS production, for instance, by certain types of bacteria is responsible for activation of the immune-endocrine-nervous system and the HPA axis. On the other hand, gut colonization with helpful bacteria can reduce an exaggerated HPA response [26].

Microbial Products (Neurotransmitters, Neuromodulators)

Microorganisms' metabolism can also result in neurophysiological changes with the production of chemical substances that act locally or systemically [9]. Microbiota-derived metabolites are critical intermediaries for microbiota-gut-CNS signaling. Neuropeptides are multilateral molecules and serve as messengers in many systems (endocrine, nervous, immune) [27]. The gut microbiota can produce and emit an array of neurotransmitters, such as GABA, catecholamines, histamine, norepinephrine, 5-HT, butyric acid and dopamine [28]. These molecules are engaged in paracrine communication with the nerves, immune cells and enteroendocrine cells (influencing the hormone production of the former) and also endocrine signaling, reaching distant tissues such as the brain, and further impacting on central centers[29].

The physiology of the MGB axis allows a bidirectional communication between the brain and the gut so that both gastrointestinal and psychopathological entities could be both origin and consequence of one another. Both of the systems are mutually affected and depending on the other. This hypothesis is verified indirectly from the observation of the high co-morbidity between psychiatric and gastrointestinal diseases. Many patients suffering from gastrointestinal disorders experience mood and behavioral changes, whilst a lot of patients with psychiatric diseases suffer from gastrointestinal symptoms [30,31]. Due to this entangled relationship, the gut-brain axis forms a mean for the gut microbiota to speak indirectly to the brain. In dysbiotic states, where the composition or metabolic function of the indigenous bacteria is shifted against the benefit of the host, psychiatric and neurodevelopmental illnesses may occur [8,32,33].

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How the Microbes Influence the Brain Development

Neural development commences early in embryonic life and extends from the prenatal period to post adolescence [34], with the brain remodeling continuing into the third decade of life [35]. It involves the contribution of genetic and a long list of environmental factors [35]. The process of neurodevelopment is dynamic and spans for years which makes it vulnerable to external perturbations and thus susceptible for alteration[8]. This crucial period of neurodevelopment progresses concurrently with the establishment and growth of the gut microbiome, a vital process which guides the maturation and training of the immune system [36], the development of the neuroendocrine system [8] and the regulation of many physiological functions, regional or distal. Studies suggest that there is a crucial link between gut microbiome and CNS maturation under physiological state [8,10]. Disturbance of the gut microbiome early in life has the potential to disrupt the delicate process of neurodevelopment and can contribute to long-term mental health outcomes later in life [8,34,36,37].

Neuronal development may be modulated by the participation of the neuro-endocrino-immunological system [34] with main representative the circulating levels of hormones and cytokines. In a review of GB Rogers et al.[8], it is mentioned that a pro-inflammatory maternal state may contribute to aberrant fetal development. During pregnancy, increased levels of circulating cytokines are known to negatively impact fetal neural development affecting the gene expression of fetal brain cells [38]. This kind of disturbance may come from the disruption of the immuno-regulatory role of the maternal gut microbiome [8]. Maternal gut dysbiosis can generate an inflammatory environment that also influences blood brain barrier (BBB) formation and function which in turn exposes the microenvironment of microglia and neurons to the blood stream components[8]. Embryos of GF mice develop a deregulated BBB with reduced expression of tight junction proteins, which is shown to be significantly compromised [8,39].

The activation of the maternal hypothalamic–pituitary–adrenal axis may change the normal neurodevelopmetal trajectories and it is linked with fetal neurological defects. As GB Rogers et al.[8] reviewed, any stress factor can activate the HPA axis and contribute to a broad spectrum of neurodevelopmental abnormalities [8,40]. How the maternal HPA hyperactivation impacts the fetal development remains poorly understood, but it is believed that the maternal blood cortisol can traverse the placenta and influence the gene expression of the brain cells [8,41]. The effects of prenatal stress on offspring can be mimicked to a limited degree by giving pregnant animals a synthetic glucocorticoid hormone [8,41].

Bacteria composition is associated with neuronal connectivity development and thus the quality of neuronal circuitries during pre- and postnatal life. In the review of Rogers et al.[8] is cited that the process of neurodevelopment in utero depends on serotonin which controls the neuronal cell mitosis, differentiation and synaptogenesis. Proper neuronal morphogenesis requires quantities of 5-HT an embryo can't afford, and thus depends more on maternal plasma serotonin than its own[42]. The maternal microbiome can regulate the 5-HT biosynthesis by enterochromaffin cells in the gut and therefore affect fetal neurodevelopment by influencing the level of circulating serotonin[43].

Gut microbiota, in the postnatal period of life, mediate the epigenetic regulation of brain molecules involved in the neural development, such as neurotrophic factors (BDNF being the most studied of them) [44]. This is a result of microbiota - host chemical communication via the gut-brain or HPA axis where bacterial bioactive metabolites (SCFAs, neurotransmitters) and signaling molecules (peptides, endotoxins) make possible this interaction[16].

Germ free (GF) mice (sterile animals which are born and raised within germ free isolators) are a helpful tool for investigating the gut-brain correlation. Studies have shown that the absence of gut microbiota from birth has an impact on neural development and behavior [8,16,34]. GF mice are described with an excessive HPA response when exposed to mild stress, with elevated plasma ACTH and cortisol hormone compared to normal mice [8,26]. They also have neuroanatomical changes in brain areas such as amygdala and hip-

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pocampus [45], with reduced levels of BDNF, NMDA receptor and c-fos in the hippocampus and cortex [9,46]. The expression of BDNF, the 2A subtype of NMDA and 5-HT1a receptors in the cortex and hippocampus are microbiota regulated[26,47]. GF mice are presented with altered gene expression of myelin structural proteins in the prefrontal cortex, a brain region which has been implicated in cognitive behavior, personality expression and social behavior[48]. Specifically, it has been noticed, an increase in myelin production leading to hypermyelination of the prefrontal cortex, a process which is expected to occur later in life. These neuroanatomical changes could be correlated with the pathogenesis of emotional disorders[49].

Commensal microbes are required for programming and displaying normal social behavior, and are essential for the development of memory, repetitive behaviors and pain signaling from the body [50,51]. A dysbiotic state, with abnormal microbiota composition early in life, can result in abnormal mental development and behavior disorders which are not corrected when later microbial exposure occurs. There seems to be a maturation time window, on which exposure to microorganisms is necessary for proper CNS development, but after that, the changes in the newly formed brain remain permanent [26,52].

The association between the gut micobiota and neurodevelopment is strong. The precise nature of this relationship has yet to be unraveled mostly due to the difficulties of determining the multiple and unclear pathways that combine these systems together.

Manipulating host's brain function and behavior: An introduction to the neuropsychological disorders

Many studies experimented on GF mice or conventionally raised mice have shed light to the pathophysiological role of the gut microbiome in human brain neuropsychological diseases. GF mice receiving gut microbiome transplant from patients with depression exhibit more depression-like behaviors, in comparison with the control group of mice which were colonized with microbes from healthy donors[53]. In another experiment, the transplantation of gut microbes from a high anxiety mouse to a germ free one with low anxiety led to increased anxiety behavior in the recipient. The same experiment done in reverse showed matching results[54]. Some behavioral features seem to be transmissible via the gut microbes, and thus, the idea of brain manipulation by the gut flora appears to confirm itself.

The pathways of communication remain unclear to a certain extent. The gut-brain axis plays a significant role in mediating the intestinal events and the neurochemical alteration centrally. There have been described several paths involved in this axis (for reference see chapter 2: "How microbes talk to the brain: The Gut-Brain Axis").

Since the indigenous gut bacteria have a strong communication with the brain *via* the MGB axis [9,16,34], a disruption of the physiology of gut bacteria could be linked to the pathophysiology of psychopathologies. Any stress factor that influences the microenvironment of the gut microbes could also affect indirectly the cerebral development and function [8,34].

Depressive syndrome

It is right to presume that the gut microbiome is one of the many links between early environmental stress factors and the risk of developing depression later in life [55]. A disturbance of the immuno-regulatory role of the gut microbiome has been proposed to influence the developmental cues of the brain. An immune-endocrine activation could affect the process of neuronal configuration and function[5,34] via changes at the level of genetic expression of genes associated with brain development [55]. It is now known, with the assistance of animal studies, that limbic system's neurogenesis can be modified by indigenous gut microbiota[34,56]. A chronic gastrointestinal inflammation, for example, is associated with altered hippocampal neurogenesis [57], with swifts in the expression of neurotrophic factors, such a BDNF.

Stool samples from patients with depression show alterations in the proportion of indigenous bacteria in contrast

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to healthy individuals. Notably, there has been recorded increased concentration of Bacteroidetes, Actinobacteria and Proteobacteria (LPS-expressing)[58] and low numbers of Lactobacillus species[59].It's interesting the fact that increased levels of IgA and IgM against the LPS of Gram-negative bacteria are found in depressed patients[60]; markers that indicate bacteria translocation into the bloodstream. Patients with depression have increased volatile fatty acids such as isovaleric acid found in their stool. These molecules are microbe-derived and can travel with the bloodstream up to the brain, crossing the BBB, and affecting neurotransmitter release[61]. Whether these mechanisms are involved in the pathogenesis of the depressive syndrome, or are consequences of the neuro-immunological disarrangement resulted by the depression, remains unclear.

Gut microbes are required for normal brain function. Altering the microenvironment of our gut microbes with the supplementation of probiotics (helpful bacteria) can lead to changes in the bidirectional communication between the gut and the brain and thus influencing the mood, cognition and brain function. Probiotic consumption has been linked with anti-depressant effects on animal and human models. Bacterial species such as Lactobacillus and Bifidobacterium can alleviate depressive symptoms in maternal separation models of rats[62]. Chronic treatment with Lactobacillus rhamnosus in mice can reduce stress-induced corticosterone levels, anxiety and depressive behaviors[12]. These effects where attributed to altered GABA expression in the cortex, amygdlala and hippocampus. In a recent functional magnetic resonance imaging (fMRI) study with healthy individuals, after a 4-week consumption of probiotics (Bifidobacterium and Lactobacillus) the subjects displayed reduced neural activity in brain regions that process emotion and sensation in response to emotional attention tasks [63]. Clinical data of probiotic consumption provides a novel, potentially useful, therapeutic strategy for neuropsychiatric conditions. However, more clinical trials are required to truly determine their extent of efficacy in treating neuropsychological disorders.

Anxiety and Stress

Exposure to biological stressors or environmental stimuli can trigger stress and anxiety responses, which involve the activation of the HPA axis[9]. Gut microbe's metabolism may be implicated in the pathogenesis of mood and emotional disorders. Mice inoculated with *Campylobacter jejuni* show a decrease in exploratory phenotype (anxiety's sign) and activated brain sections implicated in anxious behavior [64]. Pathogens, such as *C. jejuni* [64,65], *Citrobacter rodentium*[66] and *Trichuris muris*[67] can induce anxiety-like behavior via immunological and metabolic mechanisms (reviewed in [10]). In contrast, beneficial bacteria in the form of probiotics have shown to ameliorate anxiety and reduce stress. Lactobacillus and Bifidobacterium consumption has been associated with anxiolytic effects, normalizing anxiety phenotypes in animal models[12,62,67]

GF mice behave differently in comparison with normal mice. They show increase motor activity, impaired cognition and demonstrate an exaggerated HPA stress response[9]. These behavioral traits are associated with altered expression of genes[55] leading to higher levels of neurotransmitters, decreased BDNF expression and reduced synaptic long-term potentiation[55]. Colonization by Bifidobacterium species can attenuate the exaggerated HPA stress response, with only condition the early life exposure for the inhibition to occur[26].

Schizophrenia

Schizophrenia is a complex mental disorder characterized by abnormal social behavior and failure to understand reality[68]. Schizophrenia is oftenly coexisting with gastrointestinal symptoms or disorders[69], however, whether this results from a deregulated brain-to-gut communication or is microbiota-derived remains unknown[16]. Even so, the correlation between gut dysbiosis and the pathogenesis of schizophrenia is well documented[8,36,70,71,72,73].

The causes of the disease include environmental and genetic factors. The genetic risk of schizophrenia relies upon genes that are involved in immune function[70,71]. This con-

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dition correlates with the clinical observation of upregulated inflammatory state in schizophrenia patients[69]. Bacteria translocation markers have been found in the blood of schizophrenic patients in significant higher levels than normal people[72], while high cytokine levels are related with the exaggeration of symptoms[73]. A breach in the intestinal epithelial barrier is thought to allow bacteria and their products to enter the bloodstream and cause an immune response[36]. Through molecular imitation, this response may trigger an attack upon host tissues, a fundamental process of autoimmune pathogenesis[9,36]. Schizophrenia patients bear a higher probability of autoimmune disorders, and have autoimmune antibodies against brain regions such as as the hippocampus, amygdala, and frontal cortex[9,74]. They are also found with a higher proportion of Th17 cells, a condition resembling an immune response emerging from gut dysbiosis[75].

Commensal microbiota is required for programming and displaying normal social behavior, and is essential for the development of memory and behavior[50,51]. The expression of BDNF, the 2A subtype of NMDA and 5-HT1a receptors in the cortex and hippocampus are microbiota regulated[26,47]. These factors have a significant role in brain development and function. Impaired BDNF expression leads to cognitive dysfunction, while NMDA antagonists mimic schizophrenia symptoms[9]. A dysbiotic state early in life, could affect the normal neurodevelopmental trajectory and lead to the genesis of psychiatric disorders, therefore the importance of a healthy gut microbiome becomes apparent.

Autism Spectrum Disorder (ASD)

Autism spectrum disorder (ASD) is the name for a group of developmental disorders characterized by impaired social interaction and communication. ASD includes a wide range, "a spectrum," of symptoms, skills, and levels of disability[76].
It is believed that the gut microbiota contributes, at least in part, in the pathogenesis of ASD. Children with ASD usually have a different gut microbiota profile when compared to same age healthy control group[29]. As mentioned in the chapter of brain development, a dysbiotic state may result in activation of the HPA axis and contributes as a risk factor for a broad range of neurodevelopmental abnormalities[8,40]. Children with ADHD, for instance, display an abnormal HPA response[77].

Colonization by strains of neurotoxin-producing bacteria, such as *Clostridia*, has been for long hypothesized as an etiology agent, at least in a subgroup of patients[78,79] (reviewed by Wang et al.[10]). A great number of *Clostridium* species, such as *Clostridium tetani*, have been found in fecal samples of autistic children.[10,80,81] Microbes' metabolic products may are also engaged in the pathogenesis of ASD[10]. Oral use of vancomycin can attenuate the symptoms of the disorder, while the interruption of the treatment leads to relapse of the autistic behavior[29,82]. In the same concept, oral treatment with probiotics (*Bacteroides fragillis*) ameliorates the defects of the disorder[83].

Microbiota composition and the pathogenesis of ASD are connected through the gut brain axis. Microbes are able to influence the synaptogenesis process, the production of neurotransmitters and the gene expression in many brain structures[8,55]. The main pathways employed by the gut microbiota are neural, endocrine and immune, as were seen in the Gut-Brain Axis section.

Eating Disorders

Eating disorders for long have been accepted as mental illnesses since the primary etiology of them seems to outset from psychopathological misrepresentation of body image and self acceptance [84]. They are defined by abnormal eating habits that negatively affect a person's physical or mental health [84]. The cause of these disorders is not clear yet. Both biological and environmental factors appear to play a role. In the last decade, there has been a growing body of literature that suggests a biological background in the etiology and progression of these conditions [4,85,86,87].

Millions of years ago, we permitted bacteria to live inside us and in turn they helped with digestion, protection from pathogens and production of useful molecules. They grow in accordance with the food we eat; nevertheless different

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bacteria have distinct nutritional demands. From an evolutionary point of view, bacteria that evolved ways of communicating with the host and enforce a feeding behavior which cultivates this kind of bacteria would impose a significant selective pressure, and thus thrive on this microbial-controlled environment [85,87]. A positive feedback loop emerges, as the host selects a specific dietary habit which nourish this kind of bacteria [4,87].

This idea of bacteria controlling their host's appetite is revolutionary. It's distrusting the fact that bacteria acquired such a capacity. However, they have had both the time and the formidable adaptive mechanism needed to fulfill this task.

Taking into account the extent of functions of the gut-brain axis and the influence of the diet on brain function, it is logical to assume the gut bacteria as an intermediate link between eating disorders and extreme feeding patterns [4,85]. Acting on the gut–brain axis, gut bacteria could affect brain function and alter the appetite control, thus considering as part of the genesis of eating disorders [4,88]. As the illness develops, abnormal eating habits can further affect the microbiota's ecosystem which potentially feeds back to the brain function, eventually creating a positive loop which maintains the disorder [4].

Studies have not been yet conducted on humans, but animal models help investigate the influence of gut microbes on host behavior. In the review of Lam YY et al. [4,] the authors cite a few plausible mechanisms. The first includes the control of gut bacteria over the production of appetite-regulating hormones. In the gut reside enteroendocrine cells which produce hormones or peptides (such as cholecystokinin) in response to various stimuli and release them into the bloodstream for systemic effect, diffuse them as local messengers, or transmit them to the enteric nervous system to activate nervous responses [89]. These cells express Toll-like Receptors which are activated by binding with bacterial products (lipopolysaccharides - LPS and flagellin) causing the modification of secretion of hormones that regulate hunger and satiety [4,90]. LPS can also enter to the bloodstream and disrupt the physiological permeability of the BBB [91], to augment the effect of circulating hormones and cytokines on central appetite systems. Other direct effect of LPS is the induction of an anorexic response by activating central pathways [91]. Lastly, in a recent experiment, prebiotic food supplementation in healthy subjects led to an increase in production of gut hormones (PYY and GLP-1) and promoted the impression of satiety, lowering hunger rates [92]. Changing the microenvironment of the gut and the microbiota composition seems to contribute to alterations in appetite sensation.

Another pivotal mechanism practiced by gut bacteria to manipulate host's food intake is by producing peptides that mimic the role of the host's appetite-regulating hormones. These peptides can regulate food intake with two major ways (mentioned in [4]). The first, and direct one, is by imitating the effect of the genuine appetite hormone on its receptor, while the second one is far more complex. The peptides produced by the gut microbiota may trigger an immune response towards themselves (since they are bacterial products) with the antibodies also cross-reacting with the host's appetite hormones since they are molecular analogues with these bacteria derived peptides [4,87]. The latter has actually been confirmed, as Fetissov et al. [93] presented a subgroup of patients with Anorexia Nervosa and Bulimia Nervosa that had autoantibodies against the α-MSH (melanocyte stimulating hormone). The circulating level of these autoantibodies was found to be related to the psychological features of these diseases [93]. Similar with the concept of interference with appetite central regulation, bacteria may also manipulate the dopaminergic rewarding system of the brain, affecting the pleasure and the desire for a specific dietary regimen [85].

Conclusion

Living in a microbe-free world is an unimaginable concept. Bacteria appeared on Earth long before the first human ever emerged. We evolved together and form a symbiotic relationship. They accompany us from our birth until the time of our departure. The microbes affect us in the most significant way, but only the last few years we became aware of such influence. The fact that they are involved in the process of

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neurodevelopment, on brain function and the pathogenesis of many systemic diseases gives them substantial authority upon us. We need to learn how to optimize this relationship and comprehend the mechanisms promoting health or disease. Novel therapeutic strategies (probiotics, microbiota transplantation, genetic engineer of indigenous microbes) may appear in the near future and replace partially effective existing treatments. The most difficult attempt is to unravel the mysteries behind this state of symbiosis. Exploring the secrets of the microworld of our microbes may finally give an answer to a significant argument that puzzles humanity for a long time: "are we really us?"

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Psychotropic medication and cataract: a review of case-control studies

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Abstract

Ocular side effects are possible to occur as side effect of psychotropic drug treatment. Antidepressants and typical antipsychotics have been associated with increased intraocular pressure, glaucoma, lenticular pigmentation, visual disturbances and cataract, whereas the risk of atypical antipsychotics and mood stabilizers remains unclear. The aim of our study was to review the case-control studies assessing the risk for cataract of three major classes of psychotropic medication: antidepressants, antipsychotics and mood stabilizers. Four studies assessed the risk of antidepressant drugs. A higher risk for cataract diagnosis or surgery was observed in three studies, especially on long-term use of antidepressants. One study could not identify a higher risk of antidepressant use in general, yet a higher risk was observed in patients younger than 65 years. Different types of antidepressant seem to carry different risks, with proposed harmful effects of dual mechanism and intermediate SERT affinity. Three studies suggested that the association of atypical antipsychotics and high potency typical antipsychotics with cataract is unlikely, or even that atypical antipsychotic drugs might be protective against cataract. However, there is inconsistency between the sparse preclinical and clinical evidence of their protective and harmful effects. Only one study suggested a possible association of mood stabilizers with cataract, despite the discrepant results on individual drugs. Concluding, these case-control studies cannot establish a harmful or protective causal relationship between psychotropic medication and development of cataract. Further research is needed in order to provide proper recommendations.

Keywords: antidepressants, antipsychotics, mood stabilizers, cataract

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Introduction

Cataract is a common cause of visual impairment with significant health consequences. Several risk factors may be associated with cataract, such as increased age, female gender, smoking, unhealthy lifestyle, diabetes mellitus, hypertension and other physical comorbidities, as well as ophthalmic comorbidities, family history of cataract and increased exposure to ultraviolet radiation. Certain drugs seem to predispose to cataract, such as systemic steroids, beta adrenergic antagonists, statins, cholinesterase inhibitors and possibly some psychotropics [1]. Antipsychotic and antidepressant medication targets mainly neurotransmitter receptors and transporters, which along with other mechanisms could play important roles in ocular physiology and cataract development [2]. Ophthalmic events, such as increased intraocular pressure, glaucoma, cataract and visual disturbances have been associated with antidepressants [3]. Typical antipsychotics, especially phenothiazines, have been associated with lenticular pigmentation and cataract, whereas the risk of atypical antipsychotics and mood stabilizers is questioned [4, 5]. As a result, psychotropic medication, i.e. antipsychotics, antidepressants and mood stabilizers, might be modifiable factor for cataract development. Herein, the most recent case-control studies of the risk of psychotropic medication for cataract development are reviewed and discussed.

Search strategy

A literature search was conducted on PubMed (21/12/2017) using the keyword cataract combined with antidepressants (antidepressant, citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, desvenlafaxine, duloxetine, levomilnacipran, milnacirpan, venlafaxine, amitriptyline, amoxapine, desipramine, dosulepin, doxepin, clomipramine, imipramine, maprotriline, nortriptyline, protriptyline, tianeptine, trimipramine, isocarboxazid, metralindole, moclobemide, phenelzine, pirlindole, selegiline, toloxatone, tranylcypromine, mianserin, mirtazapine, vilazodone, vortioxetine, agomelatine, buspirone, bupropion, reboxetine, nefazodone, trazodone), antipsychotics (antipsychotic, haloperidol, olanzapine, clozapine, ziprasidone, aripiprazole, asenapine, cariprazine, brexpiprazole, iloperidone, sertindole, risperidone, quetiapine, zotepine. lurasidone, chlorpromazine, perphenzine, amisulpride, sulpiride, butyrophene, phenothiazine, pimozide, fluphenazine, perazine, promethazine, prochlorperazine, trifluoperazine, clopenthixol, thiothixene, zuclopenthixol, loxapine, perospirone, blonanserin) and mood stabilizers (mood stabilizer, lithium, valproic, valproate, lamotrigine, carbamazepine, oxcarbamazepine). The search resulted in 176 hits. We included studies published in English with a case-control design, specified to assess the risk of psychotropic medication and using as cases patients with first cataract diagnosis and/or surgery. Four studies were identified, one of them a conference abstract [6] and three additional were added from external sources.

1. Antidepressant drugs

The first large case-control study, which assessed the association of antidepressants and cataract, included residents 65+ years with previously coronary revascularization in Canada [7]. The study included 18784 cases (73+/-8.1 years, 59.3% males) with first cataract diagnosis and 187840 age-matched controls. The association of cataract with antidepressant use was adjusted to gender, blood pressure and concomitant drugs. Current use of SSRI within 30 days from cataract diagnosis was associated with cataract (adjusted rate ratio: 1.15; 95% CI: 1.08-1.23). Regarding individual drugs, an association was observed with current use of fluvoxamine (adjusted rate ratio: 1.39; 95% CI: 1.07-1.8) and venlafaxine (adjusted rate ratio: 1.33; 95% CI: 1.14-1.5), but not current use of citalopram, fluoxetine, paroxetine and sertraline. Past use of antidepressants in general was not associated with cataract, but an association of past use of sertraline was observed (adjusted rate ratio: 1.19; 95% CI: 1.01-1.41). A secondary analysis assessed the risk for cataract surgery, and an association of current use of fluvoxamine and venlafaxine, as well as paroxetine (adjusted rate ratio: 1.23; 95% CI: 1.05-1.45) was observed. SSRI treatment

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needed 656 and 690 days on average from time of onset in order to associate with diagnosis and surgery of cataract, respectively.

Another case-control study with residents 50+ years of the Rochester Epidemiology Project (Minnesota, USA) found an association of antidepressant use and first-eye cataract surgery [8]. The study included 6024 cases, i.e. patients with first-eye cataract surgery and equal number of controls. There was no difference between cases (79+/-9 years, 40% males) and controls in terms of age and gender. Continuous prescription of SSRI for 1 or more year was associated with cataract surgery (crude odds ratio: 1.36, 95% CI: 1.23-1.51). The association remained even after adjusting for gender, diabetes and use of oral glucocorticosteroids. Regarding individual SSRI, citalopram (crude odds ratio: 1.53; 95% CI: 1.33-1.77) and sertraline (crude odds ratio: 1.27; 95% CI: 1.06-1.52) were associated with cataract-surgery, in contrast to paroxetine, fluoxetine, escitalopram and fluvoxamine. Prescription of SNRI for 1 or more year was also associated with cataract-surgery (crude odds ratio: 1.37; 95% CI: 1.11-17), with venlafaxine (crude odds ratio: 1.32; 95% CI: 1.05-1.67) and duloxetine (crude odds ratio: 1.82; 95% CI: 1.08-3.07). This association remained after adjusted for diabetes and corticosteroid use, but only in women.

A case-control study on the database of the National Health Insurance of Taiwan has also detected an association of antidepressant use and first cataract diagnosis [9]. The study included 7651 patients with schizophrenia or mood disorders and first cataract diagnosis and 6637 patients without cataract as controls. Age and gender was similar between cases (55.7 +/- 10.5 years, 35.8% males) and controls. The risk was adjusted to ophthalmic and other physical comorbidities, healthcare utilization, as well as use of antipsychotics or systemic steroids. An association with cataract diagnosis was observed for continuous use of SSRI (adjusted odds ratio: 1.26; 95% CI: 1.12-1.41), SNRI (adjusted odds ratio: 1.21; 95% CI: 1.02-1.43) and other antidepressants (adjusted odds ratio: 1.18, 95% CI: 1.18-1.34), i.e. bupropion, mirtazapine, trazodone and moclobemide. This association could be mediated by antidepressants with intermediate SERT affinity, i.e. dissociation constant 1-10 nM, (adjusted odds ratio: 1.68; 95% CI: 1.10-2.56) or use of multiple drugs with different SERT affinities (adjusted odds ratio: 1.31; 95% CI: 1.21-1.42). Regarding individual drugs, continuous use of venlafaxine (adjusted odds ratio: 1.44; 95% CI: 1.19-1.74), fluoxetine (adjusted odds ratio: 1.21; 95% CI: 1.01-1.46) and fluvoxamine (adjusted odds ratio: 147; 95% CI: 1.01-2.12) were associated with cataract, but not duloxetine, milnacirpan, paroxetine, citalopram, sertraline or their combination with other antidepressants. An association with cataract was also observed for past use, i.e. >30 days from cataract diagnosis, of SSRI, TCA (adjusted odds ratio: 1.26; 95% CI: 1.16-1.36) and other antidepressants. The cumulative dosage of antidepressants required for cataract diagnosis seems to vary. Low cumulative dosage of paroxetine, citalopram, escitalopram and sertraline, as well as high cumulative dosage of venlafaxine, sertraline and fluvoxamine might be associated with cataract.

However, a recent case-control study on the UK-based Clinical Practice Research Datalink is inconsistent to previous studies [6]. The 206931 cases were patients 40+ years with first time cataract diagnosis and equal number of age and gender matched controls were included. Long term continuous prescription of SSRI was not associated with cataract diagnosis (adjusted odds ratio: 0.99; 95% CI: 0.94-1.03) in general, but it was associated in younger patients aged from 40 to 64 years (adjusted odds ratio: 1.24; 95% CI: 1.15-1.34). The risk was adjusted to body mass index, glucocorticosteroid use, hypertension, diabetes and smoking.

The recent case-control studies have examined the association of antidepressants with cataract diagnosis or surgery. The studies have adjusted the risk for several confounding factors, such as ophthalmic comorbidities, components of metabolic syndrome and concomitant cataractogenic drug use. However, only one study adjusted for body mass index and smoking [6], and it was unable to detect an association in general. In accordance to the recent studies, the Beaver Dam Study assessed the incidence of drug-associated cataract within 5 years of follow-up, and amitriptyline was associated with an odds ratio of 2.03 (95% CI 1.09-1.39) [10]. In addition preclinical evidence suggested possible roles of

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serotonin, catecholamines and their receptors on the development of cataract [7].

Case-control studies cannot confirm a causal relationship, as well as several confounding factors could be encrypted, such as family history of cataract. Populations at risk could be younger patients on long term continuous use of antidepressants. In addition, differences among individual antidepressants could emerge and intermediate SERT affinity, along with NET inhibition could be possible mediators. Longitudinal prospective studies should further establish the association between antidepressant and cataract, but ocular examination of antidepressant users, especially younger patients with comorbidities, could be justified.

2. Antipsychotic drugs

The first case-control study assessed the risk of antipsychotics for cataract surgery using the British Columbia Ministry of Health Database [11]. The study included 162501 cases of cataract surgery and 650004 controls. The age and gender of cases (74.4 +/-11.8 years, 41.6% males) were similar to controls. The risk was adjusted to age, gender, concomitant SSRI, antidiabetics and steroids, as well as history of uveitis, vitrectomy and hypertension. Prescription of atypical antipsychotics within 90 days of cataract surgery was protective (adjusted rate ratio: 0.84; 95% CI: 0.8-0.89). In addition, current use of typical antipsychotics was also protective (adjusted rate ratio: 0.84; 95% CI: 0.74-0.96), though haloperidol has suggested to be the most common prescribed antipsychotic. A dose-response of protection was observed, with higher number of prescriptions (more than 7 within the previous year) were associated with lower rate ratio (adjusted rate ratio: 0.7; 95% CI: 0.65-0.75), in comparison to smaller number of prescriptions (adjusted rate ratio: 0.85; 95% CI: 0.79-0.91).

Two studies on the National Health Insurance of Taiwan examined the role of antipsychotics on cataract development. The first one included 2222 patients with schizophrenia and cataract diagnosis were defined as cases and 2144 patients with schizophrenia without cataract diagnosis as controls [12]. There was no difference in age and gender between cases (53.1+/- 11.3 years, 39% males) and controls. The risk was adjusted to ophthalmic and other physical comorbidities, antidepressant or steroid use, as well as utilization of the healthcare system. An association with cataract was not observed for continuous use of atypical (adjusted odds ratio: 1.1; 95% CI: 0.94-1.3) and typical antipsychotics (adjusted odds ratio: 1.08; 95% CI: 0.91-1.29), when compared to past use of antipsychotics, i.e. > 90 days before of the cataract diagnosis. Regarding individual atypical antipsychotics, none was associated with cataract. In contrast to the previous case-control study [11], a protective association, i.e. the higher boundary of odds ratio < 1, was not observed.

However, a protective association was observed in the second study using patients with bipolar disorder [2]. The cases were 1684 patients with bipolar disorder and cataract diagnosis (55.3+/-10.3 year, 36% females) and 1608 matched controls with bipolar disorder and without a cataract diagnosis. Similar to the previous study, the risk was adjusted to ophthalmic and other physical comorbidities, utilization of the health system, as well as use of steroids, antidepressants and mood stabilizers. Continuous or past use of atypical antipsychotics seem to be protective (adjusted odds ratio: 0.71; 95% CI: 0.59-0.85), whereas an association with typical antipsychotics was not observed (adjusted odds ratio: 0.97; 95% CI: 0.71-1.34). In addition, continuous or past use of individual atypical antipsychotic was not associated with cataract.

Several lines of evidence suggest the risk of typical antipsychotics, especially phenothiazines, for cataract, but the risk of atypical antipsychotics is still under question [13]. Earlier studies suggest that patients with schizophrenia have a lower risk for cataract in general, but a higher prevalence of anterior subcapsular cataract [14]. Phenothiazines seem to induce lenticular pigmentation and the associated anterior subcapsular cataract [13]. Case reports suggest that chlorpromazine-induced lenticular opacities could cause visual impairment, which could be reversed after switching to risperidone [15]. Regarding the newer antipsychotics, high doses of quetiapine has been accused for cataract in preclinical research, so that biannual ocular examination has been sug-

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gested. Though, clinical evidence was not able to replicate these results in humans. A recent 2-year randomized open label study suggested that quetiapine was not cataractogenic in comparison to risperidone [16]. However, bilateral cataract has been reported in a 27-year old male with bipolar disorder on treatment with risperidone and lithium [17].

The above case-control studies suggest a possible protective mechanism of atypical antipsychotics against cataract development. It is suggested that antagonism of serotonin receptors, as well as anti-oxidative and anti-inflammatory properties could play important roles [2]. Individual typical antipsychotics were not tested. However, haloperidol could be the most frequently prescribed typical antipsychotic, underestimating the risk [11]. Important confounding factors such as smoking, family history of cataract, obesity was also not included in these studied. The possible protective association should also be replicated in other ethnicities and prospective studies.

3. Mood stabilizers

A secondary analysis of the case-control study on the National Health Insurance Research Database of Taiwan [9], has studied the association between mood stabilizers and cataract diagnosis in patients with schizophrenia or mood disorders [5]. The study included 7651 patients with schizophrenia or mood disorders and first cataract diagnosis and 6637 patients without cataract as controls. The risk was adjusted to comorbidities, concomitant cataractogenic drugs and healthcare utilization. Use of mood stabilizers for more than 2 years was associated with cataract (adjusted odds ratio: 1.14; 95% CI: 1.01-1.29), and the risk remained only for doses higher than the half of the daily defined dose (adjusted odds ratio: 1.28; 95% CI: 1.08-1.53). Regarding individual drugs, long-term use of lithium alone (adjusted odds ratio: 1.39; 95% CI: 1.01-1.92) or combined with other mood stabilizers (adjusted odds ratio: 1.44; 95% CI: 1.13-1.85), as well as valproic acid combined with other mood stabilizers (adjusted odds ratio: 1.26; 95% CI: 1.02-1.57), but not alone, was associated with cataract. Association was not observed with long term use of carbamazepine, lamotrigine and their combinations with other mood stabilizers. However, their sample size was small and probably not sufficient.

The role of mood stabilizers on the risk of cataract is studied to a lesser degree than antidepressants and antipsychotics. Carbamazepine, lamotrigine and valproic acid are also used as anticonvulsants. A study has suggested that patients with epilepsy on carbamazepine (odds ratio: 1.4, 95% CI: 1.05–1.8) may have been in higher risk for cataract surgery, in contrast to barbiturates and valproic acid [18]. In addition, a case report of carbamazepine-induced bilateral cataract in a 14-year old boy have been reported [19]. The cataractogenic properties of mood stabilizers could lie on inhibiting anti-oxidant mechanisms or inducing other ocular side effects [5].

Conclusion

Psychotropic medication could alter ocular physiology contributing to cataract development. Use of antidepressant drugs seem to predispose to cataract, especially long-term use and probably in younger patients. However, there is discrepancy regarding the risk of individual drugs, but dual mechanism and intermediate SERT affinity has been suggested as possible risk factors. Furthermore, mood stabilizers could also associate with cataract, despite the inconsistent results on individual drugs. On the other hand, atypical antipsychotics and high potency typical antipsychotics seem not to associate with cataract. Atypical antipsychotic drugs might also be protective against cataract, despite sparse preclinical and clinical evidence of their harmful effects. Phenothiazines and low-potency typical antipsychotics could induce lenticular opacities and cataract. A harmful or protective causal relationship between psychotropic medication and development of cataract cannot be established by case-control studies, and further research is needed. However, ocular examinations should be suggested to patients on psychotropic medication, especially on antidepressant or mood stabilizers, as well as with comorbidities and concomitant use of cataractogenic drugs.

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Investigation of the interaction between cross-modal stimuli and item grouping and their potential effects on working memory

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Abstract

Working memory capacity is limited and can be affected by various factors. It is suggested that working memory can hold up to 3-4 objects. Empirical research indicates that the larger the number of visual material individuals hold in working memory, the less memory is accurate. Yet, fast and accurate recognition of objects can be achieved through haptic investigation and the storage of visual-auditory items is substantially increased when individuals are presented with spoken words. The aim of this study was to investigate the potential interaction of cross-modal stimuli (visual-auditory/visual-haptic) and item grouping, along with their separate effects, on working memory. Two item presentations were utilized; one where participants were presented with visual-auditory items and one where participants were presented with visual-haptic items. All participants were presented with both grouped and non-grouped items. The items' recognition (free recall) was measured in 135 participants, using a 2x2 factorial mixed ANOVA design. Statistically significant results were observed for the main effect of item grouping on working memory [(F(1,133)=40.179, p<0.001, two-tailed, 2=0.090]. No statistically significant results were observed for the main effect of cross-modal stimuli on working memory [F(1,133)=0.36, p=0.549, two-tailed], or for the interaction between item grouping and cross-modal stimuli on working memory [(F (1,133)=2.959, p=0.088, two-tailed]. Such results indicate that item/material grouping is a contributory factor to individuals' working memory, closely related to students' learning and memorization within a classroom environment. Future research should investigate the effects of these variables, along with item familiarity, on long-term memory.

Keywords: Working memory, item grouping, cross-modal stimuli, limitations, memory capacity, visual-haptic, visual-auditory, working memory model.

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Introduction

Working memory (WM) is illustrated as the reconstructive workspace that temporarily includes information for easier access, availability, inspection and computation [20, 42]. As soon as cognitive tasks are completed, new information triggers the process' re-initiation. WM is vital to complex cognitive tasks, as suggested by associations with measurements of intelligence [10, 11]. WM is distinctly different from short-term memory, although the two terms are commonly used interchangeably [1]. The term 'working memory' was introduced by Baddeley and Hitch (1974), in their relevant WM model [6]. Their model proposes the existence of three WM components; (1) the central executive, a modality-free controlling system of limited capacity which coordinates, controls, and manipulates material in the subsystems (2) the phonological loop, handling a series of verbal and auditory information (3) the visuospatial sketchpad, handling visual and spatial/haptic information [15]. The episodic buffer was later added to the model as an interface between the other systems, accommodating various modalities, binding features, and holding 'chunks' based on an array of diverse dimensions (e.g. verbal, visual, semantic) [5]. This addition to WM model was linked to Baars' (2002, 1997) view of the role of consciousness, serving the purpose of pulling together distinct streams of data from senses and binding them into observable objects and scenes [3, 4].

Various limitations accompany WM [14, 24]; one factor is the exposure to the items and another factor is interference, occurring when recently acquired knowledge exhibits similarity with the present one, causing limitations to the learning/memory capacity [47]. Wickens, Dalezman, and Eggemeier (1976) conducted a study with the aim to prove that release from such interference can occur when the new material is markedly different from the old one [47]. More specifically, they used semantic categories (flowers, vegetables, meat, fruit, and professions) as the new material when the original category was fruit. The results showed that the more dissimilar the categories, the less they interfered. Moreover, Oberauer, Farrell, Jarrold, and Lewandowsky (2016) demonstrated that WM capacity is affected by the set-size effect, suggesting that the larger the number of material held in WM, the less memory is accurate, thus memory capacity is reduced when individuals need to remember an increasing number of visual objects [31, 35, 38].

Appropriate tasks should be designed to effectively measure WM, relevant to the characteristics of the memory system [1]. WM is typically measured using complex span tasks/dual-tasks that add a secondary cognitive task, which does not need to exhibit a relevance with the primary one (e.g. solving mathematical operations, deciding whether a sentence is syntactically/semantically accurate) [44]. However, the differentiation between complex/dual and simple span tasks cannot be characterized as entirely accurate, as the two processes overlap and there is not adequate research on the matter [1].

Empirical research has focused on information processing based on stimuli in different formats [17]. Multisensory/cross-modal stimuli are behaviorally more advantageous, evoking an immediate response and enhanced recognition [49]. Moreover, bimodal/cross-modal representations (e.g. visual-auditory) have been repeatedly found to display improved free recall performance compared to unimodal ones (e.g. visual only/auditory only) [13, 32]. Also, the capacity of WM seems to increase for cross-modal rather than unimodal stimuli [8].

Sensory perception is largely dependent on the visual system [34], yet the fast and accurate recognition of objects/items can be also achieved through the conscious touching of them (haptic investigation/perception) [37, 40], which has been employed by humans as one of the simplest ways to acquire new information about the environment. Touching through kinesthetic receptors can relay information about the identity and the characteristics such as texture, shape and rigidity of the objects/items in question [9, 18, 43]. Research suggests that touch adds speed and accuracy in information-processing and is beneficial for one's WM [27]; in college-age adults, WM for objects was improved when it involved object/item touching [26].

Visual-auditory discrepancies have also been detected by empirical research [28]; visual patterns affect WM similarly

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to verbal memory, as memory is susceptible to effects of similarity and is limited to around 3-4 objects/items [45, 46]. However, there is evidence suggesting that the capacity for complex stimuli can be fewer than three items [2], whereas when the stimuli are simple, the estimate can be considerably higher than four [41]. According to Penney's (1989) model of verbal information, separate streams are responsible for both visual and auditory information [39]. The auditory presentation mode is suggested to be superior, commonly known as the modality effect [19], with studies indicating that the storage of auditory items substantially increased when participants were presented with spoken words [17], in accordance with WM models [7, 33]. Kellogg (2001) suggests that two memory components can account for the abovementioned effect; namely a sustained sensory memory for the auditory stimulus and a brief one that may cease to exist in the presence of another auditory stimulus similar to first one, commonly known as the suffix effect [25, 36].

WM limitations may derive from processes that are associated with grouping or chunking as they can enable encoding of information coming from more than one item, to be processed as one larger unit [21]. The concept of 'chunks' [35] theorized that items that share common qualities such as color, shape, rhythm, or meaning belong to a semantic group that long-term memory (LTM) is already familiar with; groups that create meaning to participants are easier to recall, and remain in memory during studies and testing [30]. Although the solid definition of 'chunks' and their storable number are points of criticism, the concept of information grouping in terms of semantic information as storable items is still present in relevant research literature, with Cowan (2010) arguing for a number of four storable items [12]. Familiar objects may positively affect mental grouping, occurring when multiple items are perceived as a singular one, indirectly increasing the limit capacity. Hence, item grouping may be associated with higher WM retention, enhancing the input encoding while eliminating redundant information [21]. However, research has shown that individuals can memorize more objects if they belong to different categories than to the same [48]. In one study by Endress, Korjoukov, and Bonatti (2017) the effect of category-based grouping performance for WM was compared to multiple object tracking [14]. Participants were presented with either 'pure' displays of cars or faces, or with 'mixed' displays of cars and faces. Overall, the effects of category were found to be weak, confirming previous findings suggesting that WM capacity limitations in various domains are, to some extent, due to distinct mechanisms' limitations.

Nonetheless, Li et al. (2018) conducted several experiments and a meta-analytic study to examine grouping effects in WM [29]. They grouped memory items through illusory contour and the results showed that WM was significantly improved when presented with grouped items. Consequently, there were robust and beneficial grouping effects on WM, influenced by diverse factors.

Notably, the effects of visual-haptic objects have been mainly investigated on LTM [40. and not on WM directly [23] and there has not been adequate research on the potential associations between cross-modal stimuli (deriving from two modalities) and item grouping on WM. Hence, the novel of the present study is to investigate the potential interaction of cross-modal stimuli and semantic item grouping and their effect on WM. The study builds upon the work of Li et al. (2018) [29], adding the variable of cross-modal stimuli. The research also aims at identifying the WM components in which the chosen variables are stored, based on the WM model [6].

Three hypotheses are suggested; **(H_1)** there is a main effect of cross-modal stimuli on WM **(H_2)** there is a main effect of item grouping on WM **(H_3)** there is an interaction of cross-modal stimuli and item grouping on WM.

Methodology

Design

The experimental method was employed, and a 2x2 factorial mixed design was adopted; the first independent variable (between subjects) was cross-modal stimuli and its two levels were visual-haptic stimulus and visual-auditory stimulus. The second independent variable (within subjects)

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was item grouping, along with its two levels; grouped items and ungrouped items. The dependent variable (DV) was WM, measured in items successfully freely recalled by the participants.

Participants

135 Mediterranean College students/teachers (72 females, 63 males, N=135, Mean=22,7, SD=6), aged 18-51 years of age, were recruited, indicated by the G*Power analysis [16], through opportunity sampling. Inclusion criteria stipulated that participants should be able to effectively communicate in Greek and to fall into the abovementioned age category. Participants with hearing/visual impairments or with any cognitive dysfunction were excluded from this study.

Materials

Fifteen miniature animal toys and fifteen everyday tangible objects were utilized in the experiment [47] (Appendix K); they were all selected to be easily recognizable and familiar. Two item presentations (Appendix H) were employed and shown via the College's classrooms' computers/projectors in the visual-auditory condition, which included two additional minuscule videos (Appendix I), found online, to act as interference. A stopwatch was further utilized to time the procedure. The data was screened and analysed using the Statistical Package for the Social Sciences (SPSS statistical software-version 25.0). Finally, all the necessary forms were also given to the participants (Appendices A,B,C,E).

Procedure

All participants were given the briefing and consent forms beforehand and were randomly allocated in the visual-haptic or the visual-auditory conditions. All items were randomly distributed to them. Sixty-eight participants took part in the visual-auditory condition and were presented with visual-auditory stimuli (pictures/sounds); half of them were initially presented with grouped items and the other half were presented with non-grouped items (counterbalancing). Sixty-seven participants took part in the visual-haptic condition and were asked to haptically explore items; half of them initially haptically explored grouped items and the other half haptically explored ungrouped items (counterbalancing). Participants in the visual-haptic conditions held their items for 10 seconds and then passed them to the other participants when hearing the signal "next" (Appendix J). In all conditions, participants watched an interference video before filling in their data collection forms (Appendix C). All participants were given debriefing notes at the end of each session.

Ethics

The research was conducted in accordance with the British Psychological Association Code of Ethics and Conduct (2014). All ethical guidelines were followed, by giving informed consent, briefing and debriefing forms to all participants. Confidentiality and anonymity were ensured, and participants were reminded of the withdrawal option at the start and at the end of the experiment. The research was also approved by the scientific committee of the Mediterranean College by obtaining a sign-off form (Appendix F).

Results

A Factorial 2x2 Mixed design was employed to analyze two independent variables with two corresponding levels, consisting of independent/repeated measures.

The data was screened to investigate the eligibility of the parametric assumptions. Since the researchers measured participants' items correctly recalled, the parametric assumption of the dependent variable being at the interval scale of measurement was fulfilled. The scores were transformed to z scores and indicated an outlier, which was replaced with the z scores' mean value (criterion +/-3 for sample sizes of <100, N=135), hence the parametric assumption of normal distribution was met. The skewness/kurtosis calculations did not surpass the +/-2.58 criterion (Appendix L). The Q-Q plots revealed two outliers, yet these participants

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did not deviate from the symmetrical distribution. Since N=135<200, the histograms could not display a normal looking graph because of the small data number.

The Kolmogorov-Smirnov normality test indicated that there was not a normal distribution. The Shapiro-Wilk normality test suggested that there was a normal distribution for two conditions. Consequently, the normal distribution assumption was accepted, but with caution. Since the Levene's test was not statistically significant, the assumption of homogeneity of variance was assumed. Concerning the Mauchly's test of Sphericity, sphericity was subsequently assumed.

All parametric assumptions were assumed. There were normal distribution issues, but within acceptable and non-alarming limits.

Table 1: Means and Standard Deviations of items successfully recalled.

	Visual-Auditory	Visual-Haptic	Total
Grouped	11.179 (1.757)	11.013 (2.011)	11.096 (1.884)
Ungrouped	9.642 (1.856)	10.132 (2.051)	9.889 (1.965)
Total	10.410 (1.960)	10.573 (2.071)	
(Raw Data: Appendix G)			

The table demonstrates a non-considerable difference between the visual-auditory and the visual-haptic conditions. In contrast, there are slightly notable differences between the grouped and ungrouped conditions, implying that most participants could better recall the items within the grouped and visual-haptic conditions.

The data was analyzed using a 2x2 mixed ANOVA. The results showed a significant main effect of item grouping on WM [(F(1,133)=40.179, p<0.001, two-tailed, 2=0.090], thus the second hypothesis is supported. Moreover, there was not a significant main effect of cross-modal stimuli on WM [F(1,133)=0.36, p=0.549, two-tailed], and the first hypothesis cannot be supported. Subsequently, there was not a significant interaction between cross-modal stimuli and item grouping on WM [(F (1,133)=2.959, p=0.088, two-tailed] and the third hypothesis is not supported as well.

Discussion

A Factorial Mixed Design was employed to investigate whether a main effect of cross-modal stimuli and object grouping or their potential interaction on WM is established. The first hypothesis was not supported, as there was no main effect of cross-modal stimuli on WM. The visual-haptic/visual-auditory items did not affect WM accuracy, contrary to research findings supporting that WM can be substantially enhanced with the use of touching [26] or listening [17]. This poses a profoundly interesting finding as it severely contradicts relevant research. Mastroberardino et al. (2007) claims that cross-modal stimuli enhance information storing and retrieval in WM [32]; however, most previous research on the subject compared cross-modal with unimodal stimuli, while the present study assessed differences of cross-modal stimuli on WM free recall.

Oppositely, the second hypothesis is supported since item grouping was found to have a significant effect on participants' retention/recall. This is in accordance to previous studies highlighting this effect [21, 29]. Hence, it can be argued that item grouping aids input encoding, allowing individuals to recall the desired pieces of information by eliminating redundant details that may have been included in the interference video. Grouped objects were better recalled by the participants, contrary to previous research [14, 47, 48] which found that there was less interference with WM when the category-based items were not similar. However, the findings are consistent with research by Li et al. (2018), as item grouping improved participants' WM [29]. The theory of 'chunks' as a strategy for storing and recollection is further enhanced by the findings, as it appears that item grouping had a medium to strong effect over WM.

The third hypothesis is not supported either, as there was no interaction of cross-modal stimuli and item grouping on WM. Nevertheless, this might support that different processes are associated with different areas of the brain.

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Specifically, visual-haptic information might be allocated in the visuo-spatial sketchpad, and visual-auditory information might be allocated in the phonological loop and the visuo-spatial sketchpad as proposed by Baddeley and Hitch's (1974) WM model [6, 15]. Grouping processes might be allocated within the central executive or the episodic buffer, which accesses LTM for previously organized groups of items, thus making recall easier for items that belong to the same group [5, 15]. However, item grouping might be directly correlated to specific/unique grouping or chunking processes that perceive items as one unit, hence their allocation within the model's components cannot be entirely confirmed.

Remarkably, in the present study, the vast majority of the participants recalled more than 4 items in all conditions, yet previous research indicates that individuals can hold up to 3-4 objects in WM and memory capacity is markedly reduced when the number of items is larger than 4 [45, 46]. This contradictory finding implies that recall in WM may not be as demanding as it was initially considered. It was also observed that participants were able to recall more animal toys (grouped) than everyday items (non-grouped), thus revealing that familiarity does not affect WM; more familiar objects are not better recalled. This might also suggest that the everyday objects employed did not carry a bias, as they are widely known and commonly used, hence they were not easier to be recalled.

This study attempted to eliminate any asymmetrical order effects by counterbalancing the conditions. Moreover, the main core of the experiment, which was WM and the effects of object grouping and different type of stimuli on it, has been replicated by previous research, hence there was a solid base of research. This makes the experiment easily replicable for further research on the matter. Finally, to address WM recency or primacy effects to an extent, this study further used randomization of stimuli order.

However, some limitations should be addressed. The most apparent limitation was the relatively small sample size. Moreover, some of the items belonging to the animal category posed some difficulty for participants to discern/identify. Additionally, the items that were to be haptically investigated were relayed from one participant to the next, causing variation in the retention time, which may have acted as a confounding variable. Furthermore, participants' WM may have been influenced by relevant features of the items used that favorite or hinder memorability, despite the effort made to use items that were equally common and familiar. For instance, observation of raw data revealed that several items used in the trials (e.g. a red ball) had a greater recollection rate (83%) compared to other items. It can be further assumed that participants shaped their answers according to what they believed should be remembered. For instance, the grouped items were animals, so they could have filled in items belonging to the same category, without that meaning that they really recalled which animals were utilized in the experiment, due to the reconstructive nature of WM [20].

Future research should correspondingly be able to replicate this experiment by investigating item grouping and cross-modal stimuli effects on LTM to observe whether participants' free recall works better in LTM. In addition, familiarity of objects should also be further investigated, since the present study noticed differences between familiar and non-familiar objects, but was not able to identify their actual effects on WM. It is also suggested that a pilot study be employed to examine examine whether some items are persistently and prominently more memorisable.

The present research has implications in education, presentations and learning within classroom environments as item/learning material grouping might be a sole significant contributory factor to students' WM. The usage of cross-modal stimuli (e.g. multimedia) may, in turn, be an effective strategy for enhancing binding, thus improving memorization and learning, especially for children with learning difficulties. Further research over this particular domain is also recommended, in order to devise optimal teaching strategies.

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Is lithium a universal inhibitor? Evidence arising from clinical neuroscience and oncology

Orestis Giotakos

Abstract

Lithium has been in the environment from the origin of life, interacting with almost all of the biological molecules that life invented. Lithium affects many components of intracellular signalling pathways, inhibiting more than ten cellular targets and displacing magnesium ions. Lithium modulates cell function via inhibitory effects on adenosine triphosphatase activity, cyclic adenosine monophosphate, and intracellular enzymes. Lithium is also an important inhibitor of the enzyme glycogen synthase kinase-3. Recent epidemiological findings strongly support the benefits of lithium use in both neuropsychiatry and oncology. Lithium is the first line drug used in the management of bipolar disorders, while natural lithium level intake may influence impulsiveness, a possible core factor mediating the manifestation of both suicidality and aggressiveness. Lithium is also useful in a broad range of diseases: neurological, such as epilepsy, Huntington chorea, Parkinson diseases, and headaches; endocrinological, such as hyperthyreosis, diabetes mellitus, and the inappropriate secretion of the antidiuretic hormone; haematological, such as neutropenia, and thrombocytopenia; and alergological, such as asthma. Moreover, lithium has been tested with promising results in oncology. Lithium reverts the apoptosis models by interfering receptor function, while a long-term lithium treatment has been shown to increase the expression of antiapoptotic genes. Lithium chloride has been also found to hold anticancer properties, while combination treatments with lithium can improve the efficacy of chemotherapeutic agents in apoptosis deficient cancer cells. Lithium can modulate autophagy in esophageal and colorectal cancer cells. A large retrospective study showed that lithium-exposed individuals were less likely to suffer melanoma-associated mortality and recent epidemiological findings showed a reduced overall cancer risk in bipolar patients treated with lithium. What we know about the effects of lithium seems to be a small fraction of what there is to know, but it seems that lithium has been central to survival in the process of biological evolution. Lithium demonstrates a broad range of inhibitory effects from the cell to the behavioral level. We may suggest that lithium operates like a natural universal inhibitor, helping the organisms to readjust balances and to survive, through the development of compensatory and readapting mechanisms.

Key words: *Lithium, bipolar disorder, inhibition, apoptosis, cancer, anticancer, oncology, neuropsychiatry*

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Introduction

Lithium (from Greek: *lithos*: stone), with the symbol *Li,* is a chemical element with an atomic number of 3. Lithium is at the heart of a deep, so-far-unsolved problem in cosmology. It is one of the three elements (the other two are hydrogen and helium) produced in the initial condensation of matter from energy immediately following the Big Bang **[1].** The lithium chemistry is not common, since atoms are highly polarized, very small, and have a high charge density. The lithium biochemical properties are similar to those of magnesium, and it influences magnesium-dependent processes **[2].** As a natural trace element, lithium is washed out by rain from rocks and soil, reaching the food chain via drinking water. The available evidence indicates that the recommended dietary allowance for a 70 kg adult Is 1,000 microg/day **[3].** An average intake would result in a daily dose of approximately 1% of a therapeutic lithium dose for bipolar disorders **[4].** Lithium plays an important role in embryogenesis and biochemical mechanisms of action are related to the function of many vitamins, hormones, enzymes, and growth factors **[5].** Between the 1880s and World War I, the most premium of all the mineral water brands were *lithia waters* because of their acclaimed health benefits. Research studies found that low doses of lithium demonstrate neuroprotective effects **[6]**, and improvements in mood and cognitive function **[7].**

Lithium is an integral drug used in the prophylaxis of bipolar disorders and the treatment of acute mania **[8].** Many other beneficial effects of lithium have been reported in neuropsychiatry, such as anti-suicidal and anti-aggressive properties, improvement of hyperactivity in schizophrenia, and behaviour benefits in intellectually disabled patients **[9].** The initial studies of molecular targets for lithium action were based on the assumption that this simple cation can interfere with transporting systems for sodium and potassium in the plasma membranes of neurons and alter the propagation of electrical signals. Some studies indicate that the lithium inhibition of the countertransport mechanism may be clinically significant and relevant to the lithium therapeutic action **[10].** Lithium has been found useful in a broad range of diseases from neurological (epilepsy, Huntington chorea, Parkinson diseases, and headaches) endocrinological (hyperthyreosis, diabetes mellitus, and the inappropriate secretion of the antidiuretic hormone), haematological (neutropenia, and trombocytopenia) to the alergological (asthma), **[11, 12].** Furthermore, lithium has now been tested with promising results in oncology (thyroid carcinoma) **[13],** infectious diseases (AIDS related dementia) **[14],** and dermatology, in seborrhoeic dermatitis, with topically application **[15].**

The signaling pathways Inhibition

Lithium affects many components of intracellular signalling pathways, like inhibiting of more than ten cellular targets and displacing magnesium ions. Lithium affects some enzymes involved in energy metabolism, such as hexokinase, pyruvate kinase, cholinesterase, tryptophan hydroxylase, and glycogen synthetase [**16].** Plenge (1985) **[17]** proposed the theory that lithium inhibits enzymes which have essential cofactor cations, such as Ca2+, Mg2+, Na+, K+, and Zn2+ by displacement of these cations from the enzyme. Lithium displaces magnesium ions, leading to the inability of the resulting very stable complex enzyme-phosphate-lithium to hydrolyse a further substrate molecule. Lithium competes for a magnesium binding site in glycogen synthase kinase-3, inositol polyphosphate 1-phosphatase, fructose 1,6-bisphosphatase, bisphosphate nucleotidase, and phosphoglucomutase **[18].** However, the targets inhibited by lithium at therapeutically relevant concentrations (0.6-1 mM) are inositol monophosphatase and glycogen synthase kinase 3ß. Thiruvengadam (2001) **[19]** proposed the understanding of the therapeutic effects of lithium and sodium valproate in bipolar disorder, modifying the known Goldman–Hodgkin–Katz equation in order to include a fourth ion, such as a lithium ion or a sodium ion. The author suggested that the resting membrane potential in bipolar patients may be hyperpolarized and the lithium ion depolarizes the resting membrane potential back to the normal state.

Lithium inhibits many of structurally similar magnesium–dependent phosphomonoesterases at levels within the therapeutically relevant range of concentrations, 0,6–1,2 mM.

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Most of the proposed mechanisms for lithium cellular action indicate an inhibitory effect on parts of various signaling pathways, such as cyclic GMP and cyclic AMP formation **[20].** Lithium shows also an inhibitory effect on the G proteins, a ubiquitous family of proteins that serve the critical role of transducers of information across the plasma membrane, by coupling receptors to various neurotransmitters. By inhibiting inositol monophosphatase, lithium obstructs the enzymatic degradation of inositol trisphosphate (IP3), resulting in reduced availability of the second messengers IP3 and DAG, the derivatives of the PIP cycle. Some studies identified the upstream inositol polyphosphatase as an additional target for lithium **[21].** Molecular genetic studies in model systems suggested Ins (1, 4, 5) P3 as a clinically relevant target of lithium **[22].** Another possible therapeutic effect of lithium is to attenuate brain phospholipase A2 (PLA2) activity. Lithium therapy seems to decreases the turnover of arachidonicacid (AA) in several brain phospholipids, which is correlated with a significantly decrease of PLA2 activity **[23, 24].**

The thyroid system inhibition

Lithium treatment seems to contribute to the development of hypothyroidism, goitre, hyperthyroidism and autoimmune thyroiditis **[25, 26].** A meta-analysis of the potential toxicity of long-term use of lithium showed that lithium causes a five-fold increased risk of hypothyroidism **[27].** Goiter, due to increased thyrotropin (TSH), after inhibition of thyroid hormone release, occurs at various reported incidence rates and lithium-induced goitre reveals a prevalence of 0 to 60% **[28].** When more sensitive ultrasonographic scans are used to detect increases in thyroid volumes, prevalence is higher (30–60%) **[29]**. Although thyroid hormone basal levels are not usually high, lithium therapy is associated with exaggerated response of both TSH and prolactin to TRH in 50%-100% of patients. Clinical or subclinical hypothyroidism due to lithium is associated with anti-thyroid peroxidase (TPO) antibodies, although it may occur in their absence. Immunogenetic background and Iodine exposure may contribute to the occurrence of goiter and hypothyroidism during long-term lithium therapy **[30].**

Lithium affects thyroid functioning through multiple mechanisms. Lithium inhibits thyroidal iodine uptake and iodotyrosine coupling, alters thyroglobulin structure, and inhibits thyroid hormone secretion. The lithium induced inhibition of the synthesis and release of thyroid hormones may result in an increase of TSH level, leading to enlargement of the gland and the goiter formation. At the thyroid cellular level, lithium decreases thyroid hormone synthesis and release. At the peripheral level, lithium decreases deiodination of tetraiodothyronine (T4) or thyroxine, by decreasing the activity of type I 5′ de-iodinase enzyme. Other mechanisms that explain the proliferation of thyrocytes in patients treated with lithium is the activation of tyrosine kinase by lithium ion, and lithium effects on intracellular signaling related with Wnt/ beta-catenin and adenylate cyclase **[31].** Lithium also increases the propensity to thyroid autoimmunity in vulnerable individuals, through the increased activity of B lymphocytes and the reduced ratio of circulating suppressor to cytotoxic T cells **[32].** In parallel with these anti-thyroid effects, lithium influences cell function via its inhibitory action on cyclic adenosine monophosphate (cAMP), adenosine triphosphatase (ATPase) activity, and intracellular enzymes. The known inhibitory effect of lithium on inositol phospholipid metabolism may affect signal transduction and may account for part of the effect in bipolar disorder. Lithium also alters the in vitro response of cultured cells to thyrotropin-releasing hormone (TRH) and can stimulate DNA synthesis **[33].**

However, the above described "disordered" thyroid-hypothalamic-pituitary axis seems to be temporary in most lithium treated patients, which suggests that the axis is adjusting in a new «state» during lithium therapy **[34].** Possibly, the lithium induced 'central hypothyroidism' activates a secondary action of the HPT axis, in order to re-activate and re-adapt the thyroid hormone availability and effect. This compensatory process may results to the correction of a possible peripheral resistance to thyroid hormones, as well as to the correction of an isolated CNS hypothyroidism. We may suggest that the compensatory mechanisms which operate to the final correction of the hypothyroidism, may

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represent a therapeutic process of lithium therapy in bipolar disorder, acting through the thyroid system resetting **[35].**

The GSK-3 inhibition

An important line of evidence has been connected with the discovery of the universal role of the enzyme glycogen synthase kinase-3 (GSK-3). Lithium has been found as the important inhibitor of GSK-3 **[36]** and activator of the Wnt signalling system **[37],** although this lithium effect occurs at high concentrations and may be related with a toxic effect **[38].** GSK-3 inhibition has been proposed as a therapeutic mechanism of action. Inositol is a metabolite that serves as a precursor for inositol phosphates and inositol lipids. Inhibition of inositol synthesis results in the inactivation of GSK-3α, which suggests a unifying hypothesis for the mechanism of action of mood-stabilizing drugs and that inositol synthesis and GSK 3α activity are intrinsically related. Ye & Greenberg (2015) **[39]** reported that inhibition of the rate-limiting enzyme of inositol synthesis leads to the inactivation of glycogen synthase kinase (GSK) 3α by increasing inhibitory phosphorylation of this kinase. Glycogen synthase kinase-3 (GSK3) affects over 100 known substrates, with many regulating mechanisms related to substrate priming, cellular trafficking, post-translational modifications, protein complexes, receptors and receptor-coupled signal transduction events. In addition, GSK3 is involved in many prevalent disorders, including inflammatory diseases, cancer, psychiatric and neurological diseases, and others **[40].**

The "GSK3 hypothesis of Alzheimer Disease" **[41]** integrates and extends the "amyloid cascade hypothesis" of Alzheimer Disease. This hypothesis strongly implicates GSK3 inhibitors as a novel treatment strategy for Alzheimer Disease, incorporating the known key molecular events and links these with outcomes such as memory impairment and inflammation. Phospholipase A2 (Pla2) is required for memory retrieval, while cognitive decline and memory deficits were shown to be reduced in animal models after lithium treatment, which suggests possible links between Pla2, lithium and memory. Mury et al (2016) **[42],** pointed to a significant perdurability of long-term memory after the chronic lithium treatment, which correlated with increased transcriptional and enzymatic activities of certain members of the Pla2 family (iPla2 and sPla2). The findings reinforced the possible use of low doses of lithium for the treatment of neurodegenerative conditions such as the Alzheimer's disease. On the other hand, not only is there no evidence about the increased prevalence of cancer in patients undergoing long-term lithium monotherapy **[43],** but lithium increases survival rates of patients with adenocarcinomas **[44].** Manji et al (2000) **[45]** demonstrated an increase of the cytoprotective factor Bcl-2 in the hippocampus, frontal cortex, and striatum in the brains of patients with bipolar disorder, after chronic lithium treatment. The mechanism of lithium action is not known, but its neuroprotective effect may be relevant in the long-term treatment of neurodegenerative disorders **[38].**

The apoptosis inhibition

The programmed cell death named *apoptosis*, is an evolutionarily conserved form of cell death and an important factor for tissue homeostasis. The glutamate insult induces excitotoxicity, cell death and apoptosis, while this is triggered by an exaggerated and prolonged rise in intracellular Ca2+. Neurodegeneration after a cerebral trauma is associated with glutamate efflux and overstimulating of glutamate receptors. On the other hand, growth factors and neurotrophins have been shown to promote cell survival and inhibit apoptosis, a process mediated through the phosphatidylinositol 3-kinase/Akt cascade. This signaling pathway is usually activated by insulin factors, like the insulin-like growth factor 1 (IGF-1) and growth factors, like the platelet-derived growth factor (PDGF). Apoptosis may induced also in a low potassium-containing culture, a process mediated primarily by activation of NMDA receptors. Llithium can revert these apoptosis models by interfering receptor function, while a long-term lithium treatment has been shown to increase the expression of the antiapoptotic gene bcl-2 **[46].**

Inactivation or inhibition of the GSK-3 has revealed anti-apoptotic effects. The GSK-3 inhibitors may downregu-

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late TGFBIp expression by blocking TGF-β signaling **[47].** A number of studies demonstrated that lithium inhibits GSK-3 directly **[48-53],** or indirectly, by triggering the phosphorylation of GSK-3 at ser21/ser9 **[54-56].** Research suggests that lithium elicits its neuroprotective effects by inhibiting GSK-3 **[57].** Lithium can block indirectly the GSK-3 activity through the phosphorylation of GSK-3α at ser21 and of GSK-3β at ser9 by multiple mechanisms, including the activation of PKA, phosphatidylinositol 3-kinase (PI3-K)-dependent AKT, and protein kinase C (PKC) **[58-59].** Choi et al (2011) **[60],** demonstrated also that lithium treatment reduces TGFBIp expression in a dose-dependent manner in corneal fibroblasts through the inactivation of GSK-3. Exposure of cells to glutamate induced a rapid and reversible loss of Akt-1 phosphorylation and kinase activity. Long-term lithium pretreatment suppressed glutamate-induced loss of Akt-1 activity and accelerated its recovery toward the control levels. Lithium also increased the phosphorylation of glycogen synthase kinase-3 (GSK-3), a downstream physiological target of Akt. Thus, modulation of Akt- 1 activity appears to play a key role in the mechanism of glutamate excitotoxicity and lithium neuroprotection **[61].** The PI 3-K/Akt signaling cascade has been linked to the pathogenesis of certain forms of leukemia, while lithium treatment is known to cause leukocytosis and has been used to suppress leukopenia in patients undergoing radiotherapy or chemotherapy **[61].**

Ceramide has been known as an apoptotic factor in a variety of cell types, through inhibition of the antiapoptotic kinase Akt, an enzyme that phosphorylates and inhibits GSK-3. Lithium could oppose the action of ceramide on GSK-3, as it has been shown to inhibit directly this enzyme **[62].** In another research area, glomerular renal dysfunction occurs after an average of twenty years of continuous lithium treatment, and the severity is related to the total lithium load as measured by dose and duration. Radiologically visible lithium-related microcysts are usually 1-2 mm. Khan & El-Mallakh (2015) **[63],** proposed that the mechanism of microcyst formation is related to the antiapoptotic effect of lithium. All the data mentioned above indicate that lithium may act as a neuroprotective drug whose action on GSK-3, either direct or mediated through the phosphatidylinositol-3-kinase pathway, by a yet unknown mechanism, may explain the broad range of apoptotic insults against which it is effective.

The metastasis inhibition

In 1981, Lyman et al **[64]** studied patients with small cell lung cancer who received radiation therapy, chemotherapy, with or without lithium. They found that patients who received lithium (900 mg per day) experienced significantly less mid-cycle leukocyte and neutrophil count depression, spent fewer days with leukopenia and neutropenia than control patients regardless of age or extent of disease, spent fewer days hospitalized and fewer days with fever in the presence of severe neutropenia than control patients. Although anti-angiogenic agents have been used for treating cancer, the overall survival in patients with advanced cancer has not been improved substantially, possibly because cancer metastasis occurs preferentially via lymphatic rather than hematogenous spread. There is an unmet need to develop therapies to block lymphangiogenesis as well as angiogenesis, in order to efficiently block tumor growth and metastasis. Saghiri et al (2016) **[65]** reported that the elements Li, Ti, Hg, Va, Nb, Ce, As, and Pb can promote and/or inhibit angiogenesis through different mechanisms, while lithium affects vasculogenesis but not angiogenesis. In 2016, Maeng et al **[47]** reported the *in vitro* and *in vivo* activities of lithium in inhibiting tumor lymphangiogenesis, TGFBIp expression, and metastasis. They found that lithium reduced the expression of TGFBIp in SW620 colon cancer cells via GSK3β inactivation and inhibited lymphatic endothelial cell migration induced by TGFBIp. Furthermore, lithium activity against lymphangiogenesis and angiogenesis, had no effect on the growth of a primary colon cancer tumor xenograft, and strongly inhibited its metastasis to the lungs, liver, and lymph nodes by blocking lymphangiogenesis in primary tumors.

Asgari et al (2017) **[66]** examined the association between lithium use and melanoma risk, conducting a retrospective cohort study on 2,213,848 adult white Kaiser Permanente Northern California members for the period 1997-2012.

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Melanoma incidence per 100,000 person-years among lithium-exposed individuals was 67.4, compared to 92.5 in unexposed individuals, while no lithium-exposed individuals presented with advanced-stage melanoma. Among melanoma cases, lithium-exposed individuals were less likely to suffer melanoma-associated mortality. The authors found that lithium treated patients (n=11,317) had reduced melanoma risk and associated mortality and they concluded that lithium may reduce melanoma risk and associated mortality. Wang et al (2017) **[67],** found that lithium can suppress proliferation and induces apoptosis in pancreatic cancer cells, while lithium and ESI-09 synergistically inhibit pancreatic cancer cell growth and survival. The authors suggested the lithium's ability to suppress cAMP/protein kinase A signaling as a novel mechanism for the synergistic action of lithium and ESI-09, in addition to the known inhibitory effect of lithium toward GSK3β.

The cancer cell inhibition

The supratherapeutic doses of lithium chloride (LiCl) has been shown to demonstrate anticancer properties. Novetsky et al (2013) **[68]** suggested that inhibition of glycogen synthase kinase 3β and lithium is a potential therapy for ovarian cancer. The authors pointed that the combination treatment with LiCl and cytotoxic agents can reduce ovarian cancer cell metabolism but does not appear to affect cellular proliferation. Erguven et al (2016) **[69],** investigated the effect of different concentrations of LiCl on prostate cancer stem cells, after incubating of human prostate stem cells and non-stem cells, with low and high concentrations of LiCl for 72 hours. They found that cell stimulated with low concentrations had low apoptotic indices, high proliferation, high MK levels and more healthy ultrastructure, while opposite results were obtained at high concentrations. Li et al (2015) **[70]**, reported that lithium chloride promotes apoptosis in human leukemia NB4 cells by inhibiting glycogen synthase kinase-3 beta. Moreover, in a dose-dependent manner, LiCl significantly increased the level of Ser9-phosphorylated glycogen synthase kinase 3β (p-GSK-3β), and decreased the level of Akt1 protein.

O'Donovan et al (2015) **[71],** reported that lithium modulates autophagy in esophageal and colorectal cancer cells and increases the efficacy of therapeutic agents. Mutations of the *PTEN* gene, that encodes the phosphatase and tensin homolog (PTEN) protein, are a step in the development of many cancers. Recent findings indicate lithium and PTEN as potential candidates for the identification of new therapeutic approaches for colorectal cancer treatment. de Araujo et al (2016) **[72],** reported that PTEN overexpression cooperates with lithium, increasing cell death and reducing malignancy in colorectal cancer cells. It should be noted that the role that lithium plays in cancer is controversial, since lithium can inhibit or activate survival signaling pathways, depending on the cell type. In this recent study, de Araujo et al (2016) **[72]** investigated the mechanisms by which lithium modulates the progression of the colorectal cancer and the role of the survival signaling pathways PI3K/Akt and PTEN. Lithium was found to decrease the proliferative potential of colorectal cancer cells and increased apoptosis, which was accompanied by decreasing proteins levels of Akt and PTEN.

Lithium inhibits glycogen synthase kinase (GSK)-3, which activates NFAT1/FasL signaling, while temozolomide inhibits GSK-3 and activates Fas in tumour protein wild-type (TP-53wt) glioma cells. Temozolomide, in combination with low-dose lithium, induces TP53wt glioma cell death, via NFAT1/FasL signaling, and this may represent a therapeutic strategy for the treatment of TP53wt glioma **[73].** Neurocognitive impairment is an important issue in patients with cancer, due to the direct or indirect involvement of the nervous system or due to the chemotherapy-related complications. Neuroprotection is a realistic goal in preventing these neurocognitive sequelae and lithium seems to have a neuroprotective effect in such patients **[74].** Due to apoptotic effects of the psychotropic drugs on immune and neural cells, opioid abusers are more likely to be infected. Sahebgharani et al (2008) **[75],** had been found that lithium chloride protects PC12 pheochromocytoma cell line (as a model of neural cells) from morphine-induced apoptosis. Activation of glycogen synthase kinase 3beta (GSK3beta) is thought to promote tumor growth and neuroendocrine peptide secretion, while inhibition of this signaling pathway with

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lithium, could be a potential therapeutic strategy to control tumor growth and hormone production. In the Kappes et al (2007) **[76]** study, pheochromocytoma PC-12 cells were treated with varying concentrations of lithium chloride, and the levels of active and inactive GSK3beta and NE peptides chromogranin A (CgA) and Mash1 were determined. The authors suggested that GSK3beta inhibition may be a novel strategy to treat catecholamine-producing neoplasms, like pheochromocytoma.

The regeneration of the muscle fibers of experimental hepatocarcinoma-29 transplanted into the hip in CBA mice was tested, after treatment with nanosized lithium carbonate particles on muscle tissue. The regeneration of the muscle fibers was found to be associated with a significant increase in activation of fibroblasts, number of microvessels, and recovery of the organ structure **[77].** Oxidant-antioxidant status in tumor tissue of male-mice CBA at spontaneous course of hepatocarcinoma-29 and after repeated injections of lithium carbonate nanosized particles was evaluated by Konenkov et al (2015) **[78].** The authors investigated the changes of lipid peroxidation products level reacted with 2-thiobarbituric acid, as indicator of oxidative stress and the activity of superoxide dismutase and catalase enzymes, as indicators of antioxidant defense. They concluded that lithium carbonate nanosized particles supports the balance between the oxidant and antioxidants and helps to limit the progression of precancerous condition toward malignancy **[78].**

Inhibition of impulsive behavior

Lithium, a naturally occurring element, has a wide use in psychiatric treatment. Inositol cycle has been suggested as a common action substrate of the bipolar medications lithium, valproate and carbamazepine. Berridge's (1989) **[20]**, with the 'inositol depletion hypothesis' suggested that the lithium induced inhibition of inositol monophosphatase may lead to Ins1P accumulation and inositol depletion. Regeneration of phosphatidylinositol 4,5-bisphosphate requires recycling of inositol from Ins1P, and lithium seems to dampen signaling, using a G-protein-coupled receptor linked to phospholipase C **[79].** Moreover, lithium's antidepressant action may be linked with reduced phosphoinositide cycle-coupled 5-HT_2 receptor function **[80].** Lithium also reduces 5-HT_2 receptor function in mouse as demonstrated by a 5-HT_2 agonist-evoked head-twitch response **[81].** This effect is mediated at the prefrontal cortex **[82],** where it is believed to be the target of lithium in the treatment of bipolar disorder **[83].**

Based on the findings that lithium inhibits both (α and β) glycogen synthetase kinase-3 isoenzymes, Jiménez et al (2014) **[84],** analyzed the possible association of the genetic variants located at the GSK3 α and β genes on impulsivity levels in patients with bipolar disorder. They found that genetic variability at GSK3β gene was associated with increased impulsivity levels in these patients. Evidence indicates that lithium, the most effective mood-stabilizers in bipolar patient, decreases impulsivity levels not only in bipolar patients, but also in other impulse control disorders **[85-89].** Since GSK-3β, a common target for both lithium and valproate, plays a critical role in the central nervous system, by regulating various cytoskeletal processes and long-term nuclear events, its inhibition could be the subject of future research **[45].** Although impulsivity has been associated with serotonin and dopamine dysregulation, some authors suggest that lithium could reduce impulsivity levels through the regulation of the aforementioned neurotransmitter systems **[90].** The inhibition of GSK3 has been suggested to play a key role of the therapeutic action of most of the pharmacological agents used to treat mood disorders **[91].** Lithium inhibits the glycogen synthase kinase-3 (GSK3) β isoenzyme, which in turn act as a mediator of serotonergic function **[92].** The above mechanisms may be also related to the lithium superior antisuicidal effects, in relation to other mood stabilizers **[93-94].**

A recent meta-analysis **[95]** in 48 randomized control trials, which compared lithium with active drugs or placebo for the treatment for mood disorders, has found that lithium is the more effective treatment for reducing the risk of suicide in people with mood disorders. Moreover, the authors suggested that *impulsivity* might be the mechanism that mediates this antisuicidal effect. John Cade (1949) **[96],** the Australian psychiatrist who discovered the lithium car-

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bonate effects in mood stabilizing during the treatment of bipolar disorder, had published the original paper with the title "Lithium salts in the treatment of psychotic excitement". On the other hand, lithium may increase the volume of the prefrontal cortex and the anterior cingulate gyrus **[97],** which indicates that lithium may at least partially exert its antisuicidal effect *via* reinforcing "top-down brakes" of impulsive action. Moreover, since lithium has been shown to increase the volume and function of the limbic system, such as hippocampus **[98],** we can suggest that lithium antisuicidal effects may consist of both reinforcing "top-down brakes" and decreasing "bottom-up drive."

Some ecological studies have shown an association between low lithium intakes from water supplies and suicide, as well as homicide rate. Schrauzer & Shrestha (1990) **[99],** using data from 27 Texas counties for the period 1978-1987, found that the incidence rates of suicide, homicide, and rape were significantly higher in counties whose drinking water supplies contain little or no lithium than in counties with water lithium levels ranging from 70-170 μg/l. Ohgami et al (2009) **[100]** examined lithium levels in tap water in the 18 municipalities of Oita prefecture in Japan, in relation to the suicide standardised mortality ratio in each municipality. They found that lithium levels were significantly and negatively associated with suicide standardised mortality ratio averages for 2002-2006 and suggested that even very low levels of lithium in drinking water may play a role in reducing suicide risk within the general population. Similarly, Blüml et al (2013) **[101]** evaluated the association between lithium levels in the public water supply and county-based suicide rates in 226 Texas counties, with a state-wide sample of 3123 lithium measurements from the public water supply. The findings provided evidence that higher lithium levels in the public drinking water are associated with lower suicide rates. However, Kabacs et al (2011) **[102]**, measuring lithium levels in tap water in the 47 subdivisions of the East of England and correlating these with the suicide standardised mortality ratio in each subdivision, found no association between lithium in drinking water and suicide rates across the East of England for the period 2006-2008. A recent study showed that lithium levels in drinking water were significantly and inversely associated with male but not total or female suicide standardized mortality ratios, in 274 municipalities of Kyushu Island in Japan **[103].** Another recent research by Liaugaudaite et al (2017) showed also that the higher levels of lithium in public drinking water systems from 9 cities of Lithuania were associated with lower suicide rates in men **[104].**

Following the above studies which investigated the relation between low lithium intakes from water supplies and suicide, we evaluated the association between lithium levels in the public water supply and prefecture-based suicide rates in Greece. Analysis was conducted with respect to lithium levels in 149 samples from 34, out of 52, prefectures of Greece. The average lithium level was 11.10 μg/l (range 0.1 to 121 μg/l). The results indicated a tendency for lower suicide rates in the prefectures with high levels of lithium in drinking water **[105].** Extending this study, we found a tendency of lower mean number of homicides in the prefectures with high levels of lithium in drinking water **[106].** Considering these results, we suggested that natural lithium level intake may influence *impulsiveness*, a possible core factor that mediate to the manifestation of both suicidality and aggressiveness, or even criminality. In summary, several studies had shown that low-dose lithium, such as lithium intakes from water supplies, could have anti-manic and anti-suicidal effects, or even anti-dementia effects, although, anti-psychotic or anti-cancer effects are yet to be determined. Further studies would need to determine those levels that are required to maintain mental health **[107]** or whether a "lithium deficiency state" may precipitate the above situations **[108].**

Epilogue - Is lithium a universal inhibitor?

Many lithium mechanisms are responsible for its therapeutic effect, which results in supporting neural plasticity and neuroprotection. Lithium modulates neural plasticity at multiple levels and enzymes, including glycogen synthase kinase-3β, cyclic AMP-dependent kinase, and protein kinase C. Lithium modulates neurotransmitters and readjusts balances between excitatory and inhibitory actions. Lithium

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also modulates signaling activities, through the regulation of second messengers, the transcription factors, and the gene expression. The neuroprotective effects may be derived from its modulation of gene expression, while the outcome of its inhibitive actions seems to result in limiting the magnitudes of fluctuations, contributing to a stabilizing influence **[109].** Lithium, for example, demonstrates two entirely separate actions, inhibition of phosphoinositide signaling and inhibition of GSK-3β. Through these actions, lithium is able at the same time to decrease the highest, stimulus-induced transcription factor activator protein1–DNA binding activity, and to raise the lowest, basal activator protein1-DNA binding activity, respectively. This bimodal action of lithium provide a stabilizing influence on signal fluctuations, ensuring that the activity of activator protein1 is not too low while at the same time protecting the cell from an overly extreme increase in AP1 activity **[109].**

The collapsin response mediator proteins (CRMPs) are mainly expressed in the nervous system during development and play important roles in axon formation from neuritis and in growth cone guidance. They are involved in cell migration, axonal guidance, dendritic spine development and synaptic plasticity through its phosphorylation. The intracellular phospho-proteins CRMPs mediate signals for numerous extracellular enzymes, like neurotrophins, semaphorins, and Reelin. **[110].** Tobe et al (2017) **[111],** using human-induced pluripotent stem cells from patients with bipolar disorder responsive to lithium, found that lithium alters the phosphorylation state of collapsin response mediator protein-2 (CRMP2). Especially, lithium lowers phosphorylated CRMP, which results in increasing spine area and density. Lithium therapy seems to normalize the ratios and spines of bipolar patients' brains, which have elevated ratios and diminished spine densities. The authors suggested that lithium in bipolar patients normalize the CRMP2's phosphorylation, which regulates cytoskeletal organization, particularly in spines, resulting in neural networks modulation. Recently, Ferensztajn-Rochowiak et al (2017) **[112],** investigated the effect of long-term lithium treatment on very-small embryonic-like stem cells and the mRNA expression

Table 1. A broad range of lithium inhibitory effects and readjusting balances

Signaling pathways
lithium inhibits more than ten cellular targets and displacing magnesium ions
inhibits enzymes which have essential cofactor cations, such as Ca2+, Mg2+, Na+, K+, and Zn2+ by displacement of these cations from the enzymes **[17]**
inhibits many magnesium–dependent phosphomonoesterases
competes for a magnesium binding site in glycogen synthase kinase-3, inositol polyphosphate 1-phosphatase, fructose 1,6-bisphosphatase, bisphosphate nucleotidase, and phosphoglucomutase **[18].**
modulates cell function via inhibitory effects on adenosine triphosphatase activity, cyclic adenosine monophosphate, and intracellular enzymes **[19]**
inhibitory effect on the G proteins, inhibitory effect on parts of various signaling pathways, such as cyclic GMP and cyclic AMP formation [20].
by inhibiting inositol monophosphatase, lithium obstructs the enzymatic degradation of inositol trisphosphate (IP3), resulting in reduced availability of the second messengers IP3 and DAG, the derivatives of the PIP cycle.
decreases the turnover of arachidonicacid (AA) in several brain phospholipids, which is correlated with a significantly decrease of phospholipase A2 (PLA2) activity [23, 24].
inhibitor of the enzyme glycogen synthase kinase-3
reduces TGFBIp expression and blocks indirectly the GSK-3 activity through the phosphorylation of GSK-3α at ser21 and of GSK-3β at ser9 [**59**]
Thyroid system
anti-thyroid effects, inhibition of thyroid hormone release
inhibits thyroidal iodine uptake and iodotyrosine coupling, alters thyroglobulin structure, and inhibits thyroid hormone secretion.
at the peripheral level, lithium decreases deiodination of tetraiodothyronine (T4) or thyroxine, by decreasing the activity of type I 5′ de-iodinase enzyme **[31]**
rearrange and normalize thyroid hormone secretion in the long-term therapy, acting possibly through an adaptive thyroid system resetting, which may result in a correction of an isolated CNS hypothyroidism **[35].**
Cancer cell
reverts apoptosis models, inhibition of the GSK-3 has revealed anti-apoptotic effects **[46]**
reduced overall cancer risk in lithium treated patients with bipolar **[113]**
suppress leukopenia in patients undergoing radiotherapy or chemotherapy **[61]**
reduce melanoma risk **[66]**
reduce ovarian cancer cell metabolism **[69]**
decrease the proliferative potential of colorectal [**72**]

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Neurotransmission

inhibition of the countertransport mechanism (interfere with transporting systems for sodium and potassium in the plasma membranes of neurons)

induced inhibition of inositol monophosphatase may lead to Ins1P accumulation and inositol depletion **[20]**

lithium antidepressant action may be linked with reduced phosphoinositide cycle-coupled 5-HT_2 receptor function **[80**

reduces 5-HT_2 receptor function **[81]**

inhibits the glycogen synthase kinase-3 (GSK3) β isoenzyme, which in turn act as a mediator of serotonergic function **[92].**

Impulsive behavior

mood stabilizing, prophylaxis of bipolar disorders and treatment of acute mania **[8]**

reduces the risk of suicide in people with mood disorders and superior antisuicidal effects, in relation to other mood stabilizers **[93-94]**

decreases impulsivity levels in bipolar patients and in other impulse control disorders **[85-89]**

low lithium intakes reduce suicide, as well as homicide rate **[99-105, 116]**

Readjusting balances

neuroprotective effect: neurological (epilepsy, Huntington chorea, Parkinson diseases, and headaches)

also, endocrinological effects (hyperthyreosis, diabetes mellitus, and the inappropriate secretion of the antidiuretic hormone), haematological (neutropenia, and trombocytopenia), alergological (asthma), **[11, 12],** in oncology (thyroid carcinoma) **[13],** infectious diseases (AIDS related dementia) **[14],** and dermatology, in seborrhoeic dermatitis, with topically application

"unlike other pharmaceuticals that are far more specific and inhibit or activate one gene or a small number of genes, the model for lithium action is that it alters the balance between a large number of interacting processes and pathways"**[115]**

of pluripotency and glial markers, in peripheral blood, in 15 patients with bipolar disorder not treated with lithium, in comparison with 15 patients with bipolar disorder treated with lithium and 15 controls. They found that long-term treatment with lithium may reduce the activation of regenerative processes of bipolar patients, by reducing the number of VSELs circulating.

Recent epidemiological findings strongly support the benefits of lithium use in both psychiatry and oncology. Martinsson et al (2016) **[113]** investigated the cancer risk in 5,442 lithium treated patients with bipolar, in comparison with the general population. The overall cancer risk in lithium treated bipolar patients was similar with the general population. In addition, the cancer risk in the digestive organs, in the intrathoracic organs, and in the endocrine glands, was significantly increased in not lithium treated bipolar patients in comparison with the lithium treated bipolar patients and the general population. Similar results were found in a retrospective study by Huang et al (2016) **[114],** who investigated the association between lithium and cancer risk in patients with bipolar disorder. They found a reduced overall cancer risk in lithium treated patients with bipolar disorder, while a dose-response relationship for cancer risk reduction was observed.

Recently, Ge & Jakobsson (2018) **[115]** conducted a pathway and network analysis exploring the role of lithium in multiple cancers. The results show that for the large majority of such cancers, there is high mutual enrichment between the interactomes of lithium-sensitive enzymes and the pathways associated with those diseases, indicating that lithium is very likely to affect the incidence and course of the disease. Three genes stand out as being not strongly connected to cancer pathways: BPNT1, DISC1, and PGM1. Of the cancer pathways, breast cancer stands out as being not likely to be strongly influenced by lithium levels. For the remainder of the genes and the remainder of the cancers, the relationship between the lithium-sensitive interactome and the cancer phenome is strong. Ge & Jakobsson **[115]** pointed that unlike other pharmaceuticals that are far more specific and inhibit or activate one gene or a small number of genes, the model for lithium action is that it alters the balance between a large number of interacting processes and pathways. Thus, a dose-response curve for lithium is likely to be highly nonlinear and not always monotonic. In the light of all these factors, the authors suggested that the correct question to ask with respect to lithium and a particular disease is not, "Should lithium be administered for this particular disease?" but rather, "What is the optimum blood level of lithium for this individual, given his or her disease history, status, genetic propensities, and other medications?" Table 1 summarizes

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the broad range of lithium inhibitory effects, from the cell to the behavioral level, and its therapeutic implications.

In conclusion, lithium treatment has been associated with many neurochemical and structural evidence of neuroprotection, like the synthesis of brain-derived neurotrophic factor, the increased expression of anti-apoptotic genes, and the inhibition of cellular oxidative stress, which results in cortical thickening, increased grey matter density, and hippocampal enlargement. Unlike other ions, lithium is not regulated by selective membrane transport processes. Unlike other pharmaceuticals, lithium is an essential nutrient and is wildly nonselective in its biochemical effects. The question with lithium is not whether it should be ingested or not, but rather how much. Extreme lithium deprivation results in failure to thrive, while too much lithium is toxic. From the origin of life, lithium was in the environment, interacting with all of the biological molecules that life created. Lithium demonstrates a broad range of inhibitory effects from the cell to the behavioral level. We have been suggested that even natural lithium level intake can influence *impulsivity*, a possible core factor that mediate to the manifestation of both suicidality and aggressiveness, or even criminality. Moreover, a lithium deficiency state may precipitate these situations **[107, 108, 116].** Also, other mechanisms acting in parallel, like the initial lithium induced hypothyroidism may help to rearrange and normalize thyroid hormone secretion in the long-term therapy, acting possibly through an adaptive thyroid system resetting, which may results in a correction of an isolated CNS hypothyroidism **[35].**

What we know about the effects of lithium is likely only a small fraction of what there is to know. The bulk of evidence suggests that the optimum level of lithium intake from food and drinking water is more than most people get. Weighing the benefits and the potential risks, we can pose the question of whether the prospect of adding lithium to drinking water is realistic. In general, lithium seems to operate like a universal inhibitor, helping the organism to readjust balances, through the development of new compensatory mechanisms. Biological evolution had to accommodate to the presence of lithium to survive, since, those entities that best minimized lithium toxicity and maximized the benefits of lithium action had an edge in the competition to survive and reproduce **[107].**

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