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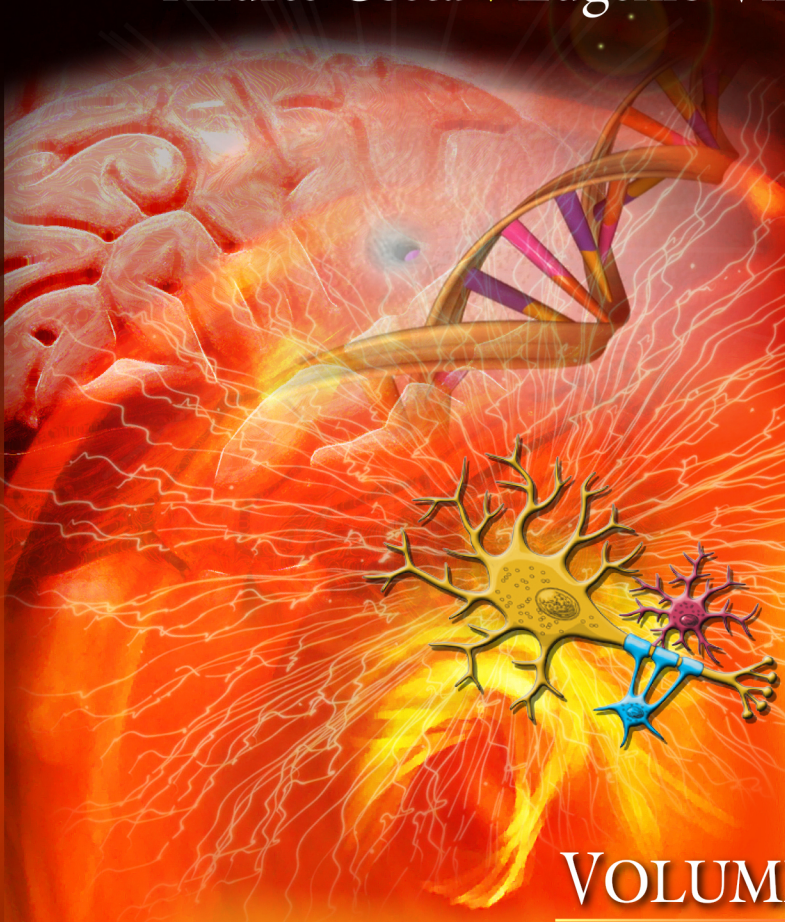

VOLUME 41

HORIZONS IN NEUROSCIENCE RESEARCH

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VOLUME 41

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CONTENTS

PREFACE

This compilation opens with a review of the clinical and preclinical studies that reproduce type 1 diabetes, prediabetes and type 2 diabetes, along with an analysis of the complications at the central level, as well as cognitive impairment and its relation with dementia.

Continuing, the authors review the typical neurocognitive deficits associated with alcohol exposure in pregnancy; the relationship between fetal alcohol spectrum disorder and attention-deficit hyperactivity disorder and autism spectrum disorder; and the combined effects of alcohol and drug abuse.

In addition, recent literature summarizing the main roles of sirtuin 2 in the central nervous system is addressed in an attempt to understand the relationship between sirtuin 2, senescence and neurodegenerative diseases.

The relationship between biological motion, emotions, and theory of mind in people with neurodevelopmental disorders is examined. Specifically, the authors use bottom-up and top-down investigations to systematically uncover behavioral and neurological patterns of biological motion perception in people with Williams syndrome, autism spectrum disorder, and Down syndrome.

One study deals with the definition of consciousness, the description of the neural substrates that have been associated with it, and the examination of the main interpretative models.

In closing, theory of mind as an implication of empowerment is explored using empirical data on social competence development among children.

Chapter 1 - Diabetes mellitus (DM) is a public health concern that affects 415 million adults and the number is projected to reach over 640 million by 2040. There are also over 47 million people living with dementia and this number is expected to affect 110 million people by 2050. The most common types of dementia are Alzheimer's disease (AD) and vascular dementia (VaD). The underlying mechanisms are not completely understood and borderlines between AD and VaD are blurry in many cases. Although age remains the main risk factor to suffer dementia, other relevant factors including metabolic diseases, and DM specifically, may predispose to suffer dementia. In this sense, DM is a chronic metabolic disease that affects peripheral organs as well as the brain.

The most common forms of DM include type 1 diabetes (T1D) and type 2 diabetes (T2D), which is largely preceded by prediabetes. T1D is characterized by β-pancreatic cell destruction, usually leading to absolute insulin deficiency and hyperglycemia. On the other hand T2D is due to a progressive insulin secretory defect associated to insulin resistance that results in increased glucose levels. The prediabetes stage precedes T2D and it is characterized by increased insulin levels that limit hyperglicemia until the balance is broken and T2D debuts. The close relation between DM and dementia has led to many studies in patients and animal models. In this chapter the authors will focus both on clinical and preclinical studies that reproduce T1D, prediabetes and T2D and the authors will analyze the complications at central level, as well as cognitive impairment and its relation with dementia.

Chapter 2 - Prenatal exposure to alcohol can create a spectrum of neurocognitive impairments in developing children. The presentation of FASD can vary considerably in severity from mild, to severe cognitive, behavioral, emotional and physical abnormalities. In this review chapter the authors will focus on neurocognitive deficits frequently present in children with FASD, and the clinical implications in relation to these deficits. The prognosis is better for affected children and young people who receive early diagnosis and appropriate support, than in those for whom support is not

provided. It is, therefore of central importance to increase knowledge and understanding of this preventable neurodevelopmental disorder and how best to manage and support children and families affected.

In this chapter, the authors will focus on reviewing the following issues:

The typical neurocognitive deficits associated with alcohol exposure in pregnancy;

The relationship between FASD and Attention-Deficit Hyperactivity Disorder (ADHD), and FASD and Autism Spectrum Disorder (ASD);

The combined effects of alcohol and drug abuse

Chapter 3 - Epigenetic changes play an important role in the ageing process and have been implicated in many age-related diseases. Sirtuins, which are nicotinamide adenine dinucleotide $(NAD)^+$-dependent class III histone deacetylases, have emerged as master regulators of metabolism and longevity. Among all sirtuins, Sirtuin 2 (SIRT2) is the most expressed in the central nervous system (CNS). It has been involved in a variety of biological processes including gene transcription, apoptosis, ageing, autophagy and inflammation. However, different groups have provided seemly contradictory results, thus, its specific functions remain unknown.

It has been described an age-dependent accumulation of SIRT2 in the brain. Moreover, its pharmacological inhibition shows neuroprotective effects in different models of Huntington, Parkinson and Alzheimer´s diseases suggesting its potential as a therapeutic target for age-related diseases. Thus, due to its possible implication in the etiology or development of neurodegenerative diseases, the elucidation of its functions in the CNS is crucial for understanding the molecular basis of these diseases and fundamental for the advancement of new therapeutic strategies.

In this context, the aim of the present chapter is to review recent literature, summarize the main roles of SIRT2 in the CNS and try to understand the relationship between SIRT2, senescence and neurodegenerative diseases.

Chapter 4 - Biological motion perception is the unique ability to perceive movement of the human body. This perception requires attention to global configurations and is an essential ability in relation to theory of mind and social cognition. Weak central coherence of global-ignoring and

local-focusing styles in visuospatial perception is characteristic of neurodevelopmental disorders. This weakness causes deficits in face processing and biological motion perception, which in turn influences development of the theory of mind, or emotional intelligence. Emotion is an essential component of social cognition. This chapter examines the relationship between biological motion, emotions, and theory of mind in people with neurodevelopmental disorders. Specifically, this chapter uses bottom-up and top-down investigations to systematically uncover behavioral and neurological patterns of biological motion perception (with and without emotions) in people with Williams syndrome, autism spectrum disorder, and Down syndrome. The results show that neurodevelopmental disorders cause changes to distinct genotypes in the early stage of life, and these changes have devastating effects on later development of phenotypes.

Chapter 5 - Among all the cognitive abilities of the human brain, the one that has most deeply interested neuroscientists is *consciousness*, which at its simplest refers to *"sentience or awareness of internal or external existence."* Several theories have been proposed to explain this phenomenon. Stuss, Picton, and Alexander (2001) and Stuss and Anderson (2004) argued that there are different types of consciousness, hierarchically organized, which need to be differentiated. The different types of consciousness are associated with distinct neural substrates, which remain the subject of intense investigation. Someone suggested that it could be a "real function" localized in a precise region of the brain, which would deal precisely with collecting and synthesizing stimuli deriving from other areas. For others, it would depend on the synchronization between sensory and mnemonic areas: critical groups of neurons, in different areas of the brain, would discharge simultaneously, thus giving rise to that integration of stimuli which is consciousness. Some neurobiological models of consciousness assume that the contents of consciousness are widely distributed in the brain.

In "The Astonishing Hypothesis" (1994), Francis Crick identifies the anterior cingulate, as a likely candidate for the center of free will in humans. The anterior cingulate cortex acts as an important interface between emotion and cognition, and more specifically in the conversion of feelings into intentions and actions. It has been implicated in 1) emotion, motivation, and

attention; 2) facial self-recognition, interceptive and emotional awareness; 3) integration of conscious experience; 4) error detection, conflict-monitoring, and self-related information monitoring. Given the above, the ACC would play an important role in both "core" and introspective self-awareness. Damasio and Mayer (2008) has previously suggested that "core consciousness" occurs when an organism becomes consciously aware of feelings associated with changes occurring to its internal bodily state; it is able to recognize that its thoughts are his own, and that they are formulated in its own perspective.

Modern neuroscience suggests that the brain's intrinsic activity may be an important process underlying consciousness. The Salience Network (SN) is an intrinsically connected large-scale network anchored in the anterior insula and dorsal anterior cingulate cortex. Together with its interconnected resting state networks, it contributes to a variety of complex brain functions. The SN has been implicated in modulating the switch between the externally directed cognition of the Central Executive Network (CES) and the internally directed cognition of the Default Mode Network (DMN). Moreover, the SN has been implicated in the detection and integration of emotional and sensory stimuli, coming for this considered responsible for self-awareness.

The chapter will deal with the definition of consciousness, the description of the neural substrates that have been associated with it, and the examination of the main interpretative models. Particular attention will be given to the role played by the cingulate cortex as a hub in functional networks involved in the emergence of consciousness.

Chapter 6 - A Theory of Mind (ToM) reflects humankind's evolution as social beings. That is, ToM implies potential energy, motivation, and empowerment. Empowerment is something that gives people hopes and dreams, brings them courage, and prompts them to be filled with the strength to live. Human beings are born with splendid abilities, and throughout their lives, they can continue to demonstrate magnificent strengths. Empowerment draws out that magnificent power and allows the vital force and potential that lie hidden deep within us to flow.

This chapter explores theory of mind as an implication of empowerment, using empirical data on social competence development among children.

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Chapter 1

COGNITIVE ALTERATIONS IN PRECLINICAL MODELS OF DIABETES MELLITUS

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ABSTRACT

Diabetes mellitus (DM) is a public health concern that affects 415 million adults and the number is projected to reach over 640 million by 2040. There are also over 47 million people living with dementia and this number is expected to affect 110 million people by 2050. The most common types of dementia are Alzheimer's disease (AD) and vascular dementia (VaD). The underlying mechanisms are not completely understood and borderlines between AD and VaD are blurry in many cases. Although age remains the main risk factor to suffer dementia, other relevant factors including metabolic diseases, and DM specifically, may predispose to suffer dementia. In this sense, DM is a chronic metabolic disease that affects peripheral organs as well as the brain.

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The most common forms of DM include type 1 diabetes (T1D) and type 2 diabetes (T2D), which is largely preceded by prediabetes. T1D is characterized by β-pancreatic cell destruction, usually leading to absolute insulin deficiency and hyperglycemia. On the other hand T2D is due to a progressive insulin secretory defect associated to insulin resistance that results in increased glucose levels. The prediabetes stage precedes T2D and it is characterized by increased insulin levels that limit hyperglicemia until the balance is broken and T2D debuts. The close relation between DM and dementia has led to many studies in patients and animal models. In this chapter we will focus both on clinical and preclinical studies that reproduce T1D, prediabetes and T2D and we will analyze the complications at central level, as well as cognitive impairment and its relation with dementia.

Keywords: type 1 diabetes (T1D), type 2 diabetes (T2D), prediabetes, Alzheimer´s disease (AD), vascular dementia (VaD)

1. INTRODUCTION

1.1. Diabetes Mellitus: General Considerations

Metabolic diseases drastically affect the life of men and women from childhood to old age in many different ways and they are a great challenge for health professionals as well as a major social issue. According to a report by World Health Organization, 108 million adults worldwide suffered diabetes mellitus (DM) in 1980, reaching 422 million in 2014. The prevalence (normalized by age) of diabetes has almost doubled since then, affecting 8.5% of the adult population. Interestingly, diabetes prevalence is increasing, particularly in developing countries (Morales et al., 2001). It also needs to be taken into consideration that metabolic disease and diabetes specifically, are affected by gender differences that determine detection, diagnosis and treatment strategies, as well as the development of complications and mortality rates. Men seem to have a higher risk of diabetes at a younger age; however, women have a dramatic increase in the risk of vascular complications associated with diabetes afterwards (Kautzky-Willer et al., 2019). DM is characterized by persistent increase in blood glucose levels for a long period of time (Laddha and Kulkarni, 2019). Insulin

mediates the clearance of glucose from blood, by activating glucose transport into the cytosol, however the absolute or relative lack of insulin and/or impaired insulin actions at its receptor significantly impairs the metabolism of circulating glucose (Ashcroft and Rorsman, 2004; Butler et al., 2003; Clark et al., 2001). The persistent increase in blood glucose is associated with frequent urination (polyuria), increased thirst (polydipsia), hunger and weight loss or related polyphagia, diabetic ketoacidosis and hyperosmolar coma (Baynes, 2015; Kitabchi et al., 2009). Moreover, DM and its associated complications are one of the leading causes of mortality worldwide. Severe hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome (American_Diabetes_Association, 2014) are some of the most severe complications associated to diabetes, which could cause the death of DM patients (Qiu et al., 2017).

Following the American Diabetes Association criteria categories (American_Diabetes_Association, 2020), diabetes can be classified into the following general categories:

- **Type 1 diabetes (T1D).** It is also known as "insulin-dependent diabetes mellitus" or "juvenile diabetes". It is related to autoimmune β-cell destruction, usually leading to absolute insulin efficiency and it is prone to ketoacidosis. This form of diabetes also includes those cases in which the etiology of β-cell destruction is unknown (American_Diabetes_Association, 2020; Goldenberg and Punthakee, 2013; Tao et al., 2015).
- **Type 2 diabetes (T2D).** This form of diabetes is also known as "non insulin-dependent diabetes mellitus" or "adult onset diabetes". T2D is provoked by a progressive loss of adequate β-cell insulin secretion, frequently on the background of insulin resistance. To compensate hyperglicemia, β-pancreatic cells respond by increasing insulin production leading to prediabetes. When β-pancreatic cells are exhausted and can no longer overproduce insulin, T2D evolves. Studies directly addressing the complications of prediabetes are limited, mostly because it is asymptomatic and largely under diagnosed in the general population. However T2D accounts for

about 90% of all the cases of diabetes and prediabetes patients are in high risk to develop T2D.

- **Gestational diabetes mellitus.** Gestational diabetes refers to glucose intolerance with onset or first recognition during pregnancy (Punthakee et al., 2018). This type of diabetes is diagnosed in the second or third trimester of pregnancy in mothers that had no overt diabetes prior to gestation (American_Diabetes_Association, 2020).
- **Diabetes due to other causes.** These types of diabetes include a wide variety of relatively uncommon conditions, including genetically defined forms of diabetes (such as neonatal diabetes and maturity-onset diabetes of the young), diabetes associated with other diseases (such as cystic fibrosis) (Moran et al., 2018), drug- or chemical-induced diabetes (associated to glucocorticoids, in the treatment of HIV/AIDS) (Eckhardt et al., 2012), or diabetes associated to organ transplantation (American_Diabetes_ Association, 2020; Shivaswamy et al., 2016).

Whereas DM classification remains an important issue to determine the best therapeutic approach, some individuals cannot be clearly classified as T1D or T2D patients at the time of diagnosis (American_Diabetes_ Association, 2020). T1D and T2D are heterogeneous diseases in which clinical presentation and disease progression may vary considerably. In fact, classical assumption that T2D only occurs in adults and T1D exclusively affects children, is no longer accepted and it is possible that T1D and T2D occur in both age-groups. Since the prevalence of T2D increases with age, the progressive aging of population seems to indicate that the "diabetes epidemic" will continue (Wild et al., 2004). In line with this idea, DM could be prevented to a certain extent. Breast feeding to babies has been shown to reduce the risk of youth onset of T2D (Taylor et al., 2005). However, since the primary cause of T2D is excessive body weight and not enough physical activity (American_Diabetes_Association, 2020; Tao et al., 2015), the World Health Organization has recommended global physical activity and healthy nutrition for children to prevent diabetes (Willumsen and Bull, 2020). Also, pharmacological approaches, like orlistat, approved for the

treatment of overweight and obese adolescents, might reduce the risk to develop diabetes (Rogovik et al., 2010).

1.1.1. Peripheral Complications Associated to Diabetes Mellitus

DM causes relatively specific long-term microvascular complications affecting the retina, kidneys and nerves that may end up provoking blindness, nephropathy and neuropathies respectively. Vascular damage can also cause foot ulcers (Noor et al., 2015) and affect gastrointestinal motility (Kishi et al., 2019). Additionally, DM increases the risk for cardiovascular disease and stroke (Goldenberg and Punthakee, 2013; Laddha and Kulkarni, 2019). The ultimate mechanisms for observed disturbances are not completely known, however, it has been shown that DM contributes to the progression of oxidative stress (Nasrallah et al., 2014), affects metabolic, genetic and hemodynamic systems by activation of polyol, protein kinase C (Sharma et al., 2017) or hexosamine pathways and increases advanced glycation end products formation (Mima, 2013). As previously stated, peripheral micro and macrovascular complications associated with T2D, including neuropathies, retinopathies or nephropathies, have been widely studied (Rosenson et al., 2011), and central complications associated with long-term metabolic alterations, have also received a great deal of attention (Craft, 2012). In this context, metabolic disease not only increases the incidence of atherosclerotic cardiovascular and peripheral arterial disease, but cerebrovascular compromise is also observed (American_Diabetes_ Association, 2014). Moreover, many recent studies have focused on the relation between diabetes and its role in neurodegeneration, dementia and Alzheimer´s disease (AD) (Batista et al., 2018; Holscher, 2018; Wakabayashi et al., 2019).

1.1.2. Brain Complications and Dementia Associated with Diabetes Mellitus: Role in Alzheimer´s Disease and Vascular Dementia

The central nervous system (CNS) is greatly affected in diabetes, as both cerebral glucose and insulin levels are frequently abnormal, even when the disease is well controlled (McCall and Figlewicz, 1997; Northam et al., 2009). Glucose is the primary energy source for cerebral metabolism, and,

given that the brain has a limited ability to store glucose or use alternative fuel sources, such as lactate, a continuous supply is essential (Amiel, 1997). Inadequate glucose availability (hypoglycemia) triggers a continuum of events, ranging from confusion through seizures, coma and ultimately death, unless appropriate action is taken (Northam et al., 2006). During a severe state of hypoglycemia intracellular calcium toxicity and excitotoxicity are observed. Acute hyperglycemia also disrupts blood-brain barrier and depresses cerebral blood flow, whereas chronic hyperglycemia is classically associated with cerebrovascular disease and neuropathy (McCall and Figlewicz, 1997). Neurotransmitter pathways may also be affected in diabetes, as insulin is involved in regulation of biogenic amines, including noradrenaline, dopamine and serotonin (McCall and Figlewicz, 1997). Diabetic ketoacidosis, as an acute, severe metabolic crisis, implies an increased risk of cerebral edema, which is thought to be precipitated by rapid changes in blood osmolarity (Carlotti et al., 2003). In line with these observations, besides the damaging effects of diabetes on different organs, cognitive alterations are also very common in diabetes patients, and include alterations in memory, executive functions, attention and academic performance (Allen et al., 2004; Biessels and Despa, 2018; Bober and Buyukgebiz, 2005; Gaudieri et al., 2008). Moreover, impairment at central level has also been detected in children with diabetes (Pourabbasi et al., 2016). At early ages, physical growth, social and emotional development are important and stimulated by formal education and schooling. Therefore, any health condition which might have an impact on cognitive function could, in turn, affect other areas of child development including cognitive, social and emotional behavior (Brown RT, 2004).

While the actual role of DM at central level, and the underlying mechanisms implicated are unclear, cognitive impairment is an important comorbidity of diabetes (Gudala et al., 2013; Koekkoek et al., 2015; Zhang et al., 2017). Among others, manifestation and prognosis of diabetes-associated cognitive dysfunction varies depending on the type of diabetes and the age of the patient (Biessels et al., 2008). In line with these observations, adult diabetes patients are at high risk to suffer dementia related disorders (Brismar et al., 2007; Northam et al., 2006; Ristow, 2004;

Selvarajah and Tesfaye, 2006; Sima, 2004). The relationship between diabetes and cognitive dysfunction was already proposed in 1922 (Miles and Root, 1922). Following this idea, different prospective population-based studies have reported comparable findings on the risk of mild cognitive impairment in patients with diabetes. A hazard ratio of 1.5-1.6% for mild cognitive impairment has been observed in diabetes patients (Roberts et al., 2014) and a hazard ratio of 1.2-1.4% for non-amnesic mild cognitive impairment (Luchsinger et al., 2007). In addition, the prognosis of mild cognitive impairment is worse in patients with diabetes than in patients without diabetes (Biessels and Despa, 2018). Moreover, two meta-analyses have reported a relative risk of conversion to dementia of 1.7% in diabetic patients with mild cognitive impairment, when compared with patients without diabetes (Li et al., 2016). Other studies have also reported similar outcomes (Biessels et al., 2006; Gudala et al., 2013; Zhang et al., 2017). Specifically, when the two most common causes of dementia, AD and vascular dementia (VaD) are analyzed, the relative risk for AD in diabetes patients increases 56%, and 127% for VaD, in comparison with non-diabetic patients (Gudala et al., 2013). Interestingly, diabetes is a risk factor to suffer AD, even after adjusting for vascular risk factors (Huang et al., 2014; Wang et al., 2012). However, recent studies have also pointed out that while the risk of AD and VaD is increased in association with diabetes, the cerebral burden of the AD pathology is not (Biessels and Despa, 2018).

AD is the most common type of dementia, causing among 50-70% of the cases (Alzheimer's_Association, 2016). The prevalence of AD reaches 10-30% in the population over 65 years old (Masters et al., 2015) and increases with age. It has been estimated that there are over 47 million people worldwide suffering dementia, and by 2050 there will be 131 million patients (Roehr et al., 2017). AD is not only a first rate health issue but also a great societal and economic burden. In addition, it should be borne in mind that AD is a sporadic disease in 95% of cases, with a multifactorial and elusive origin (Martin-Maestro et al., 2017), and therefore it is still a pathology of unknown cause in most patients. The main neuropathological characteristics of AD are: i) amyloid-β (Aβ) peptide accumulation, which can be deposited extracellularly forming senile plaques or around blood

vessels as cerebral amyloid angiopathy, ii) neurofibrillary tangles formation due to intraneuronal deposits of abnormal phosphorylated tau protein and iii) neuronal and synaptic loss, the best correlated characteristic with the duration and severity of the disease, especially in the last stages (Fulop et al., 2018; Serrano-Pozo et al., 2011). While these are the classical neuropathological features associated with AD, the concept of neurodegeneration has expanded in later years to include earlier alterations such as synaptic and dendritic lesions (Pozueta et al., 2013; Ziegler-Waldkirch and Meyer-Luehmann, 2018), as well as disorders in adult brain neurogenesis (Crews et al., 2010; Jin et al., 2004; Li et al., 2008) circuit dysfunction and aberrant innervation (Palop and Mucke, 2016), or chronic state of neuroinflammation (Nizami et al., 2019).

VaD is the second most common cause of dementia, responsible of approximately 15% of the cases (Kalaria, 2018). VaD is an heterogeneous pathology, that can range from multiple microinfarcts to small vessel ischemic disease or microvascular injury (Craft, 2009), all of which can be triggered by Aβ deposition as amyloid angiopathy in cerebral blood vessels (Greenberg et al., 2008). Although there is a well established relationship between vascular disease and degenerative Alzheimer's pathology, amyloid angiopathy can also occur independently of AD (O'Brien and Thomas, 2015), and it has been proposed that amyloid angiopathy contribution to cognitive decline might be independent of parenchymal amyloidosis (Boyle et al., 2015). Likewise, microglia/macrophages are found around vessels affected by amyloid pathology, which suggest extensive inflammation (Schrag and Kirshner, 2016). While it is possible to find a predictable pattern of disease progression in AD, there is no agreement on the pathological scheme for VaD (O'Brien and Thomas, 2015). Moreover, the borderlines between AD and VaD are blurred, and in many patients markers of vascular injury coexist with traditional AD hallmarks. In some cases AD features might be promoted by a specific form of vascular injury; on one hand blood brain barrier dysfunction may affect Aβ transport between brain and periphery, and thereby contribute to parenchymal and neurovascular Aβ deposition (Craft, 2009). On the other hand, AD and VaD pathology may cause vascular injury, as when Aβ-induced inflammation can lead to

endothelial dysfunction. The pathological consequences of vascular damage include also alteration of functional markers, such as increased reactive oxygen species or matrix-metalloproteinase activity (Garcia-Alloza et al., 2009), all of which have been related, to a different extent, to neuronal death (Brown et al., 2009; Kim et al., 2017; Zhang and Murphy, 2007).

Apart from clinical and epidemiological studies linking DM and dementia (Luchsinger et al., 2007; Luchsinger et al., 2004; Ott et al., 1996), some biochemical links between DM and AD and VaD have contributed to the association of metabolic disorders and central complications (for review (Baglietto-Vargas et al., 2016)) (Figure 1). Among others: i) insulin levels and insulin resistance are the parameters that best correlate with a higher risk to develop AD (Schrijvers et al., 2010). Insulin modulates many central and peripheral physiological processes and insulin resistance might relate to cognitive dysfunction and reduced glucose use at central level (Baker et al., 2011). CNS insulin receptors are highly expressed in the basal forebrain, and in regions relevant for learning and memory, such as cortex and hippocampus. This is consistent with evidence showing that insulin influences memory (Craft, 2009), likely due to modulation of synaptic structure and function, long-term potentiation and CNS levels of neurotransmitters such as acetylcholine, of special relevance in AD (Schliebs and Arendt, 2006). ii) DM progression correlates with pancreatic amylin deposition, in a similar way to Aβ deposition in AD brains. Moreover, insulin, amylin and Aβ are degraded by neprilysin and insulin degrading enzyme. It has been postulated that an imbalance of substrates can affect the degradation rates and possibly influence the pathogenesis of AD and diabetes (Gotz et al., 2009); iii) on one hand Aβ oligomers may affect insulin signalling in hippocampal neurons (Zhao et al., 2008). Also, central Aβ oligomers may trigger peripheral glucose intolerance (Clarke et al., 2015). On the other hand, insulin may also regulate Aβ levels by modulation of β and γ secretases (Eckman and Eckman, 2005; Farris et al., 2003). Accordingly, reduced brain insulin signalling increases tau phosphorylation and Aβ levels in mice (Jolivalt et al., 2008). iv) Extensive evidence supports Aβ toxicity in different states of aggregation (Kumar et al., 2016; Meyer-Luehmann et al., 2008) and it seems that amylin, similar to Aβ, can induce

apoptotic cell death (Konarkowska et al., 2006; Matveyenko and Butler, 2006). It is likely that amylin and Aβ aggregates alter cellular function by similar mechanisms, such as inducing reactive oxygen species (Craft, 2009) and inflammation. Albeit all the circumstantial links mentioned above, experimental data supporting a direct relationship between DM, EA and VaD are still limited, mostly because studying the mechanistic relationship of insulin resistance to AD and VaD is extremely complex and because there are no ideal animal models.

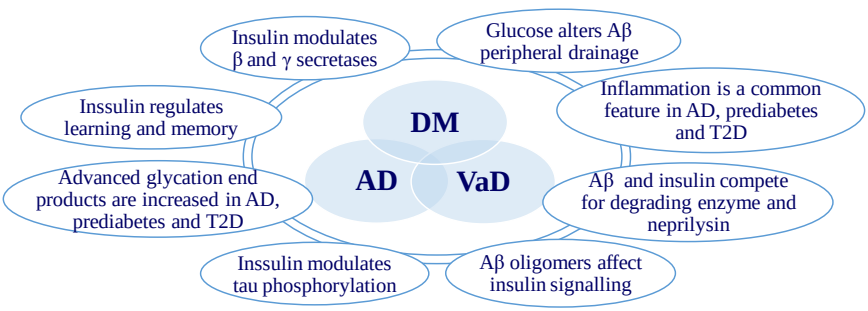

Figure 1. Possible links between T2D, AD and VaD.

2. Type 1 Diabetes Mellitus

2.1. Pathophysiology of Type 1 Diabetes Mellitus

T1D is one of the most common endocrine and metabolic conditions (Katsarou et al., 2017), also known as an autoimmune diabetes. It has been estimated that approximately 49.000 children worldwide are affected (Desai and Deshmukh, 2019). T1D imposes enormous public health costs (Herman et al., 2018; Scott et al., 2019; Shumway et al., 2019), profoundly affects individual quality of life (Gutefeldt et al., 2020; Henriquez-Tejo and Cartes-Velasquez, 2018; Wake et al., 2000) and it is associated with significant psychological morbidity (Northam et al., 2005; Sharif et al., 2018).

T1D is a chronic disease characterized by insulin deficiency due to pancreatic β-cells loss that results in hyperglycemia (Atkinson, 2012;

Houlden, 2018; Katsarou et al., 2017). The destruction of β-cells is usually the result of an autoimmune process (70-90%), which is concomitant with the formation of T1D-associated autoantibodies (Janez et al., 2020; Katsarou et al., 2017). However, in a smaller subset of patients, no immune response or autoantibodies are detected, and the cause of β-cell destruction is unknown (idiopathic T1D or type 1b diabetes) (Janez et al., 2020; Katsarou et al., 2017). In T1D the presence of autoantibodies occurs months or even years before symptoms onset. These islet-targeting autoantibodies may affect insulin, 65 KDa glutamatic acid decarboxylase, insulinoma-associated protein 2 or zinc transporter 8 (Ilonen et al., 2013; Krischer et al., 2015; Ziegler et al., 1999), all of which are proteins associated with secretory granules in β-cells. Therefore, these are biomarkers related to the autoimmune response of the disease (Katsarou et al., 2017) that can be used to identify and study individuals at risk of developing T1D (Katsarou et al., 2017). Pathogenesis of T1D is also thought to involve T cells-mediated destruction of β-cells within the pancreatic islets (Katsarou et al., 2017; Kent et al., 2017). Hybrid insulin peptides act as key antigens for autoreactive T cells and cause loss of self-tolerance in patients (Desai and Deshmukh, 2019). However, what triggers the first-appearing β-cell-targeting autoantibody is unclear (Katsarou et al., 2017). The etiology of β-cell-targeted auto-immunity is not completely understood, and it probably includes a combination of environmental and genetic factors that trigger or permit the autoimmune response against the β-cells (Katsarou et al., 2017). In this sense, many etiological factors may contribute to the observed increase in the incidence of T1D; it has been suggested that geographical location may determine both, genetic predisposition and environmental factors, although the ultimate cause remains elusive (Desai and Deshmukh, 2019; Pugliese, 2014).

The pathogenesis and evolution of T1D have been suggested to be a continuum that can be divided into three stages: detection of autoantibodies and progress to β-cells destruction, dysglycemia and finally hyperglicemia-associated symptoms (Insel et al., 2015). It has been classically assumed that T1D is mainly developed during childhood or adolescence (Atkinson, 2012), but it seems certain that T1D can develop at any time along lifespan, even

in individuals aged >80 years old (Thomas et al., 2018; Thunander et al., 2008). Most patients with T1D are adults, partly because children diagnosed with T1D survive to adulthood, but also because of new cases diagnosed in adults (Skyler et al., 2017). The identification of T1D may be challenging, and T1D may be misdiagnosed as T2D in adults aged >30 year (Atkinson, 2012; Thomas et al., 2018). Proper identification of T1D is extremely important because these patients need to receive immediate insulin therapy (Pourabbasi et al., 2016) and reach optimal glycemic control. Early control of T1D delays microvascular and macrovascular complications (Katsarou et al., 2017) and prevents the onset of diabetic ketoacidosis, which is associated with an increased risk of death. Although intensive glycemic control has reduced the incidence of microvascular and macrovascular complications, the majority of patients with T1D are still developing these complications. The use of insulin pumps and techniques to reduce injection pain have improved therapeutic success and compliance in children. Nevertheless, such approaches are not always available (Alsaleh et al., 2014). At present, T1D has no cure, and patients require frequent blood glucose monitoring along with insulin therapy (Aleppo and Webb, 2018). Major research efforts are needed to achieve early diagnosis, prevent β-cell loss and develop better treatment options to improve the quality of life and prognosis of those affected (Janez et al., 2020; Katsarou et al., 2017).

2.2. Brain Complications Associated with Type 1 Diabetes in Patients

There is a large body of literature documenting pathophysiological CNS changes and neurocognitive deficits in adults with T1D (Brands et al., 2005; Geissler et al., 2003; Makimattila et al., 2004; Musen et al., 2006; Wessels et al., 2006), associated to long-term severe hypoglycemia or chronic hyperglycemia. Among them, lower density of cortical gray matter and white matter lesions have been described (Bednarik et al., 2017; Pell et al., 2012; Sima, 2010; van Duinkerken et al., 2018). Following this idea, different brain mapping studies of neural structure, with magnetic resonance,

have demonstrated changes in the overall brain architecture of patients with T1D (Aye et al., 2011; Melberg et al., 1999; Pell et al., 2012). Studies that follow participants across childhood and into adulthood may be particularly informative in documenting the impact of T1D on brain development. The Royal Children´s Hospital, Melbourne Cohort Study recruited newly diagnosed T1D, together with a healthy control group, and 12 years later a subset of the cohort underwent neuroimaging with magnetic resonance imaging to document structural changes in the CNS. In T1D patients, they observed an age-related volume loss and T2 relaxation time changes in the thalamus and the lentiform nuclei (Pell et al., 2012). Using single photon emission computed tomography Tupola et al. (Tupola et al., 2004) observed a negative left-right index in cerebral blood flow, in a sample of prepubertal patients with diabetes, in contrast to the left-greater-than-right-hemispheric asymmetry, usually observed in healthy children, suggesting that the left hemisphere is preferentially affected in diabetes. In addition, early-onset severe hypoglycemia may have an effect on gray matter volume (Ho et al., 2008) and magnetic resonance imaging studies have reported some structural abnormalities in children, particularly mesial temporal sclerosis, suggesting hippocampal damage (Ho et al., 2008). Increased rates of cortical atrophy have been described in a number of reports (Ferguson et al., 2005; Frokjaer et al., 2013; Lobnig et al., 2006), including a study of young adults with relatively short disease duration (Lunetta et al., 1994). Similar atrophic changes in adults with T1D have been interpreted as a form of accelerated aging (Northam et al., 2006). Brain metabolite profile has also been examined using magnetic resonance spectroscopy in adults with T1D. Increased myo-inositol, which is a membrane constituent that reflects proliferation or activation of glia, and choline-containing compounds, which provide an insight into the metabolism of myelin and other phospholipids cell membranes (Burtscher and Holtås, 2001), were described (Kreis and Ross, 1992; Makimattila et al., 2004). The study carried out by Makimattila *et al*. showed that N-acetyl aspartate levels in T1D patients did not differ from control levels (Makimattila et al., 2004), although they were reduced in the study of Kries and Ross (Kreis and Ross, 1992), in which some of their patients were recovering from ketoacidosis.

2.3. Cognitive Impairment in Patients with Type 1 Diabetes

The association between DM and cognitive alterations has been largely reported at different levels. The evaluation of child development and cognitive changes has been the subject of numerous studies, some of which have reported correlations between diabetes and cognitive function. However, the pattern of deficits varies across studies and patient subgroups (Desrocher and Rovet, 2004).

The number of neuroimaging studies of youth to date is limited and understanding the impact of T1D on neurodevelopment is still based largely on inferences drawn from neurocognitive studies and from adult neuroimaging reports (Northam et al., 2009). Children with T1D can display subtle changes in cognitive development, particularly if the onset of diabetes occurs before 7 years of age (Ryan et al., 2016). Gaudieri *et al.* designed a meta-analysis to evaluate diabetes and cognition in children and they detected that different domains of cognitive function are affected by diabetes. They also reported that this correlation is more prominent in patients with early onset diabetes (Gaudieri et al., 2008). Other studies have corroborated more evident cognitive difficulties in children with early-onset disease (≤ 5 years old) (Northam et al., 2006; Ryan, 2006). In this sense, children with early-onset disease may show a possible disruption of language development, before skills consolidate, due to an unusual maturing of the CNS (Northam et al., 2006).

Children with diabetes may have lower levels of intelligence, compared with children without diabetes (Hannonen et al., 2003; Northam et al., 2009). In addition, Hershey et al. evaluated the association between hypoglycemic episodes and memory changes in children with diabetes and they showed a reduction in the spatial memory of children with T1D (Hershey et al., 2005). Ly et al. have also reported an impairment of executive function in children with diabetes in comparison with their healthy counterparts (Ly et al., 2011), and these changes in executive function might be independent of glycemic control in T1D children (Ohmann et al., 2010). Controlled, longitudinal studies are particularly informative in documenting illness-related changes in the CNS (Northam et al., 2009). The Diabetes Control and Complications

Trial (Nathan, 2014) showed no deterioration of the cognitive function in either conventionally or intensively treated patients over an 18-year period. However, this study did not enroll participants at diagnosis and recruitment was limited to those >13 years of age. Thus, the trial was unable to document any illness-related effects that may have occurred before the recruitment. Other studies support that there are no differences between children with diabetes and control children when IQ, specific neurocognitive skills or academic achievement are measured (Rovet and Ehrlich, 1999). However, years after diagnosis verbal IQ declines and academic achievement is limited (Rovet and Ehrlich, 1999). In general, the pattern of deficits observed in children is broadly similar to that seen in adults with diabetes (Brands et al., 2005), with the exception of language impairment, not described in adults. Altogether, the results of several studies have clearly demonstrated that diabetes is associated with cognitive malfunction in children. Language, attention, memory, information-processing speed and executive functions are the specific skills that are most affected in children with diabetes (Desrocher and Rovet, 2004). Children have high cerebral energy needs associated with brain growth and "neuronal purring" and may be more sensitive than adults to glucose fluctuations (AL McCall, 1997; Ryan, 2006). However, the basic mechanism through which diabetes affects academic performance is not yet known. One suggestion is that diabetes causes academic disadvantages in children through absenteeism, which consequently result in different cognitive and learning impairments (Glaab et al., 2005; Parent et al., 2009; Wodrich et al., 2011).

Epidemiological data on T1D and dementia are relatively spare (Biessels and Whitmer, 2020). This is because T1D is much less common than T2D and only recently individuals with T1D have been living to old age (Petrie et al., 2016). The largest study to date in T1D is a retrospective cohort study of individuals hospitalized for T1D (Smolina et al., 2015). This study examined the risk of dementia in over 300.000 people with T1D and a reference cohort. Those with T1D had a 65% increased risk of dementia. Also a meta-analysis of neurocognitive functioning in adults with T1D provides convincing evidence of subtle cerebral impairment (Brands et al., 2005). Compared with non-diabetic groups, adults with T1D had

significantly lower scores on general intelligence, speed of information processing, psychomotor efficiency, attention, mental flexibility and visual perception (Dejgaard et al., 1991; Ferguson et al., 2005; Lobnig et al., 2006; Perros et al., 1997). These findings are consistent with preliminary findings of CNS abnormalities described above. These decrements generally remain stable over time, with little changes relative to people without diabetes (Jacobson et al., 2007). However, there might be subgroups of patients, particularly those with advanced microvascular complications, in whom severity of cognitive dysfunction can worsen substantially over time (Nunley et al., 2015; Ryan et al., 2016). On the other hand, other researchers have pointed out that diabetes can induce cognitive impairment due to an affectation of neuronal structures (Aye et al., 2011; Bober and Buyukgebiz, 2005; Pell et al., 2012). It seems that an earlier age of onset and longer duration are risk factors for worse cognitive performance in T1D population (Li et al., 2017). Meanwhile, it remains unclear whether individuals with older age of onset of T1D exhibit the same pattern of impairment seen in the typical childhood-onset population of T1D (Biessels and Whitmer, 2020).

2.4. Brain Complications in Type 1 Diabetes Animal Models

Different animal models have been developed to reproduce T1D. Among others, spontaneous models of T1D such as the non-obese diabetic (NOD) mouse (Saravia et al., 2001) or BB/W rats (Li et al., 2002), as well as pharmacological approaches such as streptozotocin- (STZ) (Dheen et al., 1994) or alloxan-induced models (Federiuk et al., 2004). These animal models present brain complications, including increased expression of hypothalamic hormones (Dheen et al., 1994; Saravia et al., 2001), intimately linked to a marked hyperactivity of the hypothalamic-pituitary-adrenal axis (Chan et al., 2003; Zelena et al., 2006) and higher susceptibility to stress (Magarinos and McEwen, 2000; Sharif et al., 2018). High-circulating glucocorticoid levels are also associated with diabetes in STZ-induced diabetes mice (Revsin et al., 2008) and rats (Stranahan et al., 2008), showing that elevated glucocorticoids contribute to the impairment of synaptic

plasticity and neurogenesis, with associated learning and memory deficits (Stranahan et al., 2008). Therefore, the hippocampus, a major limbic structure rich in gluococorticoid receptors and very sensitive to stress, seems to be strongly affected by diabetes (Eichenbaum, 2000; Kim and Diamond, 2002; Knierim, 2015; Lupien and Lepage, 2001).

Previous studies have described hippocampal astrogliosis, low proliferation rates in the dentate gyrus, poor neurogenesis and reduced number of hilar neurons in two models of T1D; NOD mice and the STZ-induced model (Beauquis et al., 2006; Revsin et al., 2005; Saravia et al., 2004; Saravia et al., 2002). Nonetheless, some changes on the brain are shared by the encephalopathy associated to aging (Saravia et al., 2007), suggesting that the diabetic brain could be considered as an aged brain. On the other hand, some studies have focused on the relationship between T1D and AD. Ramos-Rodriguez et al. showed in APP/PS-STZ diabetic mice that there was a shift in Aβ soluble/insoluble levels, and more toxic soluble species were increased, while senile plaques deposition was reduced (Ramos-Rodriguez et al., 2016). Previous studies have also reported that genetic ablation of tau mitigates cognitive impairment induced by T1D (Abbondante et al., 2014) and an increment on hyperphosphorylated tau and spontaneous bleeding is also associated with spatial cognitive dysfunction in AD mice with induced T1D (Ramos-Rodriguez et al., 2016), as a feasible link between T1D and AD.

2.5. Cognitive Impairment in Experimental Models of Type 1 Diabetes

On top of the alterations observed in the CNS, experimental diabetes is also linked to cognitive impairment and changes in behavior (Amorim et al., 2017; Wang et al., 2018). Although cognitive deficits might be connected, at least in part, to neurotoxic effects of hyperglycemia and changes in neurotransmission and neuronal functionality, the relationship between neuronal alterations and their correlation with behavior has not been fully deciphered yet. Previous studies have shown that the reduction of

hippocampal neurogenesis is a common feature to aging, stress-induced depression and diabetes (Beauquis et al., 2008; Nam et al., 2019; Vignisse et al., 2017), that may cause cognitive impairment. In fact, Alvarez et al. (Alvarez et al., 2009) have shown a correlation between the diabetic status in STZ-induced T1D, and hippocampal neurogenic alterations, accompanied by behavioral alterations. Also, Li et al. have reported in BB/W rats that hippocampal neuronal apoptosis and low expression of IGF-I are accompanied by functional cognitive impairment in the Morris water maze (Li et al., 2002).

On the other hand, classical markers of AD pathology have also been assessed in T1D models, in connection with cognitive alterations. Following this idea, cognitive decline observed in T1D could be caused by a tau-dependent mechanism. According to this, Abbondante S. et al. (Abbondante et al., 2014) showed that tau is a key molecular factor responsible for the induction of cognitive deficits observed in T1D. In line with these observations Ramos-Rodriguez et al. also showed that by inducing T1D in an AD mouse model, phospho-tau levels are increased and episodic and working memory are significantly worsened (Ramos-Rodriguez et al., 2016).

3. TYPE 2 DIABETES MELLITUS

3.1. Pathophysiology of Type 2 Diabetes Mellitus

T2D is the most prevalent form of diabetes and it preferentially affects middle-aged and elderly people. It accounts for up to 90% of all cases of diabetes. Its prevalence reaches 400 million people all over the world and by 2035 there will be 592 million patients (Xia et al., 2017). Sedentary life style and obesity are well-established risk factors (Ristow, 2004) and in western countries, with increasingly older populations, the prevalence of T2D is raising, indicating that the diabetes epidemic will continue (Zhou et al., 2016). Altogether, the etiology of T2D is multifactorial (Lau et al., 2015), so the interaction between genetic mutations, lifestyle and

environmental factors determines the likelihood of the disease (Lau et al., 2015; Zhao et al., 2015).

Clinically, T2D is a process that extends along several stages (Ripsin et al., 2009). Insulin resistance develops in early stages of the disease, due to a decreased ability of the cells to properly capture insulin, which predisposes to hyperglycemia (Kahn et al., 2014). Given the inability of the individual to use insulin, β-pancreatic cells increase the synthesis and secretion of the hormone, resulting in compensatory hyperinsulinemia. Thanks to the increase in insulin, normoglycemia is maintained in the peripheral tissues, at early stages of the disease. To account for these early alterations, prediabetes is a practical and convenient term that refers to impaired fasting glucose, impaired glucose tolerance or glycated hemoglobin, each of which places individuals at high risk of developing diabetes and its complications (Goldenberg and Punthakee, 2013). Prediabetes is a metabolic state that lies between glucose homeostasis and T2D (Pal et al., 2018). While not all individuals with prediabetes will necessarily progress to diabetes, the prevalence of prediabetes in adult population is rapidly rising, estimated as 35% in the UK and USA and up to 50% in China (Pal et al., 2018), significantly increasing the population at risk of developing over T2D.

As diabetes progresses, metabolic balance is broken when β-pancreatic cells get exhausted, are depleted and die (Gerich, 1998; Lawlor et al., 2017). Damage of β-cells leads to the secretion of specific markers like proinsulin (Lee et al., 2011) along with amyloid fibrils (Jurgens et al., 2011), as pathological markers of pancreatic destruction. Amyloid fibrils are toxic to islet cells, leading to β-cell apoptosis (Pandey et al., 2015). The decrease in β-pancreatic cells continues as the disease progresses (Rahier et al., 2008), and ultimately T2D manifests with uncontrolled increases in blood glucose levels (Kahn et al., 2014; Lawlor et al., 2017), frequently requiring exogenously administered insulin at an advanced stage.

T2D is an important cause of morbi-mortality and causes great costs to the health system. According to data from the American Diabetes Association, the total levels of diagnosed diabetes amounted to 327 billion dollars in 2017, so there is an urgent need to intervene in lifestyle and at pharmacological level, to prevent or delay T2D progression (Zhou et al.,

2016). Likewise, it is a must to improve its early detection and a large body of data produced by "-omics" technologies, such as genomics/epigenomics, transcriptomics, proteomics and metabolomics, suggest that many potential biomarkers might be helpful in the prediction and early diagnosis of T2D (Kussmann et al., 2013). At present, commonly used biomarkers for diagnosis and monitoring of T2D are glycosylated hemoglobin, fasting plasma glucose levels and the oral glucose tolerance test (Ozery-Flato et al., 2013; Vijayakumar et al., 2017). Also, the T2D risk stratification could use cardiovascular biomarkers, such as high sensitivity troponin and B-natriuretic peptide, that play an important role in the pathophysiological process (Scirica, 2017).

T2D is related to various peripheral alterations such as hypertension, obesity, arteriosclerosis, dyslipidemia or hypercholesterolemia, also leading to an increased risk of cardiovascular disease (Ahmed and Goldstein, 2006a; Ahmed and Goldstein, 2006b). In the same way, prospective and epidemiological studies have reported that prediabetes and T2D are associated with CNS complications and increased risk of dementia (Chatterjee and Mudher, 2018; Chen et al., 2015; Cui et al., 2014; Karvani et al., 2019).

3.2. Brain Complications Associated with Type 2 Diabetes in Patients

As previously described, it is plausible that typical neurodegenerative disorders and T2D share common genetic and/or biochemical features (Ristow, 2004). The brain is actively involved in systemic glucose regulation. In fact there are neurons in the CNS which respond to high or low levels of glucose, and they are especially relevant in several nuclei of the hypothalamus and the brainstem (Lopez-Gambero et al., 2019). On the other hand, chronic hyperglycemia decreases the elasticity of smooth muscle cells, reducing the ability of blood vessels to maintain sufficient supply of blood and nutrients to brain tissue (Brownlee, 2005; Ergul, 2011). As a consequence, patients with T2D present subcortical infarcts (Pruzin et al.,

2017), closely related with arteriosclerosis (Ighodaro et al., 2017). Likewise, patients with T2D present a decrease in spontaneous neuronal activities in the cingulate gyrus and bilateral thalamus/caudate (Cui et al., 2014), as well as altered functional connectivity of the thalamus in the middle temporal gyrus and other cortical regions. These alterations correlate with dysfunction of neurons and fiber tracts (Chen et al., 2015) and T2D patients present aberrant or reduced functional connectivity between the hippocampus and other regions of the brain (Karvani et al., 2019). In addition, studies on T2D patients reveal a reduction in total white matter, gray matter, cortical, subcortical and hippocampal volumes (Bernardes et al., 2018; Ogama et al., 2018), supporting the idea that the long-lasting hyperglycemia negatively affects brain structures and accelerate brain atrophy (Karvani et al., 2019).

As previously shown, T2D is highly related to dementia, particularly VaD and AD (Chatterjee and Mudher, 2018). Surprisingly, specific assessment of AD pathology in T2D has revealed that diabetic patients present fewer amyloid plaques in the cortex and hippocampus when compared with patients without T2D (Ahtiluoto et al., 2010; Beeri et al., 2005; Nelson et al., 2009). On the other hand, depression is twice as common in T2D patients (Moulton et al., 2015), and correlations between depression and insulin resistance have also been reported (Kan et al., 2013). Psychotic illnesses, including schizophrenia, also seem to be more prevalent in patients with metabolic syndrome (Newcomer, 2007). In line with these observations a 2016 meta-analysis showed that the first-episode psychosis was associated with increased insulin resistance and impaired glucose tolerance (Perry et al., 2016). Similar associations have been observed for anxiety and bipolar disorders or stress (Czepielewski et al., 2013; Krajnak, 2014; Smith et al., 2013).

On the other hand, whether prediabetes state of hyperinsulinemia can induce CNS alterations remains controversial. Nevertheless, prediabetes is also associated with an increased risk of structural brain abnormalities. Among these a study with elderly patients (65 years and older) has defined that dysglycemia, with increased HbA1c, was associated with a higher number of brain infarcts, white matter hyperintensities and a significant decline in gray matter volume (Reitz et al., 2017). These structural

abnormalities are thought to be, like in T2D, of micro- and macrovascular origin. In contrast to this hypothesis, the Atherosclerosis Risk in Communities Neurocognitive Study has reported no association of prediabetes with lacunar infarcts, white matter hyperintensities, cerebral microbleeds, or smaller brain volumes in an elderly population study (Schneider et al., 2017). However, the development of structural brain abnormalities may start at middle age, and to our knowledge no studies have focused on middle-aged population. Also, a recent study demonstrated that prediabetes is associated with cerebral small-vessel disease and brain atrophy. This study provides further evidence that prediabetes is not just an "early estate" and stresses that prediabetes provides an opportunity for the prevention of brain disease (van Agtmaal et al., 2018). With all this in mind, in recent years, more and more studies have focused on the relationship between prediabetes, T2D and CNS alterations, with special interest in neurodegeneration and dementia although the mechanisms involved have not been completely elucidated.

3.3. Cognitive Impairment in Patients with Type 2 Diabetes

Epidemiologic studies have reported that T2D is an independent risk factor for cognitive impairment (Allen et al., 2004; Stewart and Liolitsa, 1999). As explained above, the increased risk to develop cognitive deficits is related to a number of additive or synergistic factors, affecting brain structure and function, microvascular disease, adiposity, depression, cardiovascular disease, hypothalamic-pituitary-adrenal axis deregulation, inflammation, dyslipidemia, β-amyloid deposition, acetylcholine, hypertension or insulin resistance, among others (Karvani et al., 2019).

Studies evaluating the impact of T2D on cognitive skills have reported an accelerated decline over a period of 5 years (Yates et al., 2012). Cognitive decrement concerns verbal memory, executive function, and attention and information processing speed abilities. The reduction of these domains is related with longer duration of diabetes, poor glycemic control, presence of microvascular complications and comorbidities (Karvani et al., 2019).

Pasquier et al. concludes that the most common diabetes-related cognitive profile involve slowing in motor control, difficulty in retrieving learned material and impairment in attention and problem solving processes (Pasquier, 2010). A cohort study exploring cognitive changes over a 20-year period reported that middle-age individuals with T2D experienced 19% greater cognitive age-related decline when compared with non diabetic patients (Rawlings et al., 2014). On the other hand, white matter abnormalities are also correlated with the degree of impairment in information processing speed, attention and executive functioning (Zhang et al., 2019b). Interestingly, increased task difficulty and working memory load in T2D patients led more severe frontal cortex dysfunction and worse cognitive performance (Chen et al., 2014). Also, Kaur et al. reported an association between visceral fat, characteristic in T2D, and cortical thickness in the posterior cingulate cortex (Kaur et al., 2015). Deregulation of the default mode network is common in dementia and cognitive impairment, and the authors suggest that this thickening of the posterior cingulate cortex may be an early marker for potential cognitive decline.

Mechanisms of diabetes-associated cognitive dysfunction are coming under greater scrutiny, as the prevalence of diabetes and dementia are closely related to aging. The vast majority of the research is conducted in populations > 65 years, and just a handful of studies focus on cognitive complications of diabetes in midlife (Karvani et al., 2019). As already noted, T2D has been associated with impaired neurocognitive function in the form of dementia, mostly AD and VaD (Pal et al., 2018). An increased risk of dementia in T2D populations has been demonstrated in several studies and meta-analysis with a combined overall relative risk of 1.51%, while more than one fifth of subjects with dementia have T2D (Moon, 2016). The growing body of literature on the metabolic contribution of T2D to the AD neurodegenerative process has led to the description of “type 3 diabetes” (Leszek et al., 2017). However, this term remains controversial and further studies are needed.

In addition, it has also been shown that prediabetes itself may lead to a mild cognitive impairment, leading to the development of dementia in many cases. The factors that play a part in this progression are a combination of

discrete elements that reduce cognitive reserve and accelerate neurodegenerative processes. Some studies have shown that prediabetic dysglycemia is associated with lower performance in language, speed and visuospatial function in elderly patients (Reitz et al., 2017). Moreover, many longitudinal studies involving prediabetes have diagnosed mild cognitive impairment in these patients. When the risk of progression of mild cognitive impairment to AD is assessed, longer duration of diabetes seems to be associated with an increased risk of progression, while the use of statins and oral hypoglycemic agents reduce this risk. In this sense, having multiple cardiovascular risk factors also increases the risk of progression from mild cognitive impairment to dementia in people with metabolic syndrome (Pal et al., 2018).

3.4. Brain Complications in Type 2 Diabetes Animal Models

Rodents are the first choice to study human disease, due to the short generation time and economic considerations (Gheibi et al., 2017) and they are at the forefront of scientific advancements on obesity and diabetes mellitus (Kleinert et al., 2018). Diet-induced obesity is a widely used paradigm to study the interaction of diet and genes in manifest obesity and insulin resistance, developing obesity and impairment of glucose metabolism. C57Bl/6J mice are commonly used, since they are prone to develop these characteristics (Kleinert et al., 2018). Combination of high-fat diet (HFD) with low dosage injections of STZ is also used to model the transition from the pre-diabetic insulin-resistant state to overt T2D (Skovso, 2014). However, it is also possible to find genetic rodent models to reproduce T2D. Among them, functional leptin knock out (db/db) and leptin receptor knock out (ob/ob) mice (Coleman, 1973; Coleman and Hummel, 1969; Hervey, 1959) are widely used.

As previoulsy described in patients, severe brain complications have been observed in these animal models. Both ob/ob and db/db (Ma et al., 2015; Ramos-Rodriguez et al., 2013) mice present a significant reduction in brain weight and further assessment reveals a significant brain atrophy that

affects the cortex preferentially, while the hippocampus is also affected, as the disease progresses (Infante-Garcia et al., 2016). Interestingly, frontal cortex tissue from db/db mice shows changes in proteins involved in energy metabolism, cellular structure and neural functioning. Similar changes are observed in the hippocampus, accompained by additional effects on cellular signalling proteins (Ernst et al., 2013). Following these observations, db/db mice display synaptic and neuronal alterations, such as decreased number of neurons in the cortex and the hippocampus (Infante-Garcia et al., 2018; Ma et al., 2015; Ramos-Rodriguez et al., 2013; Ramos-Rodriguez et al., 2017), also observed in ob/ob mice (Bereiter and Jeanrenaud, 1979). Reactive microglia also seems to be increased after transient cerebral ischemia is induced in T2D animals, acompained by leukocyte ativation (Ma et al., 2015; Zhang et al., 2019a). Sustained inflammation, in compromised angiogenesis, is also observed in ob/ob mice (Zhao et al., 2017) and an overall incrase in cerebral oxidative stress is detected in diabetic mice (Ma et al., 2015; Zhao et al., 2017). Moreover, recent studies report a significant impairment in db/db animals to remodel the neurovascular unit (Hayden et al., 2019). Likewise, alterations in proliferation and neurogenesis in brains from T2D models have been described. While there is an overall reduction in brain proliferation and neurogenesis, associated with aging, (Ho et al., 2013; Ramos-Rodriguez et al., 2014), the results remain controversial; some studies show an increase in the number of proliferating cells and immature neurons in T2D mice, when compared with control animals (Hamilton et al., 2011; Ramos-Rodriguez et al., 2014). Meanwhile, others studies defend a decrease in these processes in the hippocampus (Stranahan et al., 2008; Tang et al., 2019; Yi et al., 2009).

Interestingly, induction of prediabetes by HFD seems to be enough to provoke a brain insult. Prediabetic state may induce synaptic loss as well as cortical thinning. However, different paradigms seem to be differentially affected and hippocampal synaptic function and long-term potentiation are preserved in C57Bl6 mice on HFD for up to ten moths (Mielke et al., 2006). In line with these observations, studies focusing on central cell proliferantion and neurogenesis have shown that these processes are not significantly impaired in C57Bl6 mice on HFD (Hierro-Bujalance et al., 2020; Ramos-

Rodriguez et al., 2014). Other studies with rats have observed that HFD completely abolishes insulin-mediated microvascular responses and protein kinase B phosphorylation but it does not alter the capillary density in the hippocampus, which is associated with a significantly decreased cognitive function (Fu et al., 2017). HFD also elicits insulin resistance, evidenced by a significant decrease in tyrosine phosphorylation of insulin receptor and an increase in serine phosphorylation of insulin receptor-1. These changes are accompanied by inflammatory (NFκB, JNK) and stress (p38 MAPK, CHOP) responses in the brain (Kothari et al., 2017), suggesting that changes in insulin sensitivity might contribute to cognitive impairment associated with the HFD administration in mice (Kothari et al., 2017).

It should be noted that chronic hyperglycemia can lead to tau-hyperphosphorylation (Huang et al., 2020), and this has also been observed in db/db mice (Infante-Garcia et al., 2018) and Zucker rats (Manning et al., 1993) that also show latter accumulation of amyloid β in the brain. Moreover, effects observed in prediabetes models are more severe when prediabetes is induced in AD mice that present a significant increase of neurite (Ramos-Rodriguez et al., 2017). These observations are worsened in mixed animal models harboring AD and overt T2D (Infante-Garcia et al., 2016; Ramos-Rodriguez et al., 2015). APP/PS1xdb/db mice, as a mixed murine model of AD and T2D that shows reduced cortical size, neuronal branching simplification and reduction of dendritic spine density, when we compared with AD or T2D mice alone, supporting that brain complications are more severe when T2D and AD coexist in long term.

3.5. Cognitive Impairment in Experimental Models of T2D

Studies on the above mentioned animal models indicate that hyperglycemia results in cognitive deficits and impairment of learning and memory abilities (Biessels et al., 1998). Cognitive problems are detected in db/db mice, when assessed in different tests. Dinel et al. (Dinel et al., 2011) have shown that db/db mice display impaired spatial-recognition memory, associated with increased levels of pro-inflammatory cytokines (IL-1β, TNF

and IL-6), suggesting an interaction between inflammation and memory impairment. In line with these observations db/db mice are also impaired in the Y-maze, supporting that memory loss is mediated by hyperglicemia-driven neuroinflammation and compromise of the blood brain barrier (Rom et al., 2019). According with these results, Ramos-Rodriguez et al. also observed an age dependent cognitive deterioration, including episodic and spatial memory, in db/db mice by 26 weeks of age (Ramos-Rodriguez et al., 2013). When db/db mice have been aged up to 36 weeks of age severe learning and memory disabilities have been detected in different cognitive tests, including the Morris water maze or the new object discrimiation task (Infante-Garcia et al., 2016).

The Western diet, high in fat and sugar, is the lead driver to the development of obesity and T2D, inexorably linked to premature cognitive decline and AD (McLean et al., 2018). Previous research in rodents has shown that a HFD induces cognitive deficits after weeks or months on treatment. These mice present extensive cognitive alterations in different behavioral tests, including the Morris water maze, the Barnes maze, the radial arm maze, the Y-maze and several variations of the novel object recognition test (Cordner and Tamashiro, 2015). McLean et al. showed that HFD induced cognitive deficits, in complex episodic and associative memories occur rapidly, demonstrating that diet-induced cognitive dysfunction in rodents starts at early stages of the disease (McLean et al., 2018). In other studies HFD was used to induce T2D in Sprague-Dawly rats, and animals showed cognitive decline that correlated with reduced neural density in CA1 and CA2 (Mehta and Banerjee, 2019). In line with these observations microvascular alterations, derived from HFD, are associated with a significant decrease in cognitive function, both in rats (Fu et al., 2017) and mice (Kothari et al., 2017). While Ramos-Rodriguez et al. showed that long term HFD is not enough to induce cognitive deficits in C57Bl/6J mice, Soares et al. have reported that adult Wistar rats drinking high-sucrose diet for 9 weeks display poor performance in hippocampal-dependent short- and long-term spatial memory tests (Soares et al., 2013).

Since T2D is a risk factor to suffer AD, animal models have also been developed to analyze this relationship. Takeda et al. (Takeda et al., 2010)

developed a mixed T2D and AD model, by crossing ob/ob and APP23 mice, that shows cognitive disturbances in the Morris water maze. Similarly, by crossing db/db with APP/PS1 mice it is also possible to reproduce a more severe version of the disease and APP/PS1xdb/db mice present cognitive dysfunction by 14 weeks of age, as observed in the Morris water maze and new object discrimination tests (Infante-Garcia et al., 2016; Ramos-Rodriguez et al., 2015), supporting once more the cross-talk between AD and T2D.

4. CONCLUSION

At present DM has reached the consideration of pandemia, exposing these patients to severe peripheral and brain complications. Neuronal loss, brain atrophy, vascular damage and inflammation, are just some of the features observed in the brain from diabetes patients and animal models. As a consequence, cognitive impairment is also observed, even at early stages of the disease, setting the conditions for increased risk to suffer dementia or accelerate the dementia process. Altogether, metabolic control might provide a relevant tool to slow down or limit associated complications in the CNS.

ABBREVIATIONS

AD	Alzheimer's disease
CNS	central nervous system
DM	diabetes mellitus
HFD	high-fat diet
NOD	non-obese diabetic
STZ	streptozotocin
T2D	type 2 diabetes mellitus
T1D	type 1 diabetes mellitus
VaD	vascular dementia

5. REFERENCES

Abbondante, S., et al., (2014). Genetic ablation of tau mitigates cognitive impairment induced by type 1 diabetes. *Am J Pathol.*, *184*, 819-26.

Ahmed, I. & Goldstein, B. (2006a). Diabetes mellitus. *Clin Dermatol. 24*, 237-46.

Ahmed, I. & Goldstein, B. J. (2006b). Cardiovascular risk in the spectrum of type 2 diabetes mellitus. *Mt Sinai J Med.*, *73*, 759-68.

Ahtiluoto, S., et al., (2010). Diabetes, Alzheimer disease, and vascular dementia: a population-based neuropathologic study. *Neurology.*, *75*, 1195-202.

AL McCall, D. F. (1997). How does diabetes mellitus produce brain dysfunction. *Diabetes Spectrum.*, *10*, 25-31.

Aleppo, G. & Webb, K. M. (2018). Integrated Insulin Pump And Continuous Glucose Monitoring Technology In Diabetes Care Today: A Perspective Of Real-Life Experience With The Minimed() 670g Hybrid Closed-Loop System. *Endocr Pract.*, *24*, 684-692.

Allen, K. V., et al., (2004). The relationship between type 2 diabetes and cognitive dysfunction: longitudinal studies and their methodological limitations. *Eur J Pharmacol.*, *490*, 169-75.

Alsaleh, F. M., et al., (2014). Insulin pump therapy: impact on the lives of children/young people with diabetes mellitus and their parents. *Int J Clin Pharm.*, *36*, 1023-30.

Alvarez, E. O., et al., (2009). Cognitive dysfunction and hippocampal changes in experimental type 1 diabetes. *Behav Brain Res.*, *198*, 224-30.

Alzheimer's_Association. (2016). 2016 Alzheimer's disease facts and figures. *Alzheimers Dement.*, *12*, 459-509.

American_Diabetes_Association. (2014). Diagnosis and classification of diabetes mellitus. *Diabetes Care.*, *37*, Suppl 1, S81-90.

American_Diabetes_Association. (2020). 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020. *Diabetes Care.*, *43*, S14-S31.

Amiel, S. A. (1997). Hypoglycaemia in diabetes mellitus--protecting the brain. *Diabetologia., 40*, Suppl 2, S62-8.

Amorim, D., et al., (2017). Minocycline reduces mechanical allodynia and depressive-like behaviour in type-1 diabetes mellitus in the rat. *Behav Brain Res., 327*, 1-10.

Ashcroft, F. & Rorsman, P. (2004). Type 2 diabetes mellitus: not quite exciting enough? *Hum Mol Genet., 13*, Spec No 1, R21-31.

Atkinson, M. A. (2012). The pathogenesis and natural history of type 1 diabetes. *Cold Spring Harb Perspect Med., 2*.

Aye, T., et al., (2011). The feasibility of detecting neuropsychologic and neuroanatomic effects of type 1 diabetes in young children. *Diabetes Care., 34*, 1458-62.

Baglietto-Vargas, D., et al., (2016). Diabetes and Alzheimer's disease crosstalk. *Neurosci Biobehav Rev., 64*, 272-87.

Baker, L. D., et al., (2011). Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch Neurol., 68*, 51-7.

Batista, A. F., et al., (2018). The diabetes drug liraglutide reverses cognitive impairment in mice and attenuates insulin receptor and synaptic pathology in a non-human primate model of Alzheimer's disease. *J Pathol., 245*, 85-100.

Baynes, H. W. (2015). Classification, pathophysiology, diagnosis and management of diabetes mellitus. *J diabetes metab., 6*, 1-9.

Beauquis, J., et al., (2006). Reduced hippocampal neurogenesis and number of hilar neurones in streptozotocin-induced diabetic mice: reversion by antidepressant treatment. *Eur J Neurosci., 23*, 1539-46.

Beauquis, J., et al., (2008). Prominently decreased hippocampal neurogenesis in a spontaneous model of type 1 diabetes, the nonobese diabetic mouse. *Exp Neurol., 210*, 359-67.

Bednarik, P., et al., (2017). Type 1 Diabetes and Impaired Awareness of Hypoglycemia Are Associated with Reduced Brain Gray Matter Volumes. *Front Neurosci., 11*, 529.

Beeri, M. S., et al., (2005). Type 2 diabetes is negatively associated with Alzheimer's disease neuropathology. *J Gerontol A Biol Sci Med Sci., 60*, 471-5.

Bereiter, D. A. & Jeanrenaud, B. (1979). Altered neuroanatomical organization in the central nervous system of the genetically obese (ob/ob) mouse. *Brain Res., 165*, 249-60.

Bernardes, G., et al., (2018). Cortical and subcortical gray matter structural alterations in normoglycemic obese and type 2 diabetes patients: relationship with adiposity, glucose, and insulin. *Metab Brain Dis., 33*, 1211-1222.

Biessels, G. J., et al., (2008). Cognition and diabetes: a lifespan perspective. *Lancet Neurol., 7*, 184-90.

Biessels, G. J. & Despa, F. (2018). Cognitive decline and dementia in diabetes mellitus: mechanisms and clinical implications. *Nat Rev Endocrinol., 14*, 591-604.

Biessels, G. J., et al., (1998). Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. *Brain Res., 800*, 125-35.

Biessels, G. J., et al., (2006). Risk of dementia in diabetes mellitus: a systematic review. *Lancet Neurol., 5*, 64-74.

Biessels, G. J. & Whitmer, R. A. (2020). Cognitive dysfunction in diabetes: how to implement emerging guidelines. *Diabetologia., 63*, 3-9.

Bober, E. & Buyukgebiz, A. (2005). Hypoglycemia and its effects on the brain in children with type 1 diabetes mellitus. *Pediatr Endocrinol Rev., 2*, 378-82.

Boyle, P. A., et al., (2015). Cerebral amyloid angiopathy and cognitive outcomes in community-based older persons. *Neurology., 85*, 1930-6.

Brands, A. M., et al., (2005). The effects of type 1 diabetes on cognitive performance: a meta-analysis. *Diabetes Care., 28*, 726-35.

Brismar, T., et al., (2007). Predictors of cognitive impairment in type 1 diabetes. *Psychoneuroendocrinology., 32*, 1041-51.

Brown, C. E., et al., (2009). *In vivo* voltage-sensitive dye imaging in adult mice reveals that somatosensory maps lost to stroke are replaced over weeks by new structural and functional circuits with prolonged modes

of activation within both the peri-infarct zone and distant sites. *J Neurosci.*, *29*, 1719-34.

Brown, R. T. e., (2004). Changes in the provision of health care to children and adolescents. In: *Pediatric psychology in school setting*. London: Lawrence Elrbaum., 1-17.

Brownlee, M. (2005). The pathobiology of diabetic complications: a unifying mechanism. *Diabetes.*, *54*, 1615-25.

Burtscher, I. M. & Holtås, S. (2001). Proton MR spectroscopy in clinical routine. *Journal of Magnetic Resonance Imaging: An Official Journal of the International Society for Magnetic Resonance in Medicine.*, *13*, 560-567.

Butler, A. E., et al., (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes.*, *52*, 102-10.

Carlotti, A. P., et al., (2003). Importance of timing of risk factors for cerebral oedema during therapy for diabetic ketoacidosis. *Arch Dis Child.*, *88*, 170-3.

Chan, O., et al., (2003). Diabetes and the hypothalamo-pituitary-adrenal (HPA) axis. *Minerva Endocrinol.*, *28*, 87-102.

Chatterjee, S. & Mudher, A. (2018). Alzheimer's Disease and Type 2 Diabetes: A Critical Assessment of the Shared Pathological Traits. *Front Neurosci.*, *12*, 383.

Chen, Y., et al., (2014). Altered brain activation patterns under different working memory loads in patients with type 2 diabetes. *Diabetes Care.*, *37*, 3157-63.

Chen, Y. C., et al., (2015). Thalamic resting-state functional connectivity: disruption in patients with type 2 diabetes. *Metab Brain Dis.*, *30*, 1227-36.

Clark, A., et al., (2001). Decreased insulin secretion in type 2 diabetes: a problem of cellular mass or function? *Diabetes.*, *50*, Suppl 1, S169-71.

Clarke, J. R., et al., (2015). Alzheimer-associated Abeta oligomers impact the central nervous system to induce peripheral metabolic deregulation. *EMBO Mol Med.*, *7*, 190-210.

Coleman, D. L. (1973). Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia.*, *9*, 294-8.

Coleman, D. L. & Hummel, K. P. (1969). Effects of parabiosis of normal with genetically diabetic mice. *Am J Physiol., 217*, 1298-304.

Cordner, Z. A. & Tamashiro, K. L. (2015). Effects of high-fat diet exposure on learning & memory. *Physiol Behav., 152*, 363-71.

Craft, S. (2009). The role of metabolic disorders in Alzheimer disease and vascular dementia: two roads converged. *Arch Neurol., 66*, 300-5.

Craft, S. (2012). Alzheimer disease: Insulin resistance and AD--extending the translational path. *Nat Rev Neurol., 8*, 360-2.

Crews, L., et al., (2010). APP transgenic modeling of Alzheimer's disease: mechanisms of neurodegeneration and aberrant neurogenesis. *Brain Struct Funct., 214*, 111-26.

Cui, Y., et al., (2014). Altered spontaneous brain activity in type 2 diabetes: a resting-state functional MRI study. *Diabetes., 63*, 749-60.

Czepielewski, L., et al., (2013). Bipolar disorder and metabolic syndrome: a systematic review. *Braz J Psychiatry., 35*, 88-93.

Dejgaard, A., et al., (1991). Evidence for diabetic encephalopathy. *Diabet Med., 8*, 162-7.

Desai, S. & Deshmukh, A. (2019). Mapping Of Type 1 Diabetes Mellitus. *Curr Diabetes Rev*.

Desrocher, M. & Rovet, J. (2004). Neurocognitive correlates of type 1 diabetes mellitus in childhood. *Child Neuropsychol., 10*, 36-52.

Dheen, S. T., et al., (1994). Arginine vasopressin- and oxytocin-like immunoreactive neurons in the hypothalamic paraventricular and supraoptic nuclei of streptozotocin-induced diabetic rats. *Arch Histol Cytol., 57*, 461-72.

Dinel, A. L., et al., (2011). Cognitive and emotional alterations are related to hippocampal inflammation in a mouse model of metabolic syndrome. *PLoS One., 6*, e24325.

Eckhardt, B. J., et al., (2012). Glycated Hemoglobin A(1c) as screening for diabetes mellitus in HIV-infected individuals. *AIDS Patient Care STDS., 26*, 197-201.

Eckman, E. A. & Eckman, C. B. (2005). Abeta-degrading enzymes: modulators of Alzheimer's disease pathogenesis and targets for therapeutic intervention. *Biochem Soc Trans., 33*, 1101-5.

Eichenbaum, H. (2000). A cortical-hippocampal system for declarative memory. *Nat Rev Neurosci., 1,* 41-50.

Ergul, A. (2011). Endothelin-1 and diabetic complications: focus on the vasculature. *Pharmacol Res., 63,* 477-82.

Ernst, A., et al., (2013). Diabetic db/db mice exhibit central nervous system and peripheral molecular alterations as seen in neurological disorders. *Transl Psychiatry., 3,* e263.

Farris, W., et al., (2003). Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain *in vivo. Proc Natl Acad Sci U S A., 100,* 4162-7.

Federiuk, I. F., et al., (2004). Induction of type-1 diabetes mellitus in laboratory rats by use of alloxan: route of administration, pitfalls, and insulin treatment. *Comp Med., 54,* 252-7.

Ferguson, S. C., et al., (2005). Influence of an early-onset age of type 1 diabetes on cerebral structure and cognitive function. *Diabetes Care., 28,* 1431-7.

Frokjaer, J. B., et al., (2013). Macrostructural brain changes in patients with longstanding type 1 diabetes mellitus - a cortical thickness analysis study. *Exp Clin Endocrinol Diabetes., 121,* 354-60.

Fu, Z., et al., (2017). Long-term high-fat diet induces hippocampal microvascular insulin resistance and cognitive dysfunction. *Am J Physiol Endocrinol Metab., 312,* E89-e97.

Fulop, T., et al., (2018). Can an Infection Hypothesis Explain the Beta Amyloid Hypothesis of Alzheimer's Disease? *Front Aging Neurosci., 10,* 224.

Garcia-Alloza, M., et al., (2009). Matrix metalloproteinase inhibition reduces oxidative stress associated with cerebral amyloid angiopathy *in vivo* in transgenic mice. *J Neurochem., 109,* 1636-47.

Gaudieri, P. A., et al., (2008). Cognitive function in children with type 1 diabetes: a meta-analysis. *Diabetes Care., 31,* 1892-7.

Geissler, A., et al., (2003). Alterations of cerebral metabolism in patients with diabetes mellitus studied by proton magnetic resonance spectroscopy. *Exp Clin Endocrinol Diabetes., 111,* 421-7.

Gerich, J. E. (1998). The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev., 19*, 491-503.

Gheibi, S., et al., (2017). A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. *Biomed Pharmacother., 95*, 605-613.

Glaab, L. A., et al., (2005). School attendance in children with Type 1 diabetes. *Diabet Med., 22*, 421-6.

Goldenberg, R. & Punthakee, Z. (2013). Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Can J Diabetes., 37*, Suppl 1, S8-11.

Gotz, J., et al., (2009). Common features between diabetes mellitus and Alzheimer's disease. *Cell Mol Life Sci., 66*, 1321-5.

Greenberg, S. M., et al., (2008). Detection of isolated cerebrovascular beta-amyloid with Pittsburgh compound B. *Ann Neurol., 64*, 587-91.

Gudala, K., et al., (2013). Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. *J Diabetes Investig., 4*, 640-50.

Gutefeldt, K., et al., (2020). Low health-related quality of life is strongly linked to upper extremity impairments in type 1 diabetes with a long duration. *Disabil Rehabil.*, 1-7.

Hamilton, A., et al., (2011). Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain. *J Neurosci Res., 89*, 481-9.

Hannonen, R., et al., (2003). Neurocognitive functioning in children with type-1 diabetes with and without episodes of severe hypoglycaemia. *Dev Med Child Neurol., 45*, 262-8.

Hayden, M. R., et al., (2019). Empagliflozin Ameliorates Type 2 Diabetes-Induced Ultrastructural Remodeling of the Neurovascular Unit and Neuroglia in the Female db/db Mouse. *Brain Sci., 9*.

Henriquez-Tejo, R. & Cartes-Velasquez, R. (2018). [Psychosocial impact of type 1 diabetes mellitus in children, adolescents and their families. Literature review]. *Rev Chil Pediatr., 89*, 391-398.

Herman, W. H., et al., (2018). The 30-year cost-effectiveness of alternative strategies to achieve excellent glycemic control in type 1 diabetes: An economic simulation informed by the results of the diabetes control and complications trial/epidemiology of diabetes interventions and complications (DCCT/EDIC). *J Diabetes Complications.*, *32*, 934-939.

Hershey, T., et al., (2005). Frequency and timing of severe hypoglycemia affects spatial memory in children with type 1 diabetes. *Diabetes Care.*, *28*, 2372-7.

Hervey, G. R. (1959). The effects of lesions in the hypothalamus in parabiotic rats. *J Physiol.*, *145*, 336-52.

Hierro-Bujalance, C., et al., (2020). Cell proliferation and neurogenesis alterations in Alzheimer's disease and diabetes mellitus mixed murine models. *J Neurochem.*

Ho, M. S., et al., (2008). Prevalence of structural central nervous system abnormalities in early-onset type 1 diabetes mellitus. *J Pediatr.*, *153*, 385-90.

Ho, N., et al., (2013). Effects of diabetes on hippocampal neurogenesis: links to cognition and depression. *Neurosci Biobehav Rev.*, *37*, 1346-62.

Holscher, C. (2018). Novel dual GLP-1/GIP receptor agonists show neuroprotective effects in Alzheimer's and Parkinson's disease models. *Neuropharmacology.*, *136*, 251-259.

Houlden, R. L. (2018). Introduction. *Can J Diabetes.*, *42*, Suppl 1, S1-s5.

Huang, C. C., et al., (2014). Diabetes mellitus and the risk of Alzheimer's disease: a nationwide population-based study. *PLoS One.*, *9*, e87095.

Huang, R., et al., (2020). Chronic hyperglycemia induces tau hyperphosphorylation by downregulating OGT-involved O-GlcNAcylation *in vivo* and *in vitro. Brain Res Bull.*, *156*, 76-85.

Ighodaro, E. T., et al., (2017). Risk factors and global cognitive status related to brain arteriolosclerosis in elderly individuals. *J Cereb Blood Flow Metab.*, *37*, 201-216.

Ilonen, J., et al., (2013). Patterns of beta-cell autoantibody appearance and genetic associations during the first years of life. *Diabetes.*, *62*, 3636-40.

Infante-Garcia, C., et al., (2016). Long-term central pathology and cognitive impairment are exacerbated in a mixed model of Alzheimer's disease and type 2 diabetes. *Psychoneuroendocrinology., 65*, 15-25.

Infante-Garcia, C., et al., (2018). Antidiabetic Polypill Improves Central Pathology and Cognitive Impairment in a Mixed Model of Alzheimer's Disease and Type 2 Diabetes. *Mol Neurobiol. 55*, 6130-6144.

Insel, R. A., et al., (2015). Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care., 38*, 1964-74.

Jacobson, A. M., et al., (2007). Long-term effect of diabetes and its treatment on cognitive function. *N Engl J Med., 356*, 1842-52.

Janez, A., et al., (2020). Insulin Therapy in Adults with Type 1 Diabetes Mellitus: a Narrative Review. *Diabetes Ther*.

Jin, K., et al., (2004). Increased hippocampal neurogenesis in Alzheimer's disease. *Proc Natl Acad Sci U S A., 101*, 343-7.

Jolivalt, C. G., et al., (2008). Defective insulin signaling pathway and increased glycogen synthase kinase-3 activity in the brain of diabetic mice: parallels with Alzheimer's disease and correction by insulin. *J Neurosci Res., 86*, 3265-74.

Jurgens, C. A., et al., (2011). β-cell loss and β-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. *The American journal of pathology., 178*, 2632-2640.

Kahn, S. E., et al., (2014). Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet., 383*, 1068-83.

Kalaria, R. N. (2018). The pathology and pathophysiology of vascular dementia. *Neuropharmacology., 134*, 226-239.

Kan, C., et al., (2013). A systematic review and meta-analysis of the association between depression and insulin resistance. *Diabetes Care., 36*, 480-9.

Karvani, M., et al., (2019). Neurocognitive impairment in type 2 diabetes mellitus. *Hormones* (Athens).

Katsarou, A., et al., (2017). Type 1 diabetes mellitus. *Nat Rev Dis Primers., 3*, 17016.

Kaur, S., et al., (2015). Central Adiposity and Cortical Thickness in Midlife. *Psychosom Med.*, *77*, 671-8.

Kautzky-Willer, A., et al., (2019). [Gestational diabetes mellitus (Update 2019)]. *Wien Klin Wochenschr.*, *131*, 91-102.

Kelley, D. E., et al., (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes.*, *51*, 2944-50.

Kent, S. C., et al., (2017). Deciphering the Pathogenesis of Human Type 1 Diabetes (T1D) by Interrogating T Cells from the "Scene of the Crime". *Curr Diab Rep.*, *17*, 95.

Kim, J. H., et al., (2017). Astrocyte-derived lipocalin-2 mediates hippocampal damage and cognitive deficits in experimental models of vascular dementia. *Glia.*, *65*, 1471-1490.

Kim, J. J. & Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nat Rev Neurosci.*, *3*, 453-62.

Kishi, K., et al., (2019). Hyperglycemia in the early stages of type 1 diabetes accelerates gastric emptying through increased networks of interstitial cells of Cajal. *PLoS One.*, *14*, e0222961.

Kitabchi, A. E., et al., (2009). Hyperglycemic crises in adult patients with diabetes. *Diabetes Care.*, *32*, 1335-43.

Kleinert, M., et al., (2018). Animal models of obesity and diabetes mellitus. *Nat Rev Endocrinol.*, *14*, 140-162.

Knierim, J. J. (2015). The hippocampus. *Curr Biol.*, *25*, R1116-21.

Koekkoek, P. S., et al., (2015). Cognitive function in patients with diabetes mellitus: guidance for daily care. *Lancet Neurol.*, *14*, 329-40.

Konarkowska, B., et al., (2006). The aggregation potential of human amylin determines its cytotoxicity towards islet beta-cells. *Febs j.*, *273*, 3614-24.

Kothari, V., et al., (2017). High fat diet induces brain insulin resistance and cognitive impairment in mice. *Biochim Biophys Acta Mol Basis Dis.*, *1863*, 499-508.

Krajnak, K. M. (2014). Potential Contribution of Work-Related Psychosocial Stress to the Development of Cardiovascular Disease and Type II Diabetes: A Brief Review. *Environ Health Insights.*, *8*, 41-5.

Kreis, R. & Ross, B. D. (1992). Cerebral metabolic disturbances in patients with subacute and chronic diabetes mellitus: detection with proton MR spectroscopy. *Radiology., 184,* 123-30.

Krischer, J. P., et al., (2015). The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. *Diabetologia., 58,* 980-7.

Kumar, S., et al., (2016). Phosphorylation of the amyloid beta-peptide at Ser26 stabilizes oligomeric assembly and increases neurotoxicity. *Acta Neuropathol., 131,* 525-37.

Kussmann, M., et al., (2013). Perspective: a systems approach to diabetes research. *Front Genet., 4,* 205.

Laddha, A. P. & Kulkarni, Y. A. (2019). Tannins and vascular complications of Diabetes: An update. *Phytomedicine., 56,* 229-245.

Lau, E., et al., (2015). Beyond gut microbiota: understanding obesity and type 2 diabetes. *Hormones* (Athens)., *14,* 358-69.

Lawlor, N., et al., (2017). Genomics of Islet (Dys) function and Type 2 Diabetes. *Trends Genet., 33,* 244-255.

Lee, A. H., et al., (2011). Dual and opposing roles of the unfolded protein response regulated by IRE1alpha and XBP1 in proinsulin processing and insulin secretion. *Proc Natl Acad Sci U S A., 108,* 8885-90.

Leszek, J., et al., (2017). Type 3 Diabetes Mellitus: A Novel Implication of Alzheimers Disease. *Curr Top Med Chem., 17,* 1331-1335.

Li, B., et al., (2008). Failure of neuronal maturation in Alzheimer disease dentate gyrus. *J Neuropathol Exp Neurol., 67,* 78-84.

Li, J. Q., et al., (2016). Risk factors for predicting progression from mild cognitive impairment to Alzheimer's disease: a systematic review and meta-analysis of cohort studies. *J Neurol Neurosurg Psychiatry., 87,* 476-84.

Li, W., et al., (2017). Type 1 Diabetes Mellitus and Cognitive Impairments: A Systematic Review. *J Alzheimers Dis., 57,* 29-36.

Li, Z. G., et al., (2002). Hippocampal neuronal apoptosis in type 1 diabetes. *Brain Res., 946,* 221-31.

Lobnig, B. M., et al., (2006). Hippocampal volume and cognitive performance in long-standing Type 1 diabetic patients without macrovascular complications. *Diabet Med.*, *23*, 32-9.

Lopez-Gambero, A. J., et al., (2019). Brain Glucose-Sensing Mechanism and Energy Homeostasis. *Mol Neurobiol.*, *56*, 769-796.

Luchsinger, J. A., et al., (2007). Relation of diabetes to mild cognitive impairment. *Arch Neurol.*, *64*, 570-5.

Luchsinger, J. A., et al., (2004). Hyperinsulinemia and risk of Alzheimer disease. *Neurology.*, *63*, 1187-92.

Lunetta, M., et al., (1994). Evidence by magnetic resonance imaging of cerebral alterations of atrophy type in young insulin-dependent diabetic patients. *J Endocrinol Invest.*, *17*, 241-5.

Lupien, S. J. & Lepage, M. (2001). Stress, memory, and the hippocampus: can't live with it, can't live without it. *Behav Brain Res.*, *127*, 137-58.

Ly, T. T., et al., (2011). Neurocognitive outcomes in young adults with early-onset type 1 diabetes: a prospective follow-up study. *Diabetes Care.*, *34*, 2192-7.

Ma, M., et al., (2015). DPP-4 inhibition with linagliptin ameliorates cognitive impairment and brain atrophy induced by transient cerebral ischemia in type 2 diabetic mice. *Cardiovasc Diabetol.*, *14*, 54.

Magarinos, A. M. & McEwen, B. S. (2000). Experimental diabetes in rats causes hippocampal dendritic and synaptic reorganization and increased glucocorticoid reactivity to stress. *Proc Natl Acad Sci U S A.*, *97*, 11056-61.

Makimattila, S., et al., (2004). Brain metabolic alterations in patients with type 1 diabetes-hyperglycemia-induced injury. *J Cereb Blood Flow Metab.*, *24*, 1393-9.

Manning, C. A., et al., (1993). Glucose enhancement of memory in patients with probable senile dementia of the Alzheimer's type. *Neurobiol Aging.*, *14*, 523-8.

Martin-Maestro, P., et al., (2017). Slower Dynamics and Aged Mitochondria in Sporadic Alzheimer's Disease. *Oxid Med Cell Longev.*, 9302761.

Masters, C. L., et al., (2015). Alzheimer's disease. *Nat Rev Dis Primers.*, *1*, 15056.

Matveyenko, A. V. & Butler, P. C. (2006). Beta-cell deficit due to increased apoptosis in the human islet amyloid polypeptide transgenic (HIP) rat recapitulates the metabolic defects present in type 2 diabetes. *Diabetes.*, *55*, 2106-14.

McCall, A. & Figlewicz, D. (1997). How does diabetes mellitus produce brain dysfunction. *Diabetes Spectrum.*, *10*, 3.

McLean, F. H., et al., (2018). Rapid and reversible impairment of episodic memory by a high-fat diet in mice. *Sci Rep.*, *8*, 11976.

Mehta, B. K. & Banerjee, S. (2019). Minocycline reverses diabetes-associated cognitive impairment in rats. *Pharmacol Rep.*, *71*, 713-720.

Melberg, A., et al., (1999). Neuroimaging study in autosomal dominant cerebellar ataxia, deafness, and narcolepsy. *Neurology.*, *53*, 2190-2.

Meyer-Luehmann, M., et al., (2008). Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. *Nature.*, *451*, 720-4.

Mielke, J. G., et al., (2006). Longitudinal study of the effects of a high-fat diet on glucose regulation, hippocampal function, and cerebral insulin sensitivity in C57BL/6 mice. *Behav Brain Res.*, *175*, 374-82.

Miles, W. & Root, H. (1922). Psychologic tests applied to diabetic patients. *Archives of Internal Medicine.*, *30*, 767-777.

Mima, A. (2013). Inflammation and oxidative stress in diabetic nephropathy: new insights on its inhibition as new therapeutic targets. *Journal of diabetes research.*, 2013.

Moon, J. H. (2016). Endocrine Risk Factors for Cognitive Impairment. *Endocrinol Metab* (Seoul)., *31*, 185-92.

Morales, A. E., et al., (2001). Prediction and prevention of type 1 diabetes. *Curr Diab Rep.*, *1*, 28-32.

Moran, A., et al., (2018). ISPAD Clinical Practice Consensus Guidelines 2018: Management of cystic fibrosis-related diabetes in children and adolescents. *Pediatr Diabetes*., *19*, Suppl 27, 64-74.

Moulton, C. D., et al., (2015). The link between depression and diabetes: the search for shared mechanisms. *Lancet Diabetes Endocrinol.*, *3*, 461-471.

Musen, G., et al., (2006). Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. *Diabetes., 55,* 326-33.

Nam, S. M., et al., (2019). Ascorbic Acid Mitigates D-galactose-Induced Brain Aging by Increasing Hippocampal Neurogenesis and Improving Memory Function. *Nutrients., 11*.

Nasrallah, R., et al., (2014). Chronic kidney disease: targeting prostaglandin E2 receptors. *Am J Physiol Renal Physiol., 307,* F243-50.

Nathan, D. M. (2014). The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. *Diabetes Care., 37*, 9-16.

Nelson, P. T., et al., (2009). Human cerebral neuropathology of Type 2 diabetes mellitus. *Biochim Biophys Acta., 1792*, 454-69.

Newcomer, J. W. (2007). Metabolic syndrome and mental illness. *Am J Manag Care., 13*, S170-7.

Nizami, S., et al., (2019). Microglial inflammation and phagocytosis in Alzheimer's disease: Potential therapeutic targets. *Br J Pharmacol., 176*, 3515-3532.

Noor, S., et al., (2015). Diabetic foot ulcer--A review on pathophysiology, classification and microbial etiology. *Diabetes Metab Syndr., 9*, 192-9.

Northam, E. A., et al., (2005). Psychiatric morbidity and health outcome in Type 1 diabetes--perspectives from a prospective longitudinal study. *Diabet Med., 22*, 152-7.

Northam, E. A., et al., (2006). Therapy insight: the impact of type 1 diabetes on brain development and function. *Nat Clin Pract Neurol., 2*, 78-86.

Northam, E. A., et al., (2009). Central nervous system function in youth with type 1 diabetes 12 years after disease onset. *Diabetes Care., 32*, 445-50.

Nunley, K. A., et al., (2015). Clinically Relevant Cognitive Impairment in Middle-Aged Adults With Childhood-Onset Type 1 Diabetes. *Diabetes Care., 38*, 1768-76.

O'Brien, J. T. & Thomas, A. (2015). Vascular dementia. *Lancet., 386*, 1698-706.

Ogama, N., et al., (2018). Postprandial Hyperglycemia Is Associated With White Matter Hyperintensity and Brain Atrophy in Older Patients With Type 2 Diabetes Mellitus. *Front Aging Neurosci., 10*, 273.

Ohmann, S., et al., (2010). Cognitive functions and glycemic control in children and adolescents with type 1 diabetes. *Psychol Med., 40,* 95-103.

Ott, A., et al., (1996). Association of diabetes mellitus and dementia: the Rotterdam Study. *Diabetologia., 39,* 1392-7.

Ozery-Flato, M., et al., (2013). Predictive models for type 2 diabetes onset in middle-aged subjects with the metabolic syndrome. *Diabetol Metab Syndr., 5,* 36.

Pal, K., et al., (2018). Mild cognitive impairment and progression to dementia in people with diabetes, prediabetes and metabolic syndrome: a systematic review and meta-analysis. *Soc Psychiatry Psychiatr Epidemiol., 53,* 1149-1160.

Palop, J. J. & Mucke, L. (2016). Network abnormalities and interneuron dysfunction in Alzheimer disease. *Nat Rev Neurosci., 17,* 777-792.

Pandey, A., et al., (2015). Type-2 diabetes: Current understanding and future perspectives. *IUBMB Life., 67,* 506-13.

Parent, K. B., et al., (2009). Type 1 diabetes mellitus and school: a comparison of patients and healthy siblings. *Pediatr Diabetes., 10,* 554-62.

Pasquier, F., (2010). Diabetes and cognitive impairment: how to evaluate the cognitive status? *Diabetes Metab., 36,* Suppl 3, S100-5.

Pell, G. S., et al., (2012). Age-related loss of brain volume and T2 relaxation time in youth with type 1 diabetes. *Diabetes Care., 35,* 513-9.

Perros, P., et al., (1997). Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycemia. *Diabetes Care., 20,* 1013-8.

Perry, B. I., et al., (2016). The association between first-episode psychosis and abnormal glycaemic control: systematic review and meta-analysis. *Lancet Psychiatry., 3,* 1049-1058.

Petersen, K. F., et al., (2003). Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science., 300,* 1140-2.

Petrie, D., et al., (2016). Recent trends in life expectancy for people with type 1 diabetes in Sweden. *Diabetologia., 59,* 1167-76.

Pourabbasi, A., et al., (2016). Evaluation of the correlation between type 1 diabetes and cognitive function in children and adolescents, and

comparison of this correlation with structural changes in the central nervous system: a study protocol. *BMJ Open.*, *6*, e007917.

Pozueta, J., et al., (2013). Synaptic changes in Alzheimer's disease and its models. *Neuroscience.*, *251*, 51-65.

Pruzin, J. J., et al., (2017). Diabetes, Hemoglobin A1C, and Regional Alzheimer Disease and Infarct Pathology. *Alzheimer Dis Assoc Disord.*, *31*, 41-47.

Pugliese, A. (2014). Advances in the etiology and mechanisms of type 1 diabetes. *Discov Med.*, *18*, 141-50.

Punthakee, Z., et al., (2018). Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. *Can J Diabetes.*, *42*, Suppl 1, S10-s15.

Qiu, H., et al., (2017). Ketosis and diabetic ketoacidosis in response to SGLT2 inhibitors: Basic mechanisms and therapeutic perspectives. *Diabetes Metab Res Rev.*, *33*.

Rahier, J., et al., (2008). Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diabetes Obes Metab.*, *10*, Suppl 4, 32-42.

Ramos-Rodriguez, J. J., et al., (2016). Increased Spontaneous Central Bleeding and Cognition Impairment in APP/PS1 Mice with Poorly Controlled Diabetes Mellitus. *Mol Neurobiol.*, *53*, 2685-97.

Ramos-Rodriguez, J. J., et al., (2015). Central vascular disease and exacerbated pathology in a mixed model of type 2 diabetes and Alzheimer's disease. *Psychoneuroendocrinology.*, *62*, 69-79.

Ramos-Rodriguez, J. J., et al., (2014). Central proliferation and neurogenesis is impaired in type 2 diabetes and prediabetes animal models. *PLoS One.*, *9*, e89229.

Ramos-Rodriguez, J. J., et al., (2013). Differential central pathology and cognitive impairment in pre-diabetic and diabetic mice. *Psychoneuroendocrinology.*, *38*, 2462-75.

Ramos-Rodriguez, J. J., et al., (2017). Progressive Neuronal Pathology and Synaptic Loss Induced by Prediabetes and Type 2 Diabetes in a Mouse Model of Alzheimer's Disease. *Mol Neurobiol.*, *54*, 3428-3438.

Rawlings, A. M., et al., (2014). Diabetes in midlife and cognitive change over 20 years: a cohort study. *Ann Intern Med.*, *161*, 785-93.

Reitz, C., et al., (2017). Relation of Dysglycemia to Structural Brain Changes in a Multiethnic Elderly Cohort. *J Am Geriatr Soc.*, *65*, 277-285.

Revsin, Y., et al., (2005). Neuronal and astroglial alterations in the hippocampus of a mouse model for type 1 diabetes. *Brain Res.*, *1038*, 22-31.

Revsin, Y., et al., (2008). Adrenal hypersensitivity precedes chronic hypercorticism in streptozotocin-induced diabetes mice. *Endocrinology.*, *149*, 3531-9.

Ripsin, C. M., et al., (2009). Management of blood glucose in type 2 diabetes mellitus. *Am Fam Physician.*, *79*, 29-36.

Ristow, M. (2004). Neurodegenerative disorders associated with diabetes mellitus. *J Mol Med (Berl).*, *82*, 510-29.

Roberts, R. O., et al., (2014). Association of diabetes with amnestic and nonamnestic mild cognitive impairment. *Alzheimers Dement.*, *10*, 18-26.

Roehr, S., et al., (2017). Secular trends in the incidence of dementia in high-income countries: a protocol of a systematic review and a planned meta-analysis. *BMJ Open.*, *7*, e013630.

Rogovik, A. L., et al., (2010). Pharmacotherapy and weight-loss supplements for treatment of paediatric obesity. *Drugs.*, *70*, 335-46.

Rom, S., et al., (2019). Hyperglycemia-Driven Neuroinflammation Compromises BBB Leading to Memory Loss in Both Diabetes Mellitus (DM) Type 1 and Type 2 Mouse Models. *Mol Neurobiol.*, *56*, 1883-1896.

Rosenson, R. S., et al., (2011). Does microvascular disease predict macrovascular events in type 2 diabetes? *Atherosclerosis.*, *218*, 13-8.

Rovet, J. F. & Ehrlich, R. M. (1999). The effect of hypoglycemic seizures on cognitive function in children with diabetes: a 7-year prospective study. *J Pediatr.*, *134*, 503-6.

Ryan, C. M. (2006). Why is cognitive dysfunction associated with the development of diabetes early in life? The diathesis hypothesis. *Pediatr Diabetes.*, *7*, 289-97.

Ryan, C. M., et al., (2016). Neurocognitive consequences of diabetes. *Am Psychol.*, *71*, 563-576.

Saravia, F., et al., (2007). Neuroprotective effects of estradiol in hippocampal neurons and glia of middle age mice. *Psychoneuroendocrinology.*, *32*, 480-92.

Saravia, F., et al., (2004). Oestradiol restores cell proliferation in dentate gyrus and subventricular zone of streptozotocin-diabetic mice. *J Neuroendocrinol.*, *16*, 704-10.

Saravia, F. E., et al., (2001). Diabetes increases the expression of hypothalamic neuropeptides in a spontaneous model of type I diabetes, the nonobese diabetic (NOD) mouse. *Cell Mol Neurobiol.*, *21*, 15-27.

Saravia, F. E., et al., (2002). Increased astrocyte reactivity in the hippocampus of murine models of type 1 diabetes: the nonobese diabetic (NOD) and streptozotocin-treated mice. *Brain Res.*, *957*, 345-53.

Schliebs, R. & Arendt, T. (2006). The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. *J Neural Transm (Vienna).*, *113*, 1625-44.

Schneider, A. L. C., et al., (2017). Diabetes, Prediabetes, and Brain Volumes and Subclinical Cerebrovascular Disease on MRI: The Atherosclerosis Risk in Communities Neurocognitive Study (ARIC-NCS). *Diabetes Care.*, *40*, 1514-1521.

Schrag, M. & Kirshner, H. (2016). Neuropsychological Effects of Cerebral Amyloid Angiopathy. *Curr Neurol Neurosci Rep.*, *16*, 76.

Schrijvers, E. M., et al., (2010). Insulin metabolism and the risk of Alzheimer disease: the Rotterdam Study. *Neurology.*, *75*, 1982-7.

Scirica, B. M. (2017). Use of Biomarkers in Predicting the Onset, Monitoring the Progression, and Risk Stratification for Patients with Type 2 Diabetes Mellitus. *Clin Chem.*, *63*, 186-195.

Scott, A., et al., (2019). Socioeconomic disparities in access to intensive insulin regimens for adults with type 1 diabetes: a qualitative study of patient and healthcare professional perspectives. *Int J Equity Health.*, *18*, 150.

Selvarajah, D. & Tesfaye, S. (2006). Central nervous system involvement in diabetes mellitus. *Curr Diab Rep.*, *6*, 431-8.

Serrano-Pozo, A., et al., (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med.*, *1*, a006189.

Sharif, K., et al., (2018). Psychological stress and type 1 diabetes mellitus: what is the link? *Expert Rev Clin Immunol.*, *14*, 1081-1088.

Sharma, D., et al., (2017). Diabetic nephropathy: New insights into established therapeutic paradigms and novel molecular targets. *Diabetes Res Clin Pract.*, *128*, 91-108.

Shivaswamy, V., et al., (2016). Post-Transplant Diabetes Mellitus: Causes, Treatment, and Impact on Outcomes. *Endocr Rev.*, *37*, 37-61.

Shumway, M., et al., (2019). Economic costs of implementing group interventions to reduce diabetes distress in adults with type 1 diabetes mellitus in the T1-REDEEM trial. *J Diabetes Complications.*, *33*, 107416.

Sima, A. A. (2004). Diabetes underlies common neurological disorders. *Ann Neurol.*, *56*, 459-61.

Sima, A. A. (2010). Encephalopathies: the emerging diabetic complications. *Acta Diabetol.*, *47*, 279-93.

Skovso, S. (2014). Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *J Diabetes Investig.*, *5*, 349-58.

Skyler, J. S., et al., (2017). Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. *Diabetes.*, *66*, 241-255.

Smith, K. J., et al., (2013). Association of diabetes with anxiety: a systematic review and meta-analysis. *J Psychosom Res.*, *74*, 89-99.

Smolina, K., et al., (2015). Risk of dementia in patients hospitalised with type 1 and type 2 diabetes in England, 1998-2011: a retrospective national record linkage cohort study. *Diabetologia.*, *58*, 942-50.

Soares, E., et al., (2013). Spatial memory impairments in a prediabetic rat model. *Neuroscience.*, *250*, 565-77.

Stewart, R. & Liolitsa, D. (1999). Type 2 diabetes mellitus, cognitive impairment and dementia. *Diabet Med.*, *16*, 93-112.

Stranahan, A. M., et al., (2008). Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nat Neurosci.*, *11*, 309-17.

Takeda, S., et al., (2010). Diabetes-accelerated memory dysfunction via cerebrovascular inflammation and Abeta deposition in an Alzheimer mouse model with diabetes. *Proc Natl Acad Sci U S A., 107,* 7036-41.

Tang, S. S., et al., (2019). ERalpha and/or ERbeta activation ameliorates cognitive impairment, neurogenesis and apoptosis in type 2 diabetes mellitus mice. *Exp Neurol., 311,* 33-43.

Tao, Z., et al., (2015). Epidemiological Perspectives of Diabetes. *Cell Biochem Biophys., 73,* 181-5.

Taylor, J. S., et al., (2005). A systematic review of the literature associating breastfeeding with type 2 diabetes and gestational diabetes. *J Am Coll Nutr., 24,* 320-6.

Thomas, N. J., et al., (2018). Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. *Lancet Diabetes Endocrinol., 6,* 122-129.

Thunander, M., et al., (2008). Incidence of type 1 and type 2 diabetes in adults and children in Kronoberg, Sweden. *Diabetes Res Clin Pract., 82,* 247-55.

Tupola, S., et al., (2004). Comparison of regional cerebral perfusion, EEG and cognitive functions in type 1 diabetic children with and without severe hypoglycaemia. *Eur J Pediatr., 163,* 335-6.

van Agtmaal, M. J. M., et al., (2018). Prediabetes Is Associated With Structural Brain Abnormalities: The Maastricht Study. *Diabetes Care., 41,* 2535-2543.

van Duinkerken, E., et al., (2018). Accelerated executive functions decline and gray matter structural changes in middle-aged type 1 diabetes mellitus patients with proliferative retinopathy. *J Diabetes., 10,* 835-846.

Vignisse, J., et al., (2017). Thiamine and benfotiamine prevent stress-induced suppression of hippocampal neurogenesis in mice exposed to predation without affecting brain thiamine diphosphate levels. *Mol Cell Neurosci., 82,* 126-136.

Vijayakumar, P., et al., (2017). HbA1c and the Prediction of Type 2 Diabetes in Children and Adults. *Diabetes Care., 40,* 16-21.

Wakabayashi, T., et al., (2019). Differential effects of diet- and genetically-induced brain insulin resistance on amyloid pathology in a mouse model of Alzheimer's disease. *Mol Neurodegener., 14*, 15.

Wake, M., et al., (2000). The Child Health Questionnaire in children with diabetes: cross-sectional survey of parent and adolescent-reported functional health status. *Diabet Med., 17*, 700-7.

Wang, C., et al., (2018). [Impairment of spatial learning and memory and changes of oxidative stress in hippocampus from Type 1 diabetic mice]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban., 43*, 469-474.

Wang, K. C., et al., (2012). Risk of Alzheimer's disease in relation to diabetes: a population-based cohort study. *Neuroepidemiology., 38*, 237-44.

Wessels, A. M., et al., (2006). Voxel-based morphometry demonstrates reduced grey matter density on brain MRI in patients with diabetic retinopathy. *Diabetologia., 49*, 2474-80.

Wild, S., et al., (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care., 27*, 1047-53.

Willumsen, J. & Bull, F. (2020). Development of WHO Guidelines on Physical Activity, Sedentary Behavior, and Sleep for Children Less Than 5 Years of Age. *J Phys Act Health., 17*, 96-100.

Wodrich, D. L., et al., (2011). Type 1 diabetes mellitus and school: a review. *Pediatr Diabetes., 12*, 63-70.

Xia, E. Q., et al., (2017). Marine Peptides as Potential Agents for the Management of Type 2 Diabetes Mellitus-A Prospect. *Mar Drugs., 15*.

Yates, K. F., et al., (2012). Impact of metabolic syndrome on cognition and brain: a selected review of the literature. *Arterioscler Thromb Vasc Biol., 32*, 2060-7.

Yi, S. S., et al., (2009). Effects of treadmill exercise on cell proliferation and differentiation in the subgranular zone of the dentate gyrus in a rat model of type II diabetes. *Neurochem Res., 34*, 1039-46.

Yokono, K. (2006). [Possible role of mitochondrial dysfunction in insulin resistance in the elderly]. *Nihon Rinsho., 64*, 39-44.

Zelena, D., et al., (2006). The role of vasopressin in diabetes mellitus-induced hypothalamo-pituitary-adrenal axis activation: studies in Brattleboro rats. *Brain Res Bull.*, *69*, 48-56.

Zhang, F., et al., (2019a). Augmented Brain Infiltration and Activation of Leukocytes After Cerebral Ischemia in Type 2 Diabetic Mice. *Front Immunol.*, *10*, 2392.

Zhang, J., et al., (2017). An updated meta-analysis of cohort studies: Diabetes and risk of Alzheimer's disease. *Diabetes Res Clin Pract.*, *124*, 41-47.

Zhang, S. & Murphy, T. H. (2007). Imaging the impact of cortical microcirculation on synaptic structure and sensory-evoked hemodynamic responses *in vivo*. *PLoS Biol.*, *5*, e119.

Zhang, Y., et al., (2019b). Altered brain structural topological properties in type 2 diabetes mellitus patients without complications. *J Diabetes.*, *11*, 129-138.

Zhao, H., et al., (2017). Hydrogen sulfide improves diabetic wound healing in ob/ob mice via attenuating inflammation. *J Diabetes Complications.*, *31*, 1363-1369.

Zhao, J., et al., (2015). Novel metabolic markers for the risk of diabetes development in American Indians. *Diabetes Care.*, *38*, 220-7.

Zhao, W. Q., et al., (2008). Amyloid beta oligomers induce impairment of neuronal insulin receptors. *Faseb j.*, *22*, 246-60.

Zhou, B., et al., (2016). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4· 4 million participants. *The Lancet.*, *387*, 1513-1530.

Ziegler, A. G., et al., (1999). Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes.*, *48*, 460-8.

Ziegler-Waldkirch, S. & Meyer-Luehmann, M. (2018). The Role of Glial Cells and Synapse Loss in Mouse Models of Alzheimer's Disease. *Front Cell Neurosci.*, *12*, 473.

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Chapter 2

THE NEUROPSYCHOLOGICAL PROFILE OF CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDER (FASD)

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ABSTRACT

Prenatal exposure to alcohol can create a spectrum of neurocognitive impairments in developing children. The presentation of FASD can vary considerably in severity from mild, to severe cognitive, behavioral, emotional and physical abnormalities. In this review chapter we will focus on neurocognitive deficits frequently present in children with FASD, and the clinical implications in relation to these deficits. The prognosis is better for affected children and young people who receive early diagnosis and appropriate support, than in those for whom support is not provided. It is, therefore of central importance to increase knowledge and understanding of this preventable neurodevelopmental disorder and how best to manage and support children and families affected.

In this chapter, we will focus on reviewing the following issues:

- The typical neurocognitive deficits associated with alcohol exposure in pregnancy;
- The relationship between FASD and Attention-Deficit Hyperactivity Disorder (ADHD), and FASD and Autism Spectrum Disorder (ASD);
- The combined effects of alcohol and drug abuse

1. FASD: THE SCALE OF THE PROBLEM

Fetal Alcohol Spectrum Disorder (FASD) may be regarded as one of a kind in the neurodevelopmental world, such that it is a disorder which is entirely preventable, and yet remains one of the leading causes of neurodevelopmental disability across the globe [1]. There appears to be a common misconception that due to the socially acceptable nature of alcohol use, it has been assumed to be relatively benign in its effects in comparison with more illicit substances. The reality however, as reported in the American Academy of Pediatrics (2013), is that of all recreational and medically prescribed drugs, alcohol has by far the most significant and teratogenic impact upon the development of the human fetus [2].

Alcohol consumption in women of childbearing age is common worldwide [3]. The Institute of Alcohol Studies in the UK, providing data from the 2014 Health Survey for England, indicates that 80% of women have

drunk alcohol in the last twelve months, compared with 87% of men. While alcohol use and associated harm have historically been more prevalent in men than in women, this gender gap appears to have closed in recent years. A 2016 analysis of 68 international studies, combined with a sample size of over four million people, found that male to female ratios of alcohol use and related harm have shrunk dramatically over time to a more equal picture across both genders.

In 2019 SIGN 156, the National Clinical Guideline in Scotland for children and young people prenatally exposed to alcohol [4], reported that in Scotland most women of childbearing age drink alcohol regularly, although there has been a drop from 87% in 2003 to 82% in 2017. The abstinence rate among women between the ages of sixteen and thirty-four is reported to be 18%, falling to 13% among thirty-five to forty-four-year old. SIGN 156 Guideline has also estimated that approximately 3.2% of babies born in the UK are affected by FASD, which is roughly 3-4 times the prevalence of autism [4].

Popova et al. suggest that while the prevalence of prenatal alcohol exposure (PAE) is very high in many western countries, the UK has one of the highest rates of drinking during pregnancy, at over 40% [3]. This is consistent with data described within the SIGN 156 Guideline. The SIGN Guidelines refer to a recent study in Glasgow, analyzing the meconium of neonates. It was established that 42% of meconium samples showed evidence of prenatal exposure to alcohol, with 15% of those pregnancies having been exposed to very high levels.

There is some evidence that women's drinking behaviour prior to becoming pregnant may predict alcohol use during pregnancy. A study within the International Journal of Obstetrics and Gynaecology is suggestive of this pattern. Of 1969 women in the study, 82% continued to consume some alcohol during pregnancy [5]. It was established that women who drank weekly prior to becoming pregnant were 50% more likely to continue drinking during pregnancy, compared with those who drank less frequently. Where women had experienced fertility problems however, there was a 36% reduced likelihood of alcohol consumption during pregnancy. This would suggest that despite a generally poor understanding the risks involved in

prenatal alcohol exposure, when achieving a pregnancy has been more challenging, the willingness to take any level of perceived risk, including alcohol consumption, may be lower. A recent study [6] analyzed risk perception of alcohol use during pregnancy in a random sample of 426 women in their mid-trimester of pregnancy and found that only 48.1% of the women were aware that any damage caused by prenatal alcohol exposure (PAE) is permanent. There was also a misconception that drinks such as wine and beer are less 'risky' than their stronger counterparts. Younger age and lower educational level predicted a lower risk perception of alcohol use during pregnancy, particularly regarding drinking beer.

McQuire et al. (2019), in a population-based birth-cohort study using an FASD screening algorithm based upon Canadian Guidelines for diagnosis in one region of the UK, found that 6% of children screened positive for FASD in analysis using a single imputation method, 7.2% in complete case analysis, and 17% in the analysis with multiple imputed data [7]. A positive FASD screen was more likely in children of lower socio-economic status and for those whose pregnancies were unplanned. The results from this study indicate that many previous efforts at establishing prevalence, based upon complete case and single imputation methods, may have provided a considerable under-estimate of the prevalence of FASD.

One population of children who may be at particular risk of FASD are children in care and those who go on to be adopted. This is supported by Popova et al. [3], whose systemic review and meta-analysis found that children in care (in addition to those in other subpopulations, such as those in special educational provisions and young offenders' institutions) were at a 10-40 times higher risk of having FASD than children within the general population. A 2018 article published on the website *AdoptionUK*, refers to 'a hidden epidemic,' quoting that 'three quarters of looked after children are at risk' of having FASD, with those children who have an established diagnosis being the 'tip of the iceberg' in terms of the true extent of the problem within this group of children [8].

Nonetheless it must also be considered that the 'middle-class wine-drinking' population are also at risk of unwittingly harming their unborn babies through inaccurate perceptions of risk with regards to their wine-

drinking behaviour [6]. Some studies, such as Anderson et al. [5], in fact found that women of lower economic status may be less likely to drink alcohol during pregnancy compared with women of higher economic status, which contradicts other findings.

Despite the high levels of PAE reported across a variety of populations, there has historically been an absence of high-quality prevalence studies, which has meant the true prevalence of FASD in the UK (and in other countries) has remained an unknown.

The key message is that alcohol use during pregnancy affects women and babies across all socio-economic groups, which indicates a need for healthcare professionals within antenatal services to be explicit and consistent in their message about PAE to women from all walks of life. The figures that have been evolving in recent years demonstrate that FASD is a significant public health concern at a global level.

2. FASD AS ORGANIC BRAIN DAMAGE

Alcohol consumed during pregnancy enters the mother's bloodstream and passes directly through the placenta and into the fetus' bloodstream and amniotic fluid [9]. The enzymes that metabolize alcohol during adulthood are not sufficiently functional in a developing fetus, and therefore the main method of elimination available to the fetus is to transfer alcohol back into the mother's bloodstream. However, this method is restricted by alcohol-associated vasoconstriction, and therefore fetal elimination of alcohol only occurs at 3-4% of the mother's, leading to prolonged exposure in the fetus [10].

Alcohol disrupts fetal development in a number of ways, including inducing direct apoptotic cell death [9] and also by altering gene expression [11] as will be described in a later section.

The diagnostic criteria for FASD include evidence of prenatal alcohol exposure, the demonstration of structural or functional central nervous system abnormalities, a specific pattern of three facial features (if present), and possibly also prenatal and/or postnatal growth impairment. The variable

pattern of these effects creates the spectrum of physical, neurocognitive and behavioural impairment which is commonly known as Fetal Alcohol Spectrum Disorder. It is important to note that roughly only one in ten affected children will present with the sentinel facial features associated with alcohol exposure, and therefore neurocognitive and behavioural deficits should be prioritised over presence or absence of facial features during assessment [12].

A recent and widely used algorithm for assessing FASD has been created by the Scottish Intercollegiate Guidelines Network (SIGN), which was formed in 1993. The SIGN Guideline for assessing and diagnosing FASD (SIGN 156) has mirrored older Canadian Guidelines for FASD diagnosis by creating two key diagnostic titles under the FASD umbrella, namely FASD with sentinel facial features and FASD without sentinel facial features. Sentinel facial features, as described in the SIGN Guideline, are palpebral fissures measuring $<5^{th}$ centile, and lip and philtrum at either grade 4 or 5 on the University of Washington Lip-Philtrum Guide [13]. Any other facial markers are regarded as secondary and are not relevant diagnostically. Facial features are only considered to be of clinical significance when FASD is strongly suspected but alcohol exposure is unknown. In this case, where there is the presence of all three sentinel facial features, diagnosis may be made regardless of unconfirmed history due to the specificity of all three features together to alcohol exposure [4].

The neurocognitive profile of FASD is not in itself unique or specific, which is likely to be why it is so commonly missed or misdiagnosed [14]. One of the most challenging aspects and preclusions to accurate and timely diagnosis is that its presentation is a great mimicker of many other neurodevelopmental and behavioural conditions [15]. For this reason, many prenatally exposed children and young people can end up collecting multiple diagnoses over their childhoods that each perhaps explain some aspects of their symptomatology, but never quite fit entirely.

FASD must be understood as a diagnosis of exclusion, with any possible confounding genetic, environmental or organic factors established as a part of the assessment process [16]. This can create its own challenges, because statistically children with FASD tend to have experienced more adverse

childhood events than other children [17]. Therefore, their cognitive and behavioural difficulties can often be attributed solely to attachment related difficulties, which then shuts down other possible organic aetiological avenues for further exploration.

Common diagnoses that children with FASD can often be suspected of prior to accurate diagnosis are attachment disorder, dyslexia, dyscalculia, unspecified learning difficulties, autism and ADHD. Many of these individual diagnoses may well apply, particularly in the case of autism and ADHD, but with the aetiological diagnosis being FASD. Sometimes other diagnoses may be given by health professionals and allied health professionals, with the hope of a child accessing support by having them, and in the absence of any other means of explaining the child's difficulties. However, these can sometimes prove misleading if they are given without a broader assessment of the full neurocognitive profile in relation to the child's history. For example, dyscalculia may be used to explain a child's problems with mathematics, when in fact this may be due to wider working memory and executive functioning deficit caused by PAE.

Autism spectrum disorder and ADHD are common comorbidities in children with FASD. Of those with FASD, up to 74% meet the criteria for ADHD and up to 68% meet the criteria for secondary autism spectrum disorder or social communication disorder [18]. The reason these are significant comorbidities is that both conditions are comprised of disorder in the functioning of the prefrontal cortex, which houses inhibitory control and self-monitoring systems. This will be discussed further.

FASD must be understood as organic brain damage in order to appreciate the permanency of its neurocognitive effects and, therefore, to enable families and services to plan appropriately for the diagnosed child's future. While much can be done to improve and enable those with FASD to lead rich and fulfilling lives, this will largely depend upon the successful adaptation of the environment and scaffolding around the individual's deficits in order to keep them safe and to enable them to function optimally.

Although prenatal alcohol exposure causes permanent brain damage, neuroimaging for the quantification of this is not currently indicated as a necessity in the diagnosis of FASD [19]. This is not only due to financial

constraints with regards to fund-limited public services, but also due to the fact that where there is quantifiable structural abnormality in relation to PAE, the pattern and nature of the abnormality seen is diverse, nonspecific and at times misleading. Therefore, further work would need to be completed in this area before MRI can be used successfully to at least augment FASD diagnosis [19]. For the majority of individuals with FASD, their brain structure at a macro structural level will appear normal on structural MRI, even if brain connectivity and white matter integrity is implicated. Therefore, the structural MRI most commonly offered in public health services would not prove informative for families, and nor would it affect planning for future support.

Amongst the most consistent findings in brain imaging studies of FASD is of overall volume reduction [20]. Some studies also document reduction in frontal, temporal, parietal and to a lesser extent, occipital lobes in children in FASD compared with controls [19]. Frontal and parietal lobes appear to be most sensitive to the teratogenic effects of alcohol exposure. This is consistent with domains of greatest neurocognitive deficit, such as executive functioning impairment (whose neural substrate lies predominantly within the prefrontal cortex) and sensory, visual spatial and mathematical processing, originating primarily in the parietal lobes [21].

In addition to the analysis of the entire cortex, more focused studies have identified specific structures that are differentially affected by prenatal alcohol exposure. Abnormalities of the corpus callosum, the major white matter tract connecting the two cerebral hemispheres, have been reported in individuals with FASD, including both volume reduction particularly in the most anterior and posterior sections, displacement, in a minority of cases, complete agenesis [22]. Abnormalities in the corpus callosum have been associated with several domains of neuropsychological function that are frequently impaired in those with FASD, such as motor function, attention, verbal learning and executive functioning [23,24].

The cerebellum appears to be another structure that is particularly vulnerable to the teratogenic effects of prenatal alcohol exposure, again with decreased volume observed in studies in individuals with FASD compared with controls [25]. The caudate nucleus within the basal ganglia has also

been implicated in FASD structural abnormality and observed volume reduction has predicted performance in neuropsychological measures of inhibition and verbal learning [25]. These findings may be suggestive of abnormal fronto-subcortical networks in individuals with FASD which are consistent with observed neuropsychological and behavioural manifestations of executive functioning deficit [26]. The basal ganglia also maintain connections with motor cortices, which may also have implications for commonly observed fine motor skill impairment in children with FASD [27].

Diffusion Tensor Imaging (DTI) offers a detailed examination of white matter fibres within the brains of individuals with FASD. Diffusion offers information at a microstructural level by characterising white matter tissue organisation [21]. Some studies [24,28] have demonstrated alcohol-related damage to the corpus callosum at microstructural level, particularly abnormalities in white matter structure in posterior regions. DTI studies have further shown that white matter abnormality extends to wider brain regions in the alcohol-exposed population, such as damage to tracts with temporal connections involving language and visual processing [28]. A reduction in integrity within these tracts could underpin language and visuospatial impairments. Also pertinent are studies demonstrating reduced white matter integrity and organisation in superior frontal regions [29], which may again be associated with the dysexecutive presentation observed in individuals with FASD. Furthermore, tracts connecting the occipital lobe with inferior frontal and parietal lobes have been observed, which may be associated with deficits in visual spatial skills, attention, and visual working memory in those with FASD [30].

Functional MRI, measuring the blood oxygen level dependent signal, which is an indirect measure of neural activity, provides another means of accessing microstructural damage in those with prenatal alcohol exposure. Studies have found functional differences in terms of neural activation in the frontal lobes between those with FASD and controls in tests of working memory, verbal learning and an inhibition-related task [19,31].

3. Neurocognitive Deficits in FASD

While there is wide variation in findings regarding the level of damage sustained to neurocognitive functioning following prenatal alcohol exposure in the decades since Fetal Alcohol Syndrome was first described [32], a reasonably consistent picture of cognitive and behavioural deficits and relative strengths has emerged through the observation and psychometric assessment of children with PAE. A recent study [15] asserts that the reason FASD remains a diagnostic challenge, however, is its high rate of psychiatric comorbidity and also a profile that may be neurodevelopmentally and behaviourally indistinguishable from other neurodevelopmental disorders, at least without an accurate prenatal and family history. While findings from this study may have been limited by insufficient neuropsychological assessment measures, it nonetheless emphasises that the history-taking aspect of the diagnostic process is of vital importance in FASD.

At a macro level one of the most common deficits seen in children with FASD is a reduction in general intellectual functioning. Multiple studies have shown consistently lower IQs in children prenatally exposed to alcohol compared with controls [33]. The lower IQ observed in children with FASD is associated with lower academic attainment, which tends to translate into poorer life opportunities [34]. It has been established that children with sentinel facial features tend to have an IQ in the borderline range (70s), whereas children without sentinel facial features tend to have an IQ in the low average range (80s) [35]. However, children with PAE can have IQs across the entire bell curve, from the moderate/severe learning disability range, up into the superior range [36,37]. There are often discrepancies between domains which create a ‘spiky’ profile, although the direction of the discrepancy is not consistent. Some children present with significantly poorer verbal comprehension skills compared with nonverbal ability, while others may present in the opposite way [38]. Those children with a high IQ are generally still unable to achieve and function in the manner that such a high IQ would suggest, due to other specific deficits associated with PAE, particularly impairment in executive functioning, adaptive function, and language. Greenspan, Brown and Edwards introduced the concept of

'intellectual disability equivalence' in 2016, to emphasise that while an individual with FASD may appear to have a 'normal' IQ, their neuropsychological deficits (particularly their poor executive and adaptive functioning) means they do not have the ability to function 'normally' [39]. The term is intended to free services from the 'strait-jacket' imposed by over-reliance on full-scale IQ scores, which have served as a barrier to families for being eligible to access services and support. The term also has important implications legally, for young people with FASD who find themselves in trouble with the law.

Executive functioning is consistently described as a key impairment in children with FASD [40]. The term Executive Functioning defines a skillset whose neural substrate lies within the prefrontal cortex and may colloquially be described as the managing director, or the self-monitoring system, of the overall cortex. Clinically it can be useful to distinguish between 'hot' and 'cold' executive functions as a means of differentiating between two core domains of executive functioning, both of which are generally impaired in those with FASD. Children with hot executive functioning deficit have difficulties with emotion regulation, and therefore, present with many externalising difficulties such as disinhibited and inappropriate behaviour without insight, poor social relationships, agitation and/or hyperactivity [41]. Cold executive functioning problems include poor working memory, poor sustained attention, and difficulties with planning and organisation, multi-tasking and sequencing behaviours [42].

Executive functioning can be assessed directly using tests such as the Behavioural Assessment of the Dysexecutive Syndrome for Children (BADS-C) [43], or the Delis-Kaplan Executive Function System (D-KEFS) [44]. Parental report measures can also be used alongside direct assessments, such as the Behaviour Rating Inventory of Executive Function (BRIEF) [45]. It is important to observe executive functioning directly in addition to parental reporting. Direct assessment of executive function can be completed from around seven years onwards. Prior to this age, it is more challenging to separate out generalised learning disability from a dysexecutive presentation. Furthermore, for children with FASD, their impairment in executive functioning typically becomes increasingly explicit

from year one onwards in school, as the gap between themselves and their peers begins to diverge over time.

A key period of difficulty for many children with FASD is the point at which they move to secondary school. By this time, their typically developing peers will have made leaps in progress with their executive functioning skills, being increasingly independent in their learning, their ability to organise themselves and their belongings, and their ability to manage their timetable without support. They are more socially aware and able to build and maintain friendships independent of their parents. Those with executive functioning impairment due to FASD will lag significantly behind with these skills, and some will never fully develop. Executive functioning impairment is an enduring and permanent feature rather than a delay that will eventually 'catch up' [41]. This explains why adults with FASD frequently struggle to manage activities of daily living fully independently, and are at increased risk of unlawful behaviour due to their continuing social vulnerability and an inability to make safe and considered decisions as a result of impaired executive functioning [46].

Executive functioning drives adaptive functioning and adaptive behaviour [47]. Adaptive functioning refers to those skills that are necessary for us to navigate through the demands that are placed on us by our environments in a way that is effective. It includes skills such as the ability communicate effectively with others, and to keep oneself safe in social relationships. Young people and adults with FASD frequently have poor adaptive functioning, which makes them socially vulnerable and at risk of manipulation and coercion by others [48]. The presence of executive (and therefore adaptive) functioning impairment may be one of the most difficult aspects of parenting children with FASD for these reasons [49].

Language impairments are common in children with FASD, although the pattern of impairment is not uniform [50]. This may be reflective of the wide variation in neurobiological insult created by prenatal alcohol exposure [51]. Language problems frequently documented are poor receptive language, pragmatics, grammar, semantics, speech production and expression [38]. There is some suggestion that, like executive and adaptive functioning, language deficits may become more noticeable in relation to

typically developing peers as a child grows into young adulthood [52]. This is particularly the case for semantic and pragmatic understanding.

Deficits in spatial and verbal memory [53], visual spatial ability [54] and sensory processing [55] have also been observed, demonstrating the wide range of deficits in children with FASD.

Social cognition is a key aspect in the presentation of children with FASD, which bridges both cognitive and behavioural aspects of the condition. Children with FASD frequently struggle with peer relationships as they are limited in their understanding of and ability to respond appropriately to social cues. They tend to be described as socially and emotionally immature, with the poor social judgement and decision-making that is consistent with very young children [56]. These difficulties with social cognition tend to follow a similar trajectory over time as executive functioning does, such that as a child becomes older, the gap between themselves and their peers widens and their social deficits become increasingly apparent [57]. The two domains are inextricably linked, which is demonstrated by poor performance in tests of empathy and theory of mind and correlates with deficits in wider executive functioning [58]. Relatedly children with FASD also frequently present with a variety of other externalising behaviours, such as confabulation [59], sleep problems [60], stealing [61] and also internalising behaviours such as self-harm, depression, or anxiety [62].

Overall, the literature indicates that the combination of poor executive functioning, poor social cognition. and language difficulties represent a triad of impairments that result in children with FASD having limited good quality friendships and poor social relationships [63].

4. The Relationship between FASD, ADHD and ASD Comorbidities

4.1. ADHD

The current literature suggests that ADHD is frequently a comorbid secondary diagnosis in children with FASD, being diagnosable in roughly

74% of cases [2, 18]. The manner in which ADHD presents when it is secondary to FASD appears to have some differences to that of a primary ADHD presentation.

The evidence base suggests a considerably greater deficit in executive functioning in groups with FASD and comorbid ADHD, compared with those who have a single diagnosis or with typically developing controls [64]. Glass et al. [65] observed that alcohol exposed groups of children perform significantly less accurately on executive functioning tasks, make increased errors, had longer response latencies, and increased variability in their response times. Executive functioning deficits occur in both children with PAE and primary ADHD clinical groups, but the degree and pattern is observed to differ between alcohol-exposed and non-exposed groups when the primary aetiological diagnosis is considered. Children with PAE showed greater executive functioning impairment than children with primary ADHD when compared on measures of planning, fluency and set shifting [66]. This finding may indicate that appropriate neuropsychological measures assessing these executive functioning domains ought to contribute to accurate differential diagnosis and treatment. Glass et al. (2014) also identified that inattention may be a more defining factor than hyperactivity in children with PAE compared with children with primary ADHD [66].

The core symptoms of ADHD are inattention, hyperactivity, and impulsivity. This results in difficulties such a poor sustained attention on tasks, poor listening skills, poor planning and organisation, distractibility, overactivity, excessive talking, and poor social skills including reciprocal play and conversation. While these symptoms are also present in children FASD without comorbid ADHD, those who have combined FASD and ADHD (that is roughly three quarters of the FASD population) will be more severely affected by these symptoms, which will impact upon functioning to a greater extent.

Ware et al. found that adaptive functioning was poorer in children with FASD and comorbid ADHD, when compared with children with primary ADHD (and no FASD) and children with FASD with no ADHD diagnosis [67]. Children with FASD and ADHD scored poorer on measures of attention, somatic problems, and other psychiatric measures. Children with

ADHD caused by PAE appear to do worse in all general measures of IQ and executive functioning when compared to those with primary ADHD or typically developing peers [66].

Malisza et al. used fMRI to investigate spatial working memory in children with PAE, children with primary ADHD, and a control group of neurotypical children [68]. Children with PAE demonstrated increased activity, associated with reduced accuracy and increased response time variability, suggesting that the brains of children with PAE have to work considerably harder in order to manage a short-term memory load, compared with children with primary ADHD or typically developing children. The fMRI results demonstrated greater intra-subject variability in PAE and ADHD groups by region of interest. DTI studies also showed that the typically developing control group of children had significantly higher total tract volume and number of fibres compared to the PAE group. This study substantiates earlier discussion with regards to PAE causing loss of white matter connectivity and integrity, likely through apoptosis. Children with primary ADHD do not appear to demonstrate the same level of white matter neural damage.

In summary, children with FASD meet the criteria for a secondary diagnosis of ADHD in roughly three quarters of cases. The presentation of secondary ADHD may be different to that of children with primary ADHD and no PAE. Children with FASD and comorbid ADHD are found to have poorer neurocognitive, social, behavioural trajectories than children with primary ADHD and neurotypical peers [69]. Their executive and adaptive functioning is also poorer [70]. This again highlights the importance of seeking accurate diagnosis and support for children with PAE. It also indicates that level of functional impairment may be an effective means of providing accurate diagnosis of FASD compared with differential diagnoses.

4.2. Autism Spectrum Disorder (ASD)

The comorbidity in relation to FASD and ASD is less clear, and there are conflicting findings within the current literature, which is perhaps

reflective of the current climate of ASD diagnosis more globally. It is also compounded by the often-young age of those being diagnosed and studied. It is undeniable however, that specific ASD symptoms often occur when PAE is present. Lange et al. (2018) estimated that ASD in children with PAE is 2.6% higher than the general population [71]. Studies of a specific clinical population – such as in [2], based upon a cohort from a national FASD clinic – indicate a high comorbidity of FASD and ASD, with ASD being diagnosable in 68% of the children meeting the criteria for FASD diagnosis. In 2011 Mukherjee et al. completed a similar study, finding that of 21 children with FASD, 16 (72%) met ICD-10 criteria for childhood autism.

McGee et al., looked at social information processing skills in children with PAE and demonstrated that these are frequently poor in those with PAE compared with unexposed controls [72]. Children with PAE also demonstrate poorer personal and social skills [61] in line with generally poor social cognition.

There is some evidence that while diagnostic criteria may be met, a child with ASD where FASD is the aetiological diagnosis may present differently to a child with autism as their primary diagnosis. Larsson et al. (2005) found that children with primary ASD tended to have greater difficulties with initiating social interaction, sharing affect and use of nonverbal communication than children with PAE [73]. The social deficits in children with FASD are often more related to poor reading of social cues and over-familiarity [73]. Socially inappropriate behaviours and difficulty with peer relationships were common in both groups. Children with FASD also experience deficits in sensory processing, which is a common occurrence in ASD and suggests that children with FASD that co-occurs with a sensory processing deficit may struggle to respond adaptively to their environment [74].

Mukherjee et al. (2011) found in a nested study comparing children with FASD and ASD that although the children in the FASD group met the criteria for autism, they were not "classically" autistic, with the study finding that they were more passive, or active but unusual, in their social functioning [75]. The FASD group were also more likely to be bullied and reported to lack common sense. Significant differences were found between the alcohol

exposed group and the non-alcohol exposed group, with the non-exposed group being less likely to show co-ordination difficulties and less likely to avoid social engagement with peers or participation in activities that involved teams.

In summary, there is evidence that social communication disorders present differently to primary ASD in a similar way to the trajectory of primary ADHD compared with ADHD where PAE is present [75]. Nonetheless, if a child meets the criteria for ASD through their social communication and social interaction difficulties, then they would benefit from being understood by comorbid diagnosis.

It is pertinent to note that the comorbidities associated with FASD and indeed FASD itself appears to vary across cultures. From the current literature, it can be speculated that there are different cultural relationships with alcohol and its consumption, and this is reflected in spikes of FASD diagnoses. Studies of subpopulations have found that groups such as the 'aboriginal population' have significantly higher rates of FASD when compared with the general population [76], while FASD was also found to be extremely common in a follow up study of children adopted from Eastern Europe (95% also had comorbid neurodevelopmental and cognitive disorders, with 9% being found to have ASD [77]. It seems that the tendency of specific cultures and ethnicities to eschew alcohol consumption, for religious or other reasons, underpins global inter-cultural variations in FASD presentation. A global study of alcohol consumption indicates that the European region had the highest amount of alcohol use and also the highest rates of FASD, while regions such as the Eastern Mediterranean and South East Asia were found to have the lowest. The African region, the Americas, and the Western Pacific Region were relatively high for FASD rates, but lower than the European region, which is consistent with current cultural alcohol use [3].

5. FASD AND POLYSUBSTANCE EXPOSURE

Many babies who have been prenatally exposed to alcohol have also been exposed to other substances. It has been found in a study of stool and

hair samples of neonates who were prenatally exposed to heavy alcohol use, that these babies were also 3.3 times more likely to have also been exposed to amphetamines than those with no prenatal alcohol exposure, and twice as likely to have been exposed to opiates [78]. Similarly, Astley et al. established that, among 1400 patients with prenatal alcohol exposure attending the Washington FASD diagnostic clinic, 62% were also prenatally exposed to tobacco, 37% to cannabis, and 38% to crack cocaine [79]. Therefore for many children who go on to be diagnosed with FASD, their presentation will be complicated by poly-substance exposure.

Mukherjee et al. in 2013, highlighted that while alcohol is the most teratogenic substance to the developing fetus, the teratogenic effects are often enhanced by the use of multiple substances, such as cocaine, tobacco, and cannabis [2]. It is possible to characterise in a reasonably precise fashion the means by which a single toxin is absorbed by the body and how it is distributed, metabolised and eliminated. If another substance is consumed at the same time, these pharmacokinetic processes may be altered in unpredictable ways by each of the substances ingested. In addition, the combination of drugs can lead to the formation of active metabolites even more toxic than the substances originally consumed. It is also important to note that when a mother is engaging in substance misuse during pregnancy (alcohol and/or other drugs), it is likely that she will also present within an inappropriate environment for raising a baby, which further increases the rates of perinatal morbidity and mortality [80].

The combined effects of several substances are considered below, with regards to their teratogenic properties when combined with alcohol during pregnancy.

5.1. Caffeine

Caffeine is the most widely consumed psychoactive substance in the world, including among pregnant women. During pregnancy, the half-life of caffeine triples and concentrates in the developing brain of the embryo [81]. The consumption of two or more cups of coffee per day is believed to

generate a significant reduction in blood flow through the placenta, which can trigger an increase in the production of catecholamines, potentially interfering with cerebral development.

It has been observed in studies conducted using non-human subjects, that early exposure to high levels of caffeine affects the construction and activity of a newly born animal's cortical networks. Specifically, it has been proven that caffeine affects the construction of GABAergic neuron networks in the primary visual cortex, chosen as a neocortical zonal model. This is due to a reduction in the number of GABAergic neurons analysed in the postnatal period [82], which has been found to influence the recognition of objects in animals [83]. Because of this it is possible that chronic and high exposure to caffeine during pregnancy may alter the cognitive functionality of adult animals. If caffeine and alcohol are consumed concurrently during pregnancy, the former appears to exacerbate he deleterious effects of the latter [81]. However, there currently exists very little information in this regard. There is therefore a need for more studies to be conducted which evaluate the impact of combined caffeine and alcohol consumption during pregnancy, given that this combination of substances is likely to be extremely common in women who consume alcohol during pregnancy.

5.2. Tobacco

Recent studies have found that tobacco has neuro-modulatory and neurotoxic effects on the brain of a developing fetus, including cell loss, cell hypertrophy and the formation of neurites, which are indicators of potential neuronal damage. These alterations can cause cognitive impairments in the fetus with respect to learning, memory and general behaviour [84].

The changes caused by tobacco within the developing brain do not only occur at a cellular level but are also detectable at a macroscopic level, with this having been linked to lower cerebral volume, lower volume of grey matter and reduced cortical thickness in frontal, superior and precentral parietal areas [85]. It is calculated that the reduction in cerebral volume in children of women who smoked during pregnancy has the effect of reducing

IQ by up to fifteen points. However, these differences appear to reduce over time as the child grows and develops [86], probably due to biological mechanisms of compensation mediated by the neurotrophic system. Nonetheless a mismatch in coordination between different brain areas (frontal, parietal, temporal and cerebellar lobes) has been found in adolescents who were exposed to tobacco during their prenatal development, which is associated with difficulties with language and writing [87], indicating that some adverse effects are more enduring over time. It has also been proposed that early prenatal exposure to tobacco produces a lower response of the ventral striatum to the anticipation of reward, which makes these children and young people more at risk of substance abuse or addiction in the future [88]. It is therefore not the case that all neurocognitive difficulties associated with prenatal tobacco exposure vanish over time.

Regarding the combined consumption of alcohol and tobacco, it has been known for some time that the use of tobacco reduces the rate of absorption of alcohol into the bloodstream, which may encourage the pregnant mother to ingest more in order to experience the same effect, [89]. These results have also been confirmed at a pre-clinical level [90, 91]. In a recent study it was found that the combined consumption of alcohol and tobacco during pregnancy produces negative synergistic effects on the duration of pregnancy (greater number of preterm births), the child's parameters at the time of birth (height, weight, Apgar score for the general condition) and birth abnormalities such as heart defects, hypoxia and infections. These were found to be present to a greater extent than in the children of mothers who consumed alcohol or tobacco independently [92]. This may be due to the higher levels of oxidative stress generated by both alcohol and tobacco, which in this case would act synergistically in increasing individual risk [93].

5.3. Cocaine

Human studies show that children prenatally exposed to cocaine present with neurodevelopmental difficulties, even if their IQ is within the normal

range [94]. These deficits tend to become increasingly evident from the beginning of their school career, in the form of difficulties with concentration, impulsivity and/or aggressive behaviour. The neurobiological basis of these changes appears to lie in changes in cellular architecture and monoaminergic transmission. Follow-up studies conducted with children have found problems with sustaining concentration and of behavioural self-regulation, after controlling for confounding variables [95]. This pattern of neuropsychological difficulties are similar to the pattern seen in alcohol-exposed children.

As for tobacco, it has been known for some time that the concurrent consumption of alcohol and cocaine during pregnancy is more harmful than the consumption of either drug in isolation, due to the formation of a highly toxic metabolite called cocaethylene [96]. This metabolite creates a greater feeling of euphoria and well-being compared to the individual consumption of cocaine, and reduces the level of sedation produced by alcohol [97]. In studies in vitro, it has been observed that cocaethylene is neuro-teratogenic to the developing brain of the fetus, with this being of greater magnitude than the neuro-teratogenic effects of cocaine and alcohol consumed separately [98].

In a study performed with animals in comparable groups, it was observed that pregnant rats receiving alcohol and cocaine during pregnancy gave birth to offspring of lower birth weight compared to the group receiving alcohol or cocaine in isolation [99]. It was also noted that the former group had a greater number of fetal abnormalities than any of the other groups, although the effects on cognitive performance of exposure to both drugs in utero were not evaluated.

5.4. Opiates

It is estimated that one in every thousand babies has been exposed to opiates prenatally, which tends to be frequently accompanied by the consumption of other substances, including alcohol [81]. More evidence exists with respect to the consumption of methadone during pregnancy, with

the relevant studies being more controlled due to its consumption being legalised and protocolled. It has been observed that methadone in itself does not cause major malformation [100], although it has been found in preclinical studies to alter the cognitive performance and social capacities of offspring [101]. The offspring of rats exposed to opiates during pregnancy tended to present with cognitive alterations in the areas of memory and learning [102]. In humans, neurocognitive performance has been assessed over the first three years of the lives of children with mothers on methadone replacement therapy. The authors of this study found that the cognitive development of these children was normal throughout the 3 years of follow-up [103]. In general, there seem to be mixed results in relation to the neurocognitive effects of isolated opiate exposure during pregnancy. While some studies find significant cognitive deficits in exposed children, other authors do not report such effects, or find that they disappear when covariates were controlled for [104].

At a neurobiological level, however, the evidence is more compelling. It is known that neurons such as astroglia and oligodendrocytes express opioid receptors, and, therefore, a stimulation of these receptors can influence the proliferation of these cells and myelination processes [105]. The role of myelin goes beyond the coating of axons to facilitate synaptic transmission, since it also regulates the extension of axons and radial growth of the neuron, both very important processes for the correct connection between neurons. Neuroimaging studies of children exposed to opiates prenatally, show changes in the microstructure of white matter [106] and reduced brain volume in certain brain areas [107, 108].

There are currently very limited studies available comparing the effects of alcohol consumption by the mother on the cognitive development of a baby and the equivalent effects of the combined consumption of alcohol and opiates without the use of other substances to create confounding variables. It is known, however, that exposure of the fetus to alcohol increases the probability of their engagement in substance abuse as an adult [108] and this seems to be mediated by the endogenous opioid system [109].

5.5. Cannabis

Cannabis is one of the most widely used illegal psychoactive drugs in the world. Strangely, it is considered less harmful than other drugs, although there is ample evidence of its deleterious effects for the adult brain and even more so for the brain of the developing fetus [110]. The active substances in cannabis are called cannabinoids and the only cannabinoid with psychoactive properties is tetrahydrocannabinol (THC), which exerts its effects through the CB1 receptor located in the central nervous system. There is another type of cannabinoid receptor, CB2, but this is located in the immune system and the retina [111]. These receptors make up the so-called endocannabinoid system, and during fetal development, they are involved in processes of neuronal proliferation, neuronal migration and synapse formation. Therefore, prenatal exposure to cannabis would excessively stimulate these receptors by altering these processes. Cannabis use during pregnancy causes changes in the neurological development of the fetus, often resulting in hyperactivity, cognitive impairment and changes in dopamine receptors [112]. In two cohort studies, which conducted long-term follow-ups of children exposed to cannabis prenatally, authors found that by the age of 3, these children presented with impairments in short-term memory and both verbal and abstract reasoning [113, 114].

As cannabis-exposed children grow, impairment is most apparent in executive functioning [115]. One of the mechanisms proposed to explain the influence of cannabis consumption on the brain in formation is that it influences the expression of genes that code for a key protein in brain development, such as the L1 neural adhesion molecule that has an important role in the processes of cell proliferation, neuronal migration and synaptogenesis [115]. In addition, prenatal exposure to cannabinoids disrupts the normal development of nigrostriatal and mesolimbic dopaminergic neurons.

Few studies evaluate the effects on a child's cognition of the combined exposure of alcohol and cannabis in the womb. Those studies that do exist suggest that both substances act independently, with no apparent interaction between them [116].

5.6. Methamphetamine

In tests on animal subjects, the prenatal administration of methamphetamine has been observed to cause alterations in spatial learning and degeneration of dopaminergic nerve terminals, probably mediated by an increase in oxidative stress [117–119]. In follow-up studies of children prenatally exposed to methamphetamine, changes have been found at a cognitive level, which include problems with speech, language and mathematics, and lower overall IQ [120]. Neuroimaging studies in this regard find that the deficits in sustained attention and verbal memory in such children correlate with a lower volume in certain brain structures [121].

6. The Relationship between FASD and Developmental Trauma

Price [17], in his thesis publication on the impact of neglect on outcomes in FASD, has found that children with FASD are more likely to be exposed to adverse childhood experiences. The reasons for this are suggested below.

Children of alcoholic and/or drug-using parents are at increased risk of alcohol and substance misuse themselves, in addition to other negative emotional and behavioural outcomes, even in the absence of PAE. This has been established for some time in the literature, for example in [122]. A more recent systematic review [123] has identified that children of alcohol dependent parents demonstrate negative outcomes including internalising and externalising behavioural problems, depression and anxiety, low cognitive ability and academic achievement, ADHD, poor social and adaptive functioning, substance misuse and suicidality.

Alcohol and wider substance misuse make it more likely that children of substance misusing parents will receive neglectful and abusive care, either by omission or commission. Cash and Wilke found that mothers who misuse heroin or cocaine were twice as likely to neglect their children as mothers using other substances [124]. Friedman and Billick, in their literature review and observational study, found that the key risk factors for child neglect and

maltreatment included low socioeconomic status and education level, and even more significantly, parental alcohol and drug abuse, parental personal experiences of neglect, and parental stress [125].

The logical follow-on for many children whose parents abuse alcohol and other substances to a significant degree, are increased rates of removal into foster care and possible subsequent adoption. As has already been cited, the rates of FASD in children in care and adopted children are significantly higher than in the general population.

It is known that infants seek and require a secure base in the form of at least one primary caregiver who is able to regulate their emotional responses, provide comfort as they become older, and be a base from which to explore the world around them [126]. In the presence of a consistent and regulating primary attachment who is able to readily adapt to their infant's changing needs, a baby will continually develop, establishing new skills as it matures.

Based upon Crittenden's Dynamic Maturational Model of Attachment [125], if on the other hand the primary attachment figure is limited (cognitively or emotionally), or the demands of the context are too great, including for example the presence of domestic violence or substance misuse, the infant is left to prematurely 'fend' for themselves. Due to not being developmentally able to understand the situation they find themselves in, they are forced to take cognitive shortcuts in order to develop a 'strategy' to optimise their chances of survival in an aversive environment. For example, if a caregiver's behaviour is difficult for an infant to predict, they may rely almost exclusively on displaying their feelings in ways that demand attention, such as excessive crying, in order to increase the chances of having their needs met. Alternatively if the infant has learned that showing negative feelings such as crying or expressing need brings about either a frightening response from their caregiver or being ignored, they may learn to inhibit demonstrating their true emotions and need for nurture, and rely almost exclusively on the predictable effect of displaying a false positive affect or even no affect at all. Infants can perfect such attachment strategies as young as four months old as a self-protective mechanism. When such strategies are required, an infant's social and emotional developmental pathways begin to

diverge away from those of infants who have more balanced or secure attachment relationships with their primary caregivers [127].

More recently, neurobiological and hormonal mechanisms involved in attachment have been established to support the processes described above. Animal studies have suggested a role for endorphins, dopamine and oxytocin in the development of attachment [128] and this has been supported in human neuroimaging studies [129]. The absence of a primary caregiver or experience of neglectful parenting has been found to alter the production of and interplay of these systems, which may have long term consequences for social development and emotion regulation [130]. Abusive and frightening parenting also creates a chronic stress response in infants and children, resulting in increased release of cortisol and activation of the HPA axis. Tarullo and Gunnar suggest that prolonged activation of the HPA axis in childhood shapes the long-term behaviour of this system, leading to chronic problems for an individual concerning their stress response, ability to manage their emotions, social relationships and overall mental health [131].

Until very recently, it has often been assumed that when a child has suffered significant neglect or developmental trauma, it would then not be possible to attribute causality to a child's neurocognitive difficulties in terms of separating out the trauma they have suffered, and any organic effects of prenatal alcohol and/or other substance exposure. The English Romanian Adoptee (ERA) Study [132], which offered a rare opportunity to study the effects of extreme neglect, social and emotional deprivation in infants and children in varying ages, provided some interesting findings in this respect. One of the most significant findings was that adopted children who suffered severe neglect for six months or under within Romanian orphanages, appeared to be somewhat more resilient to neuropsychological, physical and emotional adverse outcomes than those whose experiences of neglectful caregiving continued for somewhat longer than six months. While the majority of children were initially developmentally delayed as would be expected, by six years of age when compared with adopted English controls, they had caught up cognitively and behaviourally [133]. Those who were adopted at an older age did less well and had enduring problems into adulthood, particularly with behavioural and social-emotional difficulties

[134]. Other studies of children experiencing institutionalised care have found similar results. Severe and prolonged early deprivation is associated with attachment disorder, deficits in executive functioning and memory [135] and general emotional and behavioural problems. Most significantly, the duration of institutional care is positively correlated to severity of cognitive and behavioural outcomes [136].

Very few studies appear to have investigated the dual effects of both environmental factors and prenatal alcohol exposure [135]. In his literature review, Price (2019] found only six studies had considered both factors. However, within this small number of studies to which his own has contributed considerably, the emerging pattern has been that children with both developmental trauma and FASD were more similar to children with FASD in neuropsychological outcome than children with only trauma experiences. Children with trauma backgrounds in addition to FASD had a slight increase in behavioural and conduct problems, however the main conclusions were that the impact of developmental trauma on behavioural and neurocognitive outcomes in children with FASD seemed to be very subtle, particularly in terms of cognitive functioning. Therefore, where children have a history of both trauma and prenatal alcohol exposure, children should primarily be treated as children with FASD, with appropriate support and intervention for FASD, rather than a primary focus being placed on attachment. This finding is supported by the study conducted Mukherjee et al. in 2019 based upon cohort data from a national FASD clinic in the UK [18].

The key message from these studies is that the neuropsychological outcomes in children with prenatal alcohol exposure has demonstrated that the presence of developmental trauma does not significantly alter neurocognitive outcomes and deficits for children with FASD. Therefore, the effects of developmental trauma and those of prenatal alcohol exposure may be understood as acting separately upon a child's presentation. Developmental trauma does not serve as a confounding variable such that it makes it impossible to be specific about organic diagnoses such as FASD, but instead adds an additional layer over the top of the organic difficulties created by PAE. FASD will affect the type of therapeutic intervention that a

child may respond best to, again emphasising the need to treat a child with FASD primarily as a child with FASD in order to ensure they receive the appropriate support and understanding.

7. The Role of Epigenetics in FASD

While the direct exposure of a fetus to alcohol is an essential requirement for current FASD diagnostic guidelines, an increasing body of literature is evolving, suggesting factors other than maternal alcohol consumption contribute to make a fetus more or less vulnerable to the effects of prenatal alcohol exposure, which are described in this section

An area of growing interest is the potential role of fathers. The alcohol consumption of fathers has been found to affect unborn fetuses through both biological and environmental means. In a recent large-scale study [137], alcohol consumption in the week leading to IVF sperm collection was found to be predictive of both spontaneous miscarriage, and failure of the procedure to result in live birth. Teratogenic effects were also evidenced in a mouse model [138]. Researchers have also begun to investigate the transgenerational effect of a father's alcohol use, for example from parent to child to grandchild. Studies have demonstrated that the impact of a father's alcohol consumption and other substance abuse may be passed on through multiple generations via changes to male sperm [139]. Based on these studies, teratogenic effects through pre-conception alcohol consumption by fathers may result in changes to the male sperm such as count or quality, including possible fragmentation of sperm DNA in rat studies, changes to sperm DNA that is then inherited, or epigenetic changes to the gene regions that are inherited [140]. These implications may alter gene expression in terms of how they may be switched on or off in male sperm. Liyanage-Zachariah and Harding (2019) suggest that the contribution of fathers to FASD may be explained by the concept of 'paternal exposome'. This describes the process by which a father may pass toxic messages he acquires from the environment to his children, including the toxic impact of alcohol exposure.

Tunc-Ozcan suggests that both genetic and epigenetic risk factors interact to modulate both vulnerability and resilience to FASD [141]. This has been supported in a recent twin study by Hemingway et al. who found that identical PAE can result in markedly different outcomes for nonidentical twins, indicating that fetal genetics may significantly influence their vulnerability to severity of FASD outcome [142]. Dick and Foroud describe that certain genes have been identified in relation to susceptibility to FASD, which may be inherited [143]. Furthermore, paternally inherited genes that contribute to thyroid hormone production in the fetus have also been found to make a fetus more vulnerable to maternal PAE [144]. Other gene variants have been suggested to contribute to increased resilience of the fetus to maternal PAE. These genes carry the genetic messages required to general alcohol metabolizing enzymes which remove alcohol faster, thus reducing its potential harm [141]. The question is the magnitude of the genetic effects touched upon, and also how genetic variation interacts with environmental factors such as maternal/paternal nutrition [11].

Epigenetic changes are modifications on the DNA and to the proteins that are bound to DNA, referred to as DNA methylation and histone modifications. Without changing the DNA sequence, these modifications determine whether a gene is switched on or off, and therefore may contribute to some of the symptoms and deficits in FASD. Liyanage-Zachariah and Harding provide an example of the Dopamine Transporter (DAT) gene [145]. In a study by Lee et al., DNA methylation of the DAT gene was decreased in fathers with heavy alcohol consumption and in their offspring, suggesting that the epigenetic changes caused by pre-conception paternal alcohol consumption may be inherited [146].

DNA regions known as Imprinting Control Regions regulate imprinted genes which are switched on or off in children based on the parental origin of inheritance. H19 and Rasgrf1 are two examples of control regions. DNA methylation changes at both have been reported in the offspring of male rats who ingested alcohol before mating, resulting in reduced postnatal growth, which was more pronounced in the male offspring of male rats exposed to alcohol [147]. It is possible that some of the visible symptoms associated with FASD are caused by these changes.

These studies, and those like them, demonstrate that despite an emphasis on maternal alcohol consumption and direct PAE as the major driver for FASD diagnosis, the role of the father's alcohol intake pre-conception and its effect on offspring, even in the absence of PAE, ought to be recognised even if it currently does not contribute toward diagnostic indicators. The main reason for their lack of contribution currently is that the mechanisms remain poorly understood and, therefore, are difficult to quantify [11].

Pre-conception effects in women are more difficult to study as they are invariably influenced by alcohol consumption behaviour that continues into conception and pregnancy. They are also heavily influenced by malnutrition and external factors such as socioeconomic status (Haycock, 2009). However, controlled mouse studies of alcohol exposure for ten weeks prior to conception but not during pregnancy, generated genetic changes similar to those seen in PAE, including retarded growth [148, 149].

In summary, transgenerational effects of alcohol must be mediated through the process of gametogenesis and modulated by the maternal environment during pregnancy. There is compelling evidence that in addition to direct PAE, genetic variation and epigenetic remodelling are important risk factors.

CONCLUSION

FASD may be regarded as a national and international public health crisis. Work needs to be done to increase public understanding of the impact of PAE in order to reduce prevalence over time. This requires an unambiguous message from Governmens and health organisations about the dangers of drinking any amount of alcohol during pregnancy. In the meantime, affected children, and particularly children within high risk populations such as children in care and those who are adopted, need timely access to specialist assessment services in order to secure diagnosis, thereby opening the doors for appropriate support. Children with FASD have better outcomes the earlier they receive accurate diagnosis. Children who receive appropriate diagnosis at eight years old or younger present with fewer

behavioural difficulties and other adverse outcomes than those diagnosed at a later age [150, 151). While the perceived stigma of an FASD may explain the reluctance to diagnose in some clinicians [152), these results demonstrate the need to meet these misperceptions head-on with facts, for the good of subsequent generations.

REFERENCES

[1] May PA1, Gossage JP, Kalberg WO, Robinson LK, Buckley D, Manning M, Hoyme HE. Prevalence and Epidemiologic Characteristics of FASD From Various Research Methods With an Emphasis on Recent In-School Studies. *Dev Disabil Res Rev.* 2009;15(3):176-92.

[2] Mukherjee, R, Wray, E., Commers, M., Hollins, S., Curfs, L. The impact of raising a child with FASD upon carers: findings from a mixed methodology study in the UK. *Adoption and Fostering.* 2013;37(1):43–56.

[3] Popova S, Lange S, Probst C, Gmel G, Rehm J. Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: a systematic review and meta-analysis. *Lancet Glob Health.* 2017;5(3):e290–9.

[4] *SIGN 156 Children and young people exposed prenatally to alcohol* [Internet]. [cited 2020 Jan 31]. Available from: https://www.sign.ac.uk/sign-156-children-and-young-people-exposed-prenatally-to-alcohol.html.

[5] Anderson AE, Hure AJ, Forder P, Powers JR, Kay-Lambkin FJ, Loxton DJ. Predictors of antenatal alcohol use among Australian women: a prospective cohort study. *BJOG.* 2013 Oct;120(11):1366–74.

[6] Corrales-Gutierrez I, Mendoza R, Gomez-Baya D, Leon-Larios F. Pregnant Women's Risk Perception of the Teratogenic Effects of Alcohol Consumption in Pregnancy. *J Clin Med.* 2019 Jun 25;8(6).

[7] McQuire C, Mukherjee R, Hurt L, Higgins A, Greene G, Farewell D, et al. Screening prevalence of fetal alcohol spectrum disorders in a region of the United Kingdom: A population-based birth-cohort study. *Prev Med.* 2019;118:344–51.

[8] *FASD: The hidden epidemic [Internet]*. Adoption UK Charity. [cited 2020 Jan 31]. Available from: https://www.adoptionuk.org//fasd-the-hidden-epidemic.

[9] Gupta KK, Gupta VK, Shirasaka T. An Update on Fetal Alcohol Syndrome-Pathogenesis, Risks, and Treatment. *Alcohol Clin Exp Res.* 2016;40(8):1594–602.

[10] Heller M, Burd L. Review of ethanol dispersion, distribution, and elimination from the fetal compartment. *Birth Defects Res Part A Clin Mol Teratol.* 2014 Apr;100(4):277–83.

[11] Ramsay M. Genetic and epigenetic insights into fetal alcohol spectrum disorders. *Genome Med*. 2010 Apr 28;2(4):27.

[12] *NOFAS UK - Dedicated to supporting people affected by Foetal Alcohol Spectrum Disorders (FASD)* [Internet]. NOFAS UK. [cited 2020 Jan 31]. Available from: http://www.nofas-uk.org/

[13] *Lip-Philtrum Guides* [Internet]. [cited 2020 Jan 31]. Available from: https://depts.washington.edu/fasdpn/htmls/lip-philtrum-guides.htm.

[14] Chasnoff IJ, Wells AM, King L. Misdiagnosis and missed diagnoses in foster and adopted children with prenatal alcohol exposure. *Pediatrics.* 2015 Feb;135(2):264–70.

[15] Lange S, Shield K, Rehm J, Anagnostou E, Popova S. Fetal alcohol spectrum disorder: neurodevelopmentally and behaviorally indistinguishable from other neurodevelopmental disorders. *BMC Psychiatry*. 2019 Oct 28;19(1):322.

[16] Brown JM, Bland R, Jonsson E, Greenshaw AJ. The Standardization of Diagnostic Criteria for Fetal Alcohol Spectrum Disorder (FASD): Implications for Research, Clinical Practice and Population Health. *Can J Psychiatry.* 2019;64(3):169–76.

[17] Price A, Cook PA, Norgate S, Mukherjee R. Prenatal alcohol exposure and traumatic childhood experiences: A systematic review. *Neurosci Biobehav Rev*. 2017 Sep;80:89–98.

[18] Mukherjee RAS, Cook PA, Norgate SH, Price AD. Neurodevelopmental outcomes in individuals with fetal alcohol spectrum disorder (FASD) with and without exposure to neglect: Clinical cohort data from a national FASD diagnostic clinic. *Alcohol.* 2019;76:23–8.

[19] Astley SJ, Aylward EH, Olson HC, Kerns K, Brooks A, Coggins TE, et al. Magnetic resonance imaging outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res.* 2009 Oct;33(10):1671–89.

[20] Willoughby KA, Sheard ED, Nash K, Rovet J. Effects of prenatal alcohol exposure on hippocampal volume, verbal learning, and verbal and spatial recall in late childhood. *J Int Neuropsychol Soc.* 2008 Nov;14(6):1022–33.

[21] Norman AL, Crocker N, Mattson SN, Riley EP. Neuroimaging and fetal alcohol spectrum disorders. *Dev Disabil Res Rev.* 2009; 15(3):209–17.

[22] Swayze VW, Johnson VP, Hanson JW, Piven J, Sato Y, Giedd JN, et al. Magnetic resonance imaging of brain anomalies in fetal alcohol syndrome. *Pediatrics.* 1997 Feb;99(2):232–40.

[23] Bookstein FL, Streissguth AP, Sampson PD, Connor PD, Barr HM. Corpus callosum shape and neuropsychological deficits in adult males with heavy fetal alcohol exposure. *Neuroimage*. 2002 Jan;15(1):233–51.

[24] Sowell ER, Mattson SN, Thompson PM, Jernigan TL, Riley EP, Toga AW. Mapping callosal morphology and cognitive correlates: effects of heavy prenatal alcohol exposure. *Neurology.* 2001 Jul 24; 57(2):235–44.

[25] Archibald SL, Fennema-Notestine C, Gamst A, Riley EP, Mattson SN, Jernigan TL. Brain dysmorphology in individuals with severe prenatal alcohol exposure. *Dev Med Child Neurol.* 2001 Mar;43(3):148–54.

[26] Kodituwakku PW, May PA, Clericuzio CL, Weers D. Emotion-related learning in individuals prenatally exposed to alcohol: an investigation of the relation between set shifting, extinction of responses, and behavior. *Neuropsychologia.* 2001;39(7):699–708.

[27] Connor PD, Sampson PD, Streissguth AP, Bookstein FL, Barr HM. Effects of prenatal alcohol exposure on fine motor coordination and balance: A study of two adult samples. *Neuropsychologia.* 2006;44(5):744–51.

[28] Lebel C, Rasmussen C, Wyper K, Walker L, Andrew G, Yager J, et al. Brain diffusion abnormalities in children with fetal alcohol spectrum disorder. *Alcohol Clin Exp Res.* 2008 Oct;32(10):1732–40.

[29] Fryer SL, Schweinsburg BC, Bjorkquist OA, Frank LR, Mattson SN, Spadoni AD, et al. Characterization of white matter microstructure in fetal alcohol spectrum disorders. *Alcohol Clin Exp Res.* 2009 Mar; 33(3):514–21.

[30] Corbetta M, Shulman GL. Human cortical mechanisms of visual attention during orienting and search. Philos Trans R Soc Lond, B, *Biol Sci.* 1998 Aug 29;353(1373):1353–62.

[31] Malisza KL, Allman A-A, Shiloff D, Jakobson L, Longstaffe S, Chudley AE. Evaluation of spatial working memory function in children and adults with fetal alcohol spectrum disorders: a functional magnetic resonance imaging study. *Pediatr Res.* 2005 Dec; 58(6):1150–7.

[32] Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. *Lancet.* 1973 Nov 3;302(7836):999–1001.

[33] Spohr H-L, Willms J, Steinhausen H-C. Fetal alcohol spectrum disorders in young adulthood. *J Pediatr.* 2007 Feb;150(2):175–179, 179.e1.

[34] Howell KK, Lynch ME, Platzman KA, Smith GH, Coles CD. Prenatal alcohol exposure and ability, academic achievement, and school functioning in adolescence: a longitudinal follow-up. *J Pediatr Psychol.* 2006 Feb;31(1):116–26.

[35] Ervalahti N, Korkman M, Fagerlund A, Autti-Rämö I, Loimu L, Hoyme HE. Relationship between dysmorphic features and general cognitive function in children with fetal alcohol spectrum disorders. *Am J Med Genet A.* 2007 Dec 15;143A(24):2916–23.

[36] Mukherjee RAS, Hollins S, Turk J. Fetal alcohol spectrum disorder: an overview. *J R Soc Med.* 2006 Jun;99(6):298–302.

[37] Chasnoff IJ, Wells AM, Telford E, Schmidt C, Messer G. Neurodevelopmental functioning in children with FAS, pFAS, and ARND. *J Dev Behav Pediatr.* 2010 Apr; 31(3):192–201.

[38] Mattson SN, Crocker N, Nguyen TT. Fetal alcohol spectrum disorders: neuropsychological and behavioral features. *Neuropsychol Rev.* 2011 Jun; 21(2):81–101.

[39] Greenspan, S., Brown, N.N., Edwards, W. FASD and the Concept of "Intellectual Disability Equivalence". In: *Fetal Alcohol Spectrum Disorders in Adults: Ethical and Legal Perspectives.* Switzerland: Springer International; 2016.

[40] Khoury JE, Milligan K, Girard TA. Executive Functioning in Children and Adolescents Prenatally Exposed to Alcohol: A Meta-Analytic Review. *Neuropsychol Rev.* 2015 Jun; 25(2):149–70.

[41] Rasmussen C, Bisanz J. Executive functioning in children with Fetal Alcohol Spectrum Disorders: profiles and age-related differences. *Child Neuropsychol.* 2009 May;15(3):201–15.

[42] Fuglestad AJ, Whitley ML, Carlson SM, Boys CJ, Eckerle JK, Fink BA, et al. Executive functioning deficits in preschool children with Fetal Alcohol Spectrum Disorders. *Child Neuropsychol.* 2015; 21(6):716–31.

[43] Engel-Yeger B, Josman N, Rosenblum S. Behavioural assessment of the Dysexecutive Syndrome for Children (BADS-C): an examination of construct validity. *Neuropsychol Rehabil.* 2009 Oct;19(5):662–76.

[44] Homack S, Lee D, Riccio CA. Test review: Delis-Kaplan executive function system. *J Clin Exp Neuropsychol.* 2005 Jul;27(5):599–609.

[45] Gioia, G.A., Isquith, P.K., Guy, S.C., Kenworthy, L. Test review behavior rating inventory of exectuvie function. *Child Neuropsychology.* 2000;6(3):235–8.

[46] Hughes, N., Clasby, B., Chitsabesan, P., Williams, H. A systematic review of the prevalence of foetal alcohol syndrome disorders among young people in the criminal justice system. *Cogent Psychology.* 2016;3(1):1–8.

[47] Clark C, Prior M, Kinsella G. The relationship between executive function abilities, adaptive behaviour, and academic achievement in

children with externalising behaviour problems. *J Child Psychol Psychiatry.* 2002 Sep;43(6):785–96.

[48] Fagerlund Å, Åse F, Autti-Rämö I, Ilona A-R, Kalland M, Mirjam K, et al. Adaptive behaviour in children and adolescents with foetal alcohol spectrum disorders: a comparison with specific learning disability and typical development. *Eur Child Adolesc Psychiatry.* 2012 Apr;21(4):221–31.

[49] Paley B, O'connor MJ, Frankel F, Marquardt R. Predictors of stress in parents of children with fetal alcohol spectrum disorders. *J Dev Behav Pediatr.* 2006 Oct;27(5):396–404.

[50] McCabe, M. Fetal alcohol spectrum disorder in Aboriginal children: Does a language profile emerge? *Doctoral disertation [Internet]*. 2009. Available from: http://uwo.ca/fhs/csd/ebp/reviews/2008 09/McCabe.pdf.

[51] Wyper KR, Rasmussen CR. Language impairments in children with fetal alcohol spectrum disorders. *J Popul Ther Clin Pharmacol.* 2011;18(2):e364-376.

[52] Proven, S., Ens, C., Beaudin, P.G. The Language Profile of School-Aged Children with Fetal Alcohol Spectrum Disorder (FASD). *Canadian Journal of Speech-Language Pathology & Audiology.* 2014;37(4):268–79.

[53] Manji S, Pei J, Loomes C, Rasmussen C. A review of the verbal and visual memory impairments in children with foetal alcohol spectrum disorders. *Dev Neurorehabil.* 2009 Aug;12(4):239–47.

[54] Crocker N, Riley EP, Mattson SN. Visual-spatial abilities relate to mathematics achievement in children with heavy prenatal alcohol exposure. *Neuropsychology.* 2015 Jan;29(1):108–16.

[55] Fjeldsted B, Xue L. Sensory Processing in Young Children with Fetal Alcohol Spectrum Disorder. *Phys Occup Ther Pediatr.* 2019; 39(5):553–65.

[56] Greenbaum RL, Steven SA, Nash K, Koren G, Rovet, J. Social Cognitive and Emotion Processing Abilities of Children With Fetal Alcohol Spectrum Disorders: A Comparison With Attention Deficit

Hyperactivity Disorder. *Alcoholism: Clinical and Experimental Research.* 2009;33(10):1656–70.

[57] Rangmar J, Hjern A, Vinnerljung B, Strömland K, Aronson M, Fahlke C. Psychosocial outcomes of fetal alcohol syndrome in adulthood. *Pediatrics.* 2015 Jan;135(1):e52-58.

[58] Lindinger NM, Malcolm-Smith S, Dodge NC, Molteno CD, Thomas KGF, Meintjes EM, et al. Theory of Mind in Children with Fetal Alcohol Spectrum Disorders. *Alcoholism: Clinical and Experimental Research.* 40(2):367–76.

[59] Brown, J. Fetal alcohol spectrum disorder and confabulation: a clinical, forensic and judicial dilemma. *Ther Journal of Special Populations.* 2017;1(2):1–11.

[60] Chen ML, Olson HC, Picciano JF, Starr JR, Owens J. Sleep problems in children with fetal alcohol spectrum disorders. *J Clin Sleep Med.* 2012 Aug 15;8(4):421–9.

[61] Rasmussen C, Andrew G, Zwaigenbaum L, Tough S. Neurobehavioural outcomes of children with fetal alcohol spectrum disorders: A Canadian Perspective. *Paediatrics & child health.* 2008;13(3):185–91.

[62] Khoury JE, Jamieson B, Milligan K. Risk for Childhood Internalizing and Externalizing Behavior Problems in the Context of Prenatal Alcohol Exposure: A Meta-Analysis and Comprehensive Examination of Moderators. *Alcohol Clin Exp Res.* 2018 May 31;

[63] Kully-Martens K, Denys K, Treit S, Tamana S, Rasmussen C. A review of social skills deficits in individuals with fetal alcohol spectrum disorders and prenatal alcohol exposure: profiles, mechanisms, and interventions. *Alcohol Clin Exp Res.* 2012 Apr; 36(4):568–76.

[64] Aronson M, Hagberg B, Gillberg C. Attention deficits and autistic spectrum problems in children exposed to alcohol during gestation: a follow-up study. *Developmental Medicine & Child Neurology.* 2008;39(9):583–7.

[65] Glass L, Graham DM, Deweese DM, Jones KL, Riley EP, Matteson SN. Correspondence of parent report and laboratory measures of

inattention and hyperactivity in children with heavy prenatal alcohol exposure. *Neurotoxicology and Teratology.* 2014;42:43–50.

[66] Glass L, Ware AL, Crocker N, Deweese BN, Coles CD, Matteson SN. Neuropsychological deficits associated with heavy prenatal alcohol exposure are not exacerbated by ADHD. *Neuropsychology.* 27(6):713–24.

[67] Ware AL, Glass L, Crocker N, Deweese BN, Coles CD, Kable JA. Effects of Prenatal Alcohol Exposure and Attention-Deficit/Hyperactivity Disorder on Adaptive Functioning. *Alcoholism: Clinical and Experimental Research.* 2014;38(5):1439–47.

[68] Malisza KL, Buss JL, Bolster RB, de Gervai PD, Woods-Frohlich L, Summers R, et al. Comparison of spatial working memory in children with prenatal alcohol exposure and those diagnosed with ADHD; A functional magnetic resonance imaging study. *Journal of Neurodevelopmental Disorders*. 2012;4(1).

[69] Gardener H, Speigelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive Meta-Analysis. *Pediatrics.* 2011;

[70] Crocker N, Vario L, Riley EP, Matteson SN. Comparison of Adaptive Behavior in Children With Heavy Prenatal Alcohol Exposure or Attention-Deficit/Hyperactivity Disorder. *Alcoholism: Clinical and Experimental Research.* 2009;33(11):2015–23.

[71] Lange S, Anagnostou E, Rehm J, Popova S. Prevalence of externalizing disorders and Autism Spectrum Disorders among children with Fetal Alcohol Spectrum Disorder: systematic review and meta-analysis. *Biochemistry and Cell Biology.* 2018;96 (2):241–251.

[72] McGee CL, Bjorkquist OA, Price JM, Matteson SN, Riley EP. Social Information Processing Skills in Children with Histories of Heavy Prenatal Alcohol Exposure. *Journal of Abnormal Child Psychology.* 2009;37(6):817–30.

[73] Larsson HJ, Eaton WW, Madsen KM, Vestergaard M, Olesen AV, Agerbo E, et al. Risk Factors for Autism: Perinatal Factors, Parental Psychiatric History, and Socioeconomic Status. *American Journal of Epidemiology.* 2005;161(10):916–25.

[74] Franklin L, Dietz J, Jirikowic T, Astley S. Children With Fetal Alcohol Spectrum Disorders: Problem Behaviors and Sensory Processing. *American Journal of Occupational Therapy.* 2008; 62:265–73.

[75] Mukherjee R, Layton M, Yacoub E, Turk J. Autism and autistic traits in people exposed to heavy prenatal alcohol: data from a clinical series of 21 individuals and nested case control study. *Advances in Mental Health and Intellectual Disabilities.* 2011;5:42–9.

[76] Popova S, Lange S, Shield K, Burd L, Rehm J. Prevalence of Fetal Alcohol Spectrum Disorder among Special Sub-populations: A Systematic Review and Meta-analysis. *Addiction.* 2019;

[77] Landgren M, Svensson L, Strömland K, Andersson Grönlund M. Prenatal alcohol exposure and neurodevelopmental disorders in children adopted from eastern Europe. *Pediatrics.* 2010 May; 125(5):e1178-1185.

[78] Shor S, Nulman I, Kulaga V, Koren G. Heavy in utero ethanol exposure is associated with the use of other drugs of abuse in a high-risk population. *Alcohol.* 2010 Dec;44(7–8):623–7.

[79] Astley SJ. Profile of the first 1,400 patients receiving diagnostic evaluations for fetal alcohol spectrum disorder at the Washington State Fetal Alcohol Syndrome Diagnostic & Prevention Network. *Can J Clin Pharmacol.* 2010;17(1):e132-164.

[80] Eriksson M, Larsson G, Zetterström R. Abuse of alcohol, drugs and tobacco during pregnancy--consequences for the child. *Paediatrician.* 1979;8(4):228–42.

[81] Gressens P, Mesples B, Sahir N, Marret S, Sola A. Environmental factors and disturbances of brain development. *Semin Neonatol.* 2001 Apr;6(2):185–94.

[82] Fazeli W, Zappettini S, Marguet SL, Grendel J, Esclapez M, Bernard C, et al. Early-life exposure to caffeine affects the construction and activity of cortical networks in mice. *Exp Neurol.* 2017;295:88–103.

[83] Soellner DE, Grandys T, Nuñez JL. Chronic prenatal caffeine exposure impairs novel object recognition and radial arm maze behaviors in adult rats. *Behav Brain Res.* 2009 Dec 14;205(1):191–9.

[84] Slotkin TA, Seidler FJ. Mimicking maternal smoking and pharmacotherapy of preterm labor: interactions of fetal nicotine and dexamethasone on serotonin and dopamine synaptic function in adolescence and adulthood. *Brain Res Bull.* 2010 Apr 29;82(1–2):124–34.

[85] El Marroun H, Schmidt MN, Franken IHA, Jaddoe VWV, Hofman A, van der Lugt A, et al. Prenatal tobacco exposure and brain morphology: a prospective study in young children. *Neuropsychopharmacology.* 2014 Mar;39(4):792–800.

[86] Lange S, Rovet J, Rehm J, Popova S. Neurodevelopmental profile of Fetal Alcohol Spectrum Disorder: A systematic review. *BMC Psychol.* 2017 Jun 23;5(1):22.

[87] Bublitz MH, Stroud LR. Maternal smoking during pregnancy and offspring brain structure and function: review and agenda for future research. *Nicotine Tob Res.* 2012 Apr;14(4):388–97.

[88] Scott-Goodwin AC, Puerto M, Moreno I. Toxic effects of prenatal exposure to alcohol, tobacco and other drugs. *Reprod Toxicol.* 2016;61:120–30.

[89] Johnson RD, Horowitz M, Maddox AF, Wishart JM, Shearman DJ. Cigarette smoking and rate of gastric emptying: effect on alcohol absorption. *BMJ.* 1991 Jan 5;302(6767):20–3.

[90] Gilbertson RJ, Barron S. Neonatal ethanol and nicotine exposure causes locomotor activity changes in preweanling animals. *Pharmacol Biochem Behav.* 2005 May;81(1):54–64.

[91] Parnell SE, West JR, Chen W-JA. Nicotine decreases blood alcohol concentrations in adult rats: a phenomenon potentially related to gastric function. *Alcohol Clin Exp Res.* 2006 Aug;30(8):1408–13.

[92] Hamułka J, Zielińska MA, Chądzyńska K. The combined effects of alcohol and tobacco use during pregnancy on birth outcomes. *Rocz Panstw Zakl Hig.* 2018;69(1):45–54.

[93] Odendaal HJ, Steyn DW, Elliott A, Burd L. Combined effects of cigarette smoking and alcohol consumption on perinatal outcome. *Gynecol Obstet Invest.* 2009;67(1):1–8.

[94] Hurt H, Malmud E, Betancourt L, Braitman LE, Brodsky NL, Giannetta J. Children with in utero cocaine exposure do not differ from control subjects on intelligence testing. *Arch Pediatr Adolesc Med.* 1997 Dec;151(12):1237–41.

[95] Ackerman JP, Riggins T, Black MM. A review of the effects of prenatal cocaine exposure among school-aged children. *Pediatrics.* 2010 Mar;125(3):554–65.

[96] Maia AF da S, Martins FT, Silva Neto L da, Alves RB, De Fátima Â. Cocaethylene, the in vivo product of cocaine and ethanol, is a narcotic more potent than its precursors. *Acta Crystallogr C Struct Chem.* 2017 Oct 1;73(Pt 10):780–3.

[97] Farré M, de la Torre R, González ML, Terán MT, Roset PN, Menoyo E, et al. Cocaine and alcohol interactions in humans: neuroendocrine effects and cocaethylene metabolism. *J Pharmacol Exp Ther.* 1997 Oct;283(1):164–76.

[98] Chen WJ, West JR. Cocaethylene exposure during the brain growth spurt period: brain growth restrictions and neurochemistry studies. *Brain Res Dev Brain Res.* 1997 Jun 18;100(2):220–9.

[99] Randall CL, Cook JL, Thomas SE, White NM. Alcohol plus cocaine prenatally is more deleterious than either drug alone. *Neurotoxicol Teratol.* 1999 Dec;21(6):673–8.

[100] Blinick G, Wallach RC, Jerez E, Ackerman BD. Drug addiction in pregnancy and the neonate. *Am J Obstet Gynecol.* 1976 May 15; 125(2):135–42.

[101] Chen H-H, Chiang Y-C, Yuan ZF, Kuo C-C, Lai M-D, Hung T-W, et al. Buprenorphine, methadone, and morphine treatment during pregnancy: behavioral effects on the offspring in rats. *Neuropsychiatr Dis Treat.* 2015;11:609–18.

[102] Wang Y, Han T-Z. Prenatal exposure to heroin in mice elicits memory deficits that can be attributed to neuronal apoptosis. *Neuroscience.* 2009 May 5;160(2):330–8.

[103] Kaltenbach K, O'Grady KE, Heil SH, Salisbury AL, Coyle MG, Fischer G, et al. Prenatal exposure to methadone or buprenorphine:

Early childhood developmental outcomes. *Drug Alcohol Depend.* 2018 01;185:40–9.

[104] Nygaard E, Slinning K, Moe V, Walhovd KB. Cognitive function of youths born to mothers with opioid and poly-substance abuse problems during pregnancy. *Child Neuropsychol.* 2017;23(2):159–87.

[105] Caritis SN, Panigrahy A. Opioids affect the fetal brain: reframing the detoxification debate. *Am J Obstet Gynecol.* 2019 Dec;221(6):602–8.

[106] Walhovd KB, Moe V, Slinning K, Due-Tønnessen P, Bjørnerud A, Dale AM, et al. Volumetric cerebral characteristics of children exposed to opiates and other substances in utero. *Neuroimage.* 2007 Jul 15;36(4):1331–44.

[107] Sirnes E, Oltedal L, Bartsch H, Eide GE, Elgen IB, Aukland SM. Brain morphology in school-aged children with prenatal opioid exposure: A structural MRI study. *Early Hum Dev*. 2017 Apr;106–107:33–9.

[108] Chotro MG, Arias C. Prenatal exposure to ethanol increases ethanol consumption: a conditioned response? *Alcohol.* 2003 May;30(1):19–28.

[109] Miranda-Morales RS, Molina JC, Spear NE, Abate P. Participation of the endogenous opioid system in the acquisition of a prenatal ethanol-related memory: effects on neonatal and preweanling responsiveness to ethanol. *Physiol Behav.* 2010 Aug 4;101(1):153–60.

[110] Gunn JKL, Rosales CB, Center KE, Nuñez A, Gibson SJ, Christ C, et al. Prenatal exposure to cannabis and maternal and child health outcomes: a systematic review and meta-analysis. *BMJ Open.* 2016 Apr 5;6(4):e009986.

[111] Mackie K. Cannabinoid receptors: where they are and what they do. *J Neuroendocrinol.* 2008 May;20 Suppl 1:10–4.

[112] Metz TD, Stickrath EH. Marijuana use in pregnancy and lactation: a review of the evidence. *Am J Obstet Gynecol.* 2015 Dec;213(6):761–78.

[113] Fried PA, Watkinson B. 36- and 48-month neurobehavioral follow-up of children prenatally exposed to marijuana, cigarettes, and alcohol. *J Dev Behav Pediatr.* 1990 Apr;11(2):49–58.

[114] Day NL, Richardson GA, Goldschmidt L, Robles N, Taylor PM, Stoffer DS, et al. Effect of prenatal marijuana exposure on the cognitive development of offspring at age three. *Neurotoxicol Teratol.* 1994 Apr;16(2):169–75.

[115] Huizink AC, Mulder EJH. Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. *Neurosci Biobehav Rev*. 2006;30(1):24–41.

[116] Goldschmidt L, Richardson GA, Cornelius MD, Day NL. Prenatal marijuana and alcohol exposure and academic achievement at age 10. *Neurotoxicol Teratol.* 2004 Aug;26(4):521–32.

[117] Slamberová R, Pometlová M, Syllabová L, Mancusková M. Learning in the Place navigation task, not the New-learning task, is altered by prenatal methamphetamine exposure. *Brain Res Dev Brain Res.* 2005 Jun 30;157(2):217–9.

[118] Williams MT, Blankenmeyer TL, Schaefer TL, Brown CA, Gudelsky GA, Vorhees CV. Long-term effects of neonatal methamphetamine exposure in rats on spatial learning in the Barnes maze and on cliff avoidance, corticosterone release, and neurotoxicity in adulthood. *Brain Res Dev Brain Res.* 2003 Dec 30;147(1–2):163–75.

[119] Jeng W, Wong AW, Ting-A-Kee R, Wells PG. Methamphetamine-enhanced embryonic oxidative DNA damage and neurodevelopmental deficits. *Free Radic Biol Med.* 2005 Aug 1;39(3):317–26.

[120] Cernerud L, Eriksson M, Jonsson B, Steneroth G, Zetterström R. Amphetamine addiction during pregnancy: 14-year follow-up of growth and school performance. *Acta Paediatr.* 1996 Feb;85(2):204–8.

[121] Chang L, Smith LM, LoPresti C, Yonekura ML, Kuo J, Walot I, et al. Smaller subcortical volumes and cognitive deficits in children with prenatal methamphetamine exposure. *Psychiatry Res.* 2004 Dec 15;132(2):95–106.

[122] Berkowitz A, Perkins HW. Personality characteristics of children of alcoholics. *J Consult Clin Psychol.* 1988 Apr;56(2):206–9.

[123] Park, S., Schepp, K.G. A systematic review of research on children of alcoholics. *Journal of Child and Family Studies.* 2015;24(5):1222–31.

[124] Cash SJ, Wilke DJ. An ecological model of maternal substance abuse and child neglect: issues, analyses, and recommendations. *Am J Orthopsychiatry*. 2003 Oct;73(4):392–404.

[125] Friedman E, Billick SB. Unintentional child neglect: literature review and observational study. *Psychiatr Q*. 2015 Jun;86(2):253–9.

[126] Ainsworth, M. D. S.,, owlby, J. An ethological approach to personality development. *American Psychologist*. 1991;46:331–41.

[127] Crittenden, P.M. *Raising Parents: Attachment, Parenting and Child Safety*. Routledge; 2011.

[128] Insel TR. Oxytocin and the neurobiology of attachment. *Behav Brain Sci*. 1992 Sep;15(3):515–6.

[129] Swain JE, Kim P, Spicer J, Ho SS, Dayton CJ, Elmadih A, et al. Approaching the biology of human parental attachment: brain imaging, oxytocin and coordinated assessments of mothers and fathers. *Brain Res*. 2014 Sep 11;1580:78–101.

[130] Strathearn L. Maternal neglect: oxytocin, dopamine and the neurobiology of attachment. *J Neuroendocrinol*. 2011 Nov; 23 (11):1054–65.

[131] Tarullo AR, Gunnar MR. Child maltreatment and the developing HPA axis. *Horm Behav*. 2006 Nov;50(4):632–9.

[132] Rutter M. Developmental catch-up, and deficit, following adoption after severe global early privation. English and Romanian Adoptees (ERA) Study Team. *J Child Psychol Psychiatry*. 1998 May; 39 (4):465–76.

[133] Rutter M, Andersen-Wood L, Beckett C, Bredenkamp D, Castle J, Groothues C, et al. Quasi-autistic patterns following severe early global privation. English and Romanian Adoptees (ERA) Study Team. *J Child Psychol Psychiatry*. 1999 May;40(4):537–49.

[134] Sonuga-Barke EJS, Kennedy M, Kumsta R, Knights N, Golm D, Rutter M, et al. Child-to-adult neurodevelopmental and mental health trajectories after early life deprivation: the young adult follow-up of the longitudinal English and Romanian Adoptees study. *Lancet*. 2017 15;389(10078):1539–48.

[135] Bos KJ, Fox N, Zeanah CH, Nelson Iii CA. Effects of early psychosocial deprivation on the development of memory and executive function. *Front Behav Neurosci.* 2009;3:16.

[136] Hawk B, McCall RB. CBCL behavior problems of post-institutionalized international adoptees. *Clin Child Fam Psychol Rev.* 2010 Jun;13(2):199–211.

[137] Czeizel AE, Czeizel B, Vereczkey A. The participation of prospective fathers in preconception care. *Clin Med Insights Reprod Health.* 2013 Jan 22;7:1–9.

[138] Lee, H.J., Ryu, J-S, Choi, N. Y., Park, Y. S., Kim, Y. I., Han, D. W., Ko, K., Shin, C. Y., Hwuang, H. S., Kang, K.-S., Ko, K. Transgenerational effects of paternal alcohol exposure in mouse offspring. *Animal Cells and Systems.* 2013;17(6):429–34.

[139] Yohn NL, Bartolomei MS, Blendy JA. Multigenerational and transgenerational inheritance of drug exposure: The effects of alcohol, opiates, cocaine, marijuana, and nicotine. *Prog Biophys Mol Biol.* 2015 Jul;118(1–2):21–33.

[140] Abel EL. Paternal and maternal alcohol consumption: effects on offspring in two strains of rats. *Alcohol Clin Exp Res.* 1989 Aug;13(4):533–41.

[141] Tunc-Ozcan E, Sittig LJ, Harper KM, Graf EN, Redei EE. Hypothesis: genetic and epigenetic risk factors interact to modulate vulnerability and resilience to FASD. *Front Genet.* 2014;5:261.

[142] Hemingway, S.J.A., Bledsoe, J., Davies, J., Brooks, A., Jirikowic, T.M., Olson, E., et al. Twin study confirms virtually identical prenatal alcohol exposures can lead to markedly different fetal alcohol spectrum disorder outcomes-fetal genetics influences fetal vulnerability. *Advances in Pediatric Research.* 2018;5(3).

[143] Dick DM, Foroud T. Candidate genes for alcohol dependence: a review of genetic evidence from human studies. *Alcohol Clin Exp Res.* 2003 May;27(5):868–79.

[144] Sittig LJ, Redei EE. Paternal genetic contribution influences fetal vulnerability to maternal alcohol consumption in a rat model of fetal alcohol spectrum disorder. *PLoS ONE.* 2010 Apr 7;5(4):e10058.

[145] Liyanage-Zachariach, V., Harding, K. *Genetic and epigenetic perspectives on the role of fathers in Fetal Alcohol Spectrum Disorder.* Ontario. Canada: Canada FASD Research Network. Laurentian University; 2019.

[146] Lee B-Y, Park S-Y, Ryu H-M, Shin C-Y, Ko K-N, Han J-Y, et al. Changes in the methylation status of DAT, SERT, and MeCP2 gene promoters in the blood cell in families exposed to alcohol during the periconceptional period. *Alcohol Clin Exp Res.* 2015 Feb;39(2):239–50.

[147] Knezovich JG, Ramsay M. The effect of preconception paternal alcohol exposure on epigenetic remodeling of the h19 and rasgrf1 imprinting control regions in mouse offspring. *Front Genet.* 2012;3:10.

[148] Becker HC, Randall CL. Two generations of maternal alcohol consumption in mice: effect on pregnancy outcome. *Alcohol Clin Exp Res.* 1987 Jun;11(3):240–2.

[149] Livy DJ, Maier SE, West JR. Long-term alcohol exposure prior to conception results in lower fetal body weights. *Birth Defects Res B Dev Reprod Toxicol*. 2004 Jun; 71(3):135–41.

[150] Alex K, Feldmann R. Children and adolescents with fetal alcohol syndrome (FAS): better social and emotional integration after early diagnosis. *Klin Padiatr.* 2012 Mar; 224(2):66–71.

[151] Streissguth AP, Bookstein FL, Barr HM, Sampson PD, O'Malley K, Young JK. Risk factors for adverse life outcomes in fetal alcohol syndrome and fetal alcohol effects. *J Dev Behav Pediatr.* 2004 Aug; 25(4):228–38.

[152] Mukherjee, R. Fetal alcohol spectrum disorders. *Paediatrics and Child Health.* 2015;25(12):580–6.

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Chapter 3

SIRTUIN 2: A POTENTIAL TARGET FOR AGE-RELATED NEURODEGENERATIVE DISEASES?

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ABSTRACT

Epigenetic changes play an important role in the ageing process and have been implicated in many age-related diseases. Sirtuins, which are nicotinamide adenine dinucleotide $(NAD)^+$-dependent class III histone deacetylases, have emerged as master regulators of metabolism and longevity. Among all sirtuins, Sirtuin 2 (SIRT2) is the most expressed in the central nervous system (CNS). It has been involved in a variety of biological processes including gene transcription, apoptosis, ageing, autophagy and inflammation. However, different groups have provided seemly contradictory results, thus, its specific functions remain unknown.

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It has been described an age-dependent accumulation of SIRT2 in the brain. Moreover, its pharmacological inhibition shows neuroprotective effects in different models of Huntington, Parkinson and Alzheimer´s diseases suggesting its potential as a therapeutic target for age-related diseases. Thus, due to its possible implication in the etiology or development of neurodegenerative diseases, the elucidation of its functions in the CNS is crucial for understanding the molecular basis of these diseases and fundamental for the advancement of new therapeutic strategies.

In this context, the aim of the present chapter is to review recent literature, summarize the main roles of SIRT2 in the CNS and try to understand the relationship between SIRT2, senescence and neurodegenerative diseases.

Keywords: sirtuin 2, ageing, neuroinflammation, Alzheimer's disease, Parkinson's disease, Huntington's disease, epigenetics, neurodegenerative diseases

ABBREVIATIONS

Aβ: Amyloid-β
AD: Alzheimer's disease
APP: Amyloid precursor protein
CNS: Central nervous system
fAD: Familial Alzheimer's disease
HAT: Histone acetyltransferase
HD: Huntington's disease
HDAC: Histone deacetylase
HTT: Huntingtin
LPS: Lipopolysaccharide
MT: Microtubules
NAD: Nicotinamide adenine dinucleotide
NFT: Neurofibrillary tangles
NO: Nitric oxide
PD: Parkinson's disease
sAD: Sporadic Alzheimer's disease

SAMP8: Senescence Accelerated Mouse-Prone 8
SIRT: Sirtuin
$SIRT2^{-/-}$: SIRT2 knockout mice
TNF-α: Tumor Necrosis Factor-α
WT: Wild-type

AGEING AND EPIGENETICS

Ageing is a complex multifactorial biological process shared by all organisms. It is manifested by a gradual decline of normal physiological functions in a time-dependent manner increasing the susceptibility to many diseases, including cancer, metabolic disorders, cardiovascular disorders, and neurodegenerative diseases. Over the last decades, the increase in human life expectancy and the reduction in death rates have incremented exponentially the world elderly population, increasing also the prevalence of age-related diseases. According to data from the 2019 Revision of World Population Prospects, the number of older people, those aged 65 years or over, is expected to more than double by 2050, rising to 2.5 billion [1]. Thus, understanding the molecular mechanisms involved in ageing and identifying ways to increase lifespan are intriguing areas of biogerontology research.

Although there are multiple hypotheses about aging and its causes are poorly understood, epigenetic alterations represent one crucial mechanism behind the deteriorated cellular functions. These alterations are linked with changes in gene expression which occur during aging and, thus, they are recognized as part of the ageing process implicated in many age-related diseases [2].

The term *Epigenetics* is defined as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence" [3] that is, the process which influences gene expression levels without involving changes of the primary DNA sequence [4]. Epigenetic modifications comprise four main processes: ATP-dependent chromatin-remodeling complexes, non-coding RNAs, covalent

modifications of DNA bases (such as DNA methylation) and modifications of histones (for review, see [2]).

Focusing on histone modifications, they are a variety of covalent posttranslational modifications that include acetylation, methylation, ubiquitylation, phosphorylation, sumoylation, ribosylation and citrullination [2, 5, 6]. Within these, the most studied modification is acetylation by histone acetyltransferases (HATs) which changes chromatin structure into an open and relaxed conformation facilitating the interaction between transcription factors and gene promoters, and activating gene transcription. On the contrary, the removal of acetyl groups by histone deacetylases (HDACs) induces chromatin compaction and provokes the silencing of gene expression. Thus, the balance between acetylated and deacetylated states plays a crucial role in gene expression regulation [7, 8]. In this sense, several studies have investigated the link between global histone acetylation and longevity [9]. It has been described that the longevity phenotype correlates with improved stress response and upregulated autophagy via increased histone acetylation [10]. However, there are some discrepancies in this point. While specific acetylation of histone 3 and 4 (H3K9 and H4K12) leads to the expression of genes related to synaptic growth and neural activity (for review, see [11]), it has been shown that deacetylation of some histones also contributes to lifespan extension (for review, see [12]). Thus, the relationship between histone acetylation and aging may depend on cellular status and the combination with other epigenetic modifications.

SIRTUIN FAMILY

In mammals, HDACs can be grouped into four classes based on their homology and phylogenetic relationship: class I (HDAC1, 2, 3 and 8); class II HDACs which are divided into two subclasses – IIa (HDAC4, 5, 7 and 9) and IIb (HDAC6 and 10); class III, also called sirtuins (SIRT1, 2, 3, 4, 5, 6 and 7); and class IV (HDAC11). The class I, II, and IV HDACs use zinc to catalyze hydrolysis of the acetylated lysines, whereas sirtuins (SIRTs) rely

on the cofactor nicotinamide adenine dinucleotide (NAD^+) for their function [13].

Sirtuins (SIRTs) constitute a class of deacetylases highly conserved from prokaryotes to eukaryotes. They were initially described as transcription-silencing HDACs in yeast. However, they have been associated with an increase in lifespan by a process believed to be analogous to caloric restriction [14]. Thus, they have emerged as master regulators of metabolism and longevity.

In total, seven SIRT proteins have been identified (SIRT1-7) [15]. Although all of them share a similar catalytic domain of 275 amino acids, they differ in the N-terminal and/or C-terminal sequences flanking its core [16]. They can deacetylate histone and non-histone substrates such as transcriptional factors (Forkhead box class O or FOXO family), enzymes and other proteins [17]. Interestingly, some members of the family also show different properties, like SIRT4 and SIRT6 that are primarily mono-ADP-ribosyl transferases [18, 19].

Regarding their intracellular localization, it has been described that they localize in all subcellular compartments including nucleus, cytosol, membrane and cytoskeleton [17]. While SIRT3, SIRT4 and SIRT5 are mitochondrial proteins, SIRT1, SIRT6 and SIRT7 are mainly nuclear enzymes with different sub-nuclear localization patterns: SIRT1 is largely associated with euchromatin within the nucleus, SIRT6 associates with heterochromatin and SIRT7 localizes to nucleoli [20]. In the case of SIRT2, it can be shuttled between nucleus and cytoplasm, depending on the phase of the cell cycle [12].

They are involved in a variety of biological processes including gene transcription, apoptosis, cell cycle progression, autophagy, metabolism, mitochondrial function, inflammation and ageing, among others (for review, see [12, 15, 17, 21–23]) (Table 1). Noteworthy, their presence has been described in the brain and, due to their multiple functions, it has been suggested that they could be implicated in neurodegenerative diseases [21].

Table 1. Main functions of SIRTs

Sirtuin	Biological functions
SIRT1	- Regulation of cellular metabolism, proliferation and survival - Increase lifespan through maintenance of epigenomic integrity and DNA methylation pattern - Positive regulation of mitochondrial activity, thermogenesis and insulin secretion - Regulation of autophagy - Anti-inflammatory and anti-oxidant activity - Modulation of memory formation and synaptic plasticity - Negative regulation of myogenesis
SIRT2	- Control of cell cycle, senescence and apoptosis - Regulation of cytoskeleton stability, autophagy and myelinogenesis - Modulation of synaptic plasticity - Stabilization of cell membrane receptors - Inhibition of neurite outgrowth and oligodendrocyte differentiation - Regulation of inflammation and microglia-mediated neuroinflammation - Regulation of cellular responses to oxidative stress - Regulation of adipogenesis and adipocyte differentiation - Tumor suppression
SIRT3	- Cellular metabolism and thermogenesis regulation - Apoptosis and cell survival regulation - Maintenance of mitochondrial biogenesis - Response to oxidative stress in Central Nervous System
SIRT4	- Control of insulin secretion and glial development - Regulation of mitochondrial energy metabolism - Involved in glutamate transport and protective role against excitotoxicity
SIRT5	- Urea cycle regulation - Regulation of mitochondrial energy metabolism
SIRT6	- Metabolism and cell survival regulation - Maintenance of DNA stability and promotion of DNA repair under stress conditions - Telomere maintenance - Regulation of somatic growth - Inhibition of proinflammatory gene expression
SIRT7	- DNA repair and genome stability regulation - RNA polymerase I activation - Regulation of oxidative-stress induced DNA repair

Adapted from [12, 15, 17, 21–23].

SIRTUIN 2

Among all sirtuins, SIRT2 expression is found the strongest in the brain [17]. In addition, it is also expressed in a wide range of tissues and organs including the muscle, liver, testes, heart, kidney and adipose tissue [24–26].

Related to brain cells, SIRT2 is expressed in neurons [17], oligodendrocytes [16, 27–30] and other glial cells such as astrocytes and microglia [17, 31]. Within the cell, it is mainly located in the cytoplasm although it can also be found in the nucleus and mitochondria. Moreover, it is located in cell membranes and most abundant in cytoskeleton.

Due to the growing interest that SIRT2 has aroused in recent years, a large number of new substrates have been identified. In the cytoplasm, SIRT2 is involved in cytoskeleton stabilization by targeting α-tubulin, the major component of microtubules (MT) [32]. In addition, due to its ability to migrate to the nuclei, during mitosis SIRT2 deacetylates histone 4 at lysine 16 and changes chromatin structure from a transcriptionally to a repressive active state [33]. In the same way, it controls the G_2/M phase transition during cell cycle as metaphase check-point protein and thus, it has been proposed that it could protect against the development of tumors (for review, see [16]).

SIRT2 is also implicated in the deacetylation of many transcription factors such as p53 [34], p73 [35], p300 [36], NF-κB [37], NRF2 [38], STAT3 [39] and Forkhead transcription factors of class O, FOXO1 [40] and FOXO3a [25]. Moreover, it also interacts with proteins involved in cellular metabolism like LDH-A [36], GKRP [41], LKB1 [42], GAPDH, ENO1 and ALDOA [43]. In addition to this, SIRT2 can deacetylate other substrates like CDK9 [44], MPK-1 [45], CNK1 [46], ATG5 [47], GLUA1 [48], MARCK3 [49] and α-synuclein [50] (for review, see [23]).

The wide variety of identified substrates, added to its expression in different cell types and its ubiquitous distribution within the cell, reflects the wide range of biological functions in which SIRT2 participates that include senescence [51], apoptosis [52], microtubule dynamics and cytoskeletal stabilization [24, 32, 53–55], synapsis and neurotransmission [48], oligodendrocyte differentiation (for review, see [56]) and myelin formation

[57], autophagy [47, 53, 55, 58, 59], metabolism regulation [43, 60] and inflammation (for review, see [61]) (Figure 1).

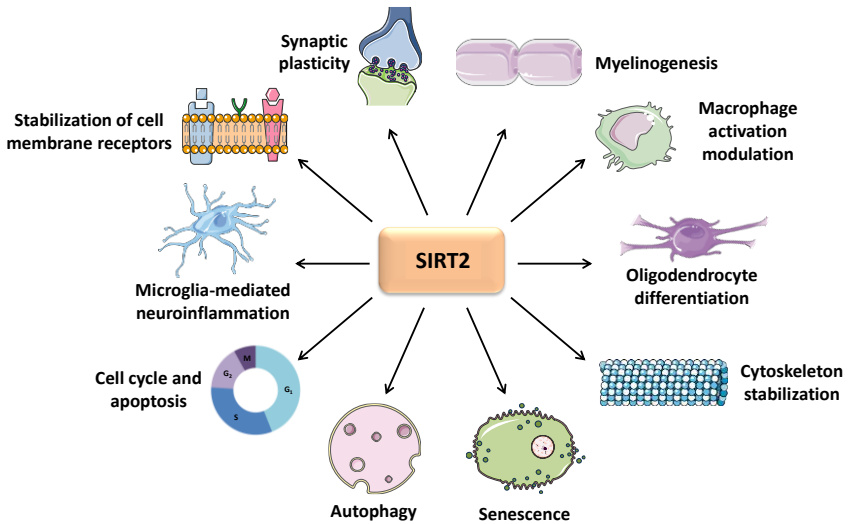


Figure 1. Biological processes modulated by SIRT2.

Role of SIRT2 in Ageing

Several studies have proposed a central role of SIRT2 in ageing, however they show contradictory data. On the one hand, it has been demonstrated that SIRT2 shows an age-dependent accumulation at cerebral [24] and plasmatic level [62]. In line with these results, Anwar et al. (2016) [51] also reported an upregulation of SIRT2 as a specific feature associated with stress induced premature senescence. However, another study indicated that the increase in SIRT2 levels in aged rat brain is specific only to occipital region and no other regions [63]. On the other hand, Kireev et al. (2013) [64] found a decrease in SIRT2 expression in the dentate gyrus of old rats. Moreover, Luo et al. (2019) [65] have recently shown a reduced SIRT2 expression and increased mitochondrial stress with age in mouse hematopoietic stem cells, supporting a previous study that had shown an

age-related decrease of SIRT2 in human peripheral blood mononuclear cells [66].

Additionally, another study has demonstrated that middle-aged SIRT2 knockout mice ($SIRT2^{-/-}$) exhibited locomotor disability due to axonal degeneration suggesting that SIRT2 is a regulator of physiological ageing [67]. In line with this hypothesis, a polymorphism (rs45592833) in the regulatory elements of *SIRT2* gene has been associated with human longevity suggesting the importance of analyzing also the gene expression regulatory mechanisms [68].

Noteworthy, the possible contribution of SIRT2 to the action of caloric restriction on metabolism and longevity has been also assessed. Caloric restriction is one of the most important dietary interventions that can improve brain health and retard ageing [69]. The notion that caloric restriction acts through the sirtuins is attractive and has been intensively investigated but has remained controversial [70]. Wang and coworkers described that SIRT2 expression was activated by caloric restriction and oxidative stress [25]. They showed that under low levels of oxidative stress, SIRT2 stopped cell death and promoted cellular repair through FOXO3a deacetylation. However, under high oxidative stress conditions, SIRT2 could facilitate cell death to clear cells damaged beyond repair. They suggested that SIRT2 is linked to caloric restriction, insulin-like signaling pathway and oxidative stress resistance which are crucial pathways in the control of ageing process [25]. In the same way, North et al. (2014) [26] described that SIRT2 overexpression increased the expression of a key protein involved in ageing and longevity, BubR1. Interestingly, BubR1 levels decrease during normal ageing, and its increase improves lifespan. Given that caloric restriction is associated with increases in NAD+, the authors suggest the possibility that SIRT2-mediated increases in BubR1 could underlie some of the health benefits associated with this procedure [26].

In conclusion, current research on the relationship between SIRT2 and ageing does not provide conclusive results and additional *in vivo* studies are needed to understand the expression of SIRT2 in the different cell types and

its implication in ageing. In any case, despite being contradictory, these reports reinforce the idea of SIRT2 acting as a powerful regulator of ageing.

Role of SIRT2 in Inflammation

Ageing is also characterized by the development of a mild pro-inflammatory state [71]. Inflammation is essential to health since it helps organisms to fight the invasion of microorganisms and it plays essential roles in repair and maintenance of organs. However, when it becomes prolonged it can lead to the accumulation of damage and pathology. This state of chronic inflammation that correlates with ageing is sometimes referred to as "inflammaging" and is a strong risk factor for the occurrence, progression and complication of many chronic diseases including obesity, cardiovascular disease, and neurodegenerative diseases [72]. Analogously, neuroinflammation is the innate immune mechanism of the CNS, where the microglia is the principal effector cell. In this sense, although microglia is essential for the support of physiological functions and cellular microenvironment, in the last years it has become clear that microglia activation contributes to the etiology of neurodegenerative and neuropsychiatric diseases [73].

Clinically, inflammaging is characterized by increased blood levels of several inflammatory biomarkers, including C-reactive protein, IL-6, IL-18 and tumor necrosis factor-α (TNF-α) [74]. Interestingly, it has been shown that SIRT2 deacetylates the p65 subunit of NF-κβ at lysine 310, resulting in a reduced expression of IL-1β, IL-6, monocyte chemoattractant protein 1 and matrix metalloproteinase 9 and 13. Accordingly, cells from $SIRT2^{-/-}$ mice show hyper-acetylation of p65 concomitantly with an increased expression of NF-κβ-dependent genes induced by TNF-α [37, 75]. In line with these results, upon inhibition or deletion of SIRT2, stimulation of the immune response by lipopolysaccharide (LPS) led to an overt production of pro-inflammatory cytokines in an experimental model of colitis and after traumatic brain injury [76, 77]. In addition, Pais and coworkers reported similar results in $SIRT2^{-/-}$ mice after intracortical injection of LPS [31].

Interestingly, Lin et al. (2013) [75] showed that inflammatory factors (LPS, collagen, and TNF-α) reduced the expression of SIRT2, which may account for the decrease in SIRT2 anti-inflammatory functions. Moreover, Zhang et al. (2018) found that SIRT2 overexpression alleviated neuropathic pain and neuroinflammation and its inhibition aggravated these two pathologies, suggesting a role for SIRT2 in inhibiting the inflammatory response [78].

However, a role of SIRT2 in promoting inflammation was also found upon LPS treatment in microglial BV2 cell line and macrophages where inhibition or silencing of SIRT2 decreased nitric oxide (NO) production and pro-inflammatory cytokine levels [79–81]. This was supported by an *in vivo* study using a lethal septic model [82]. Authors showed a reduction in cytokine levels and improved survival in those mice where the activity of SIRT2 was pharmacologically reduced [82]. Same results were reported in C57BL/6 mice treated with LPS [83]. More recently, in line with these conclusions, another study has demonstrated *in vivo* and *in vitro* that SIRT2 inhibition alleviates LPS induced neuroinflammation through regulation of mitogen-activated protein kinase phosphatase-1 and reducing the increase of phosphorylated p38, JNK, and ERK [80]. Paradoxically, after experimental stroke, ischemic brains of wild-type (WT) and $SIRT2^{-/-}$ mice were characterized by a similar induction of neutrophils and activated microglia/macrophages [84].

Thus, to summarize, the presence of SIRT2 seems to be important for inflammation, however, its specific role under the different conditions and the underlying mechanisms still remain unclear.

Role of SIRT2 in Neurodegenerative Diseases

Neurodegenerative disorders share some features in common, including (i) polygenic/complex anomalies, together with cerebrovascular alterations, epigenetic modifications and environmental risk factors; (ii) age-related onset and disease progression (an increase in prevalence in parallel with age); (iii) progressive neuronal degeneration starting in early periods of life with clinical manifestations occurring decades later; (iv) accumulation of

abnormal proteins and conformational changes in pathogenic proteins (abnormal deposits of neurotoxic products); (v) no specific biomarkers for a predictive diagnosis and unspecific clinical phenotypes for an early detection; and (vi) limited options for therapeutic intervention with no curative treatments [85]. In this context, finding a molecular substrate involved in all these common pathways underlying the neurodegenerative disease would provide a novel pharmacological target for these pathologies.

In this sense, SIRT2 has emerged as a potential target to treat neurodegenerative diseases. Firstly, although SIRT2 roles are not well described yet, mounting evidence indicates that excess SIRT2 might be deleterious to neurons [86, 87]. In agreement with this hypothesis, Maxwell and coworkers demonstrated that, among all sirtuins, isoform 3 of SIRT2, which is brain-enriched, has age-dependent accumulation in mouse brain and spinal cord [24]. In addition, it has been shown that SIRT2 overexpression inhibits lysosome-mediated autophagic turnover and induces protein accumulation under proteasome inhibition [58], suggesting its involvement in protein aggregation, a common characteristic of many neurodegenerative diseases.

Taking this into account, the deacetylase SIRT2 could have a crucial role in the etiology or development of neurodegeneratives diseases. In the next sections, the relationship between three main neurodegeneratives diseases (Parkinson, Huntington and Alzheimer's diseases) and SIRT2 is discussed.

Parkinson's Disease

Parkinson's disease (PD) is an age-associated neurodegenerative disorder characterized by the loss of dopamine-producing neurons from a region in the brain known as the substantia nigra and by the accumulation of the protein α-synuclein in intracellular clumps. Regarding the involvement of SIRT2 in its etiology, Outeiro et al. (2007) [88] demonstrated, for the first time, that inhibition of SIRT2 rescued α-synuclein toxicity and modified inclusion morphology in a cellular model of PD. Some years later, Di Fruscia and coworkers also determined this neuroprotective effect of SIRT2

inhibition in another *in vitro* model, a lactacystin-induced model of Parkinsonian neuronal cell death in the N27 cell line [89].

Afterwards, another study described that SIRT2 activity was increased in frontal and temporal cortex in postmortem tissues of PD's patients compared to control groups [90]. Interestingly, the authors showed that SIRT2 inhibition enhanced α-synuclein aggregate formation and demonstrated that its overexpression reduced the number of aggregates in SH-SY5Y cells, suggesting that SIRT2 increase in PD's tissues is a compensatory mechanism to combat oxidative stress [90]. On the other hand, the same year, De Oliveira et al. (2017) [50] found that α-synuclein acetylation is a key regulatory mechanism for α-synuclein aggregation and toxicity. In this sense, genetic deletion of SIRT2 increased α-synuclein acetylation and reduced its aggregation and its toxicity, demonstrating the potential therapeutic value of SIRT2 inhibition in synucleinopathies.

Accordingly, Esteves et al. (2018) [91] also observed that NAD^+ metabolism was altered in sporadic PD patient-derived cells, which contributed to SIRT2 activation and subsequently α-tubulin deacetylation. SIRT2 inhibition enhanced α-tubulin acetylation and facilitated the trafficking and clearance of misfolded proteins. In addition, they showed that $SIRT2^{-/-}$ mice treated with MPTP had no alterations in motor behavior, highlighting the association between SIRT2, mitochondrial metabolism, autophagy and neurodegeneration in PD [91]. In line with these results, the injection of the microRNA miR-212-5p, which selectively inhibits SIRT2, into the midbrain of mice treated with MPTP prevented dopaminergic neuron loss and promoted autophagy showing neuroprotective effects [92].

More recently, it has been described that α-synuclein is a microtubule associated protein and that its neurotoxicity is partially due to the deacetylation of α-tubulin by SIRT2 [53]. They demonstrate a functional role of α-tubulin and α-synuclein acetylation on autophagic vesicular traffic and cargo clearance. Thus, α-tubulin acetylation induced by SIRT2 inhibition improved microtubules stability and increased α-synuclein/tubulin binding reducing α-synuclein toxicity [53] indicating that microtubules can be also a promising therapeutic target in the field of

neurodegenerative disorders and that SIRT2 could play a key role in the process.

Lately, human studies have been performed concluding that PD patients have higher SIRT2 mRNA levels in peripheral blood compared with healthy controls [93]. In addition, the same group has identified a novel polymorphism (rs2015) in the 3'UTR region of *SIRT2* gene which is associated with PD risk in Chinese Han population [93].

In summary, there is a general consensus on the beneficial effects of SIRT2 inhibition in PD. However, more studies are needed to corroborate these results and to decipher the underlying mechanisms.

Huntington's Disease

Huntington's disease (HD) is a progressive, fatal hereditary autosomal dominant neurodegenerative disorder with multiple neurological manifestations. The genetic basis of HD is a CAG trinucleotide repeat (40 or more times) expansion within exon 1 of the huntingtin gene (HTT). Prominent neuropathological features include ubiquitin positive cytoplasmic aggregates and intranuclear inclusions of N-terminal mutant HTT fragments. Reduction of the striatum volume and thinning of the cortex could therefore be detected by CT/MRI in genetically diagnosed individuals before the appearance of the symptoms [94]. Interestingly, a recent study has shown that mRNA levels of SIRT2 are increased in the striatum of post mortem HD brains [95] and its negative role in the pathology appears consistent. In this sense, neuroprotective effects of pharmacological and/or genetic inhibition of SIRT2 have been demonstrated [60, 96–98]. Luthi-Carter et al. (2010) [60] suggested that these neuroprotective effects observed in cellular and invertebrate models of HD could be due to the transcriptional repression of cholesterol biosynthesis, in agreement with previous studies that showed detrimental effects of cholesterol accumulation in neurons. Moreover, Chopra et al. (2012) [96] corroborated Luthi-Carter's study observing that SIRT2 inhibition with compound AK-7 improved the motor function, extended survival, reduced brain atrophy and improved the striatal neuronal volume of two genetic mouse models of HD. Additionally,

it reduced mutant HTT polyglutamine and cholesterol aggregates, ameliorating their neuropathological phenotype [96].

In addition, similar neuroprotective effects have been described in a SIRT2 knockdown *Drosophila* model challenged with HTT [97]. Besides, Quinti and coworkers also corroborated the neuroprotective activity of SIRT2 inhibition in *ex vivo* rat corticostriatal slice explants expressing HTT exon 1 with expanded CAG repeats and in *Drosophila* model of HD [98].

Nevertheless, some contradictory results have also emerged. Bobrowska et al. (2012) [99] showed that genetic reduction or ablation of SIRT2 in a genetic mouse model of HD (R6/2 mice) had no effect on disease progression or HTT protein levels. The reason for these discrepancies is unclear, thus, more studies are needed to confirm the specific effects of SIRT2 on HD.

Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementing illness with over 45 million people suffering worldwide [100]. It is predominantly a sporadic late-onset disease with exponentially increasing prevalence starting at the age of 65 years. AD patients suffer from memory impairment and cognitive decline due to AD primarily affects hippocampus, entorhinal cortex and neocortex which are involved in learning and memory [101]. In addition, aphasia, personality and behaviour changes and performance disorders are also typical symptoms of AD which lead to the decline of patients' life quality.

AD can be categorized into two clinical subtypes: familial (fAD) and sporadic AD (sAD). Although both types develop similar pathological phenotypes, the factors triggering the neurodegenerative process are completely different. In fAD, the pathological buildup is caused by the presence of autosomal-dominant mutations (APP, PSEN1 and PSEN2 genes) [102]. However, sAD, which represents the majority of AD cases, is most commonly caused by multiple interactions regarding genetic, epigenetic and environmental factors, although, the principal risk factor is ageing (for review, see [22]).

Focusing on neuropathological characteristics, the principal hallmarks of AD are extra-neuronal senile plaques and intracellular neurofibrillary tangles (NFTs) [102]. The first lesion is formed by deposits of amyloid-β (Aβ) produced by the proteolysis of amyloid precursor proteins (APP) and their aggregation in plaques. On the other hand, NFTs consist of hyperphosphorylated and aggregated Tau protein. This Tau aggregation compromises cytoskeleton stability and impairs normal cellular functions (for review, see [103]). All of that leads to cell death and the recruitment of others cell types, such as microglia and astrocytes, leading to an inflammatory reaction which in turn leads to energy failure and synaptic dysfunction [104]. In fact, this inflammatory process has been also related with the onset and progression of AD where immune system activation contributes to AD pathogenesis (for review, see [73, 105]).

Epigenetic mechanisms regulate essential cellular functions and they are also associated with cognition, where changes in the epigenome can modify cognitive functions such as learning or memory [106, 107]. Among all epigenetic modifications, histone acetylation is crucial in memory acquisition and maintenance [108]. It has been demonstrated that the balance between HAT and HDAC is altered in ageing and deficits in age-related memory acquisition are due to a decrease in the transcription of genes involved in learning by an enhancement in HDAC activity [109]. Importantly, reduced histone acetylation correlates with age in the frontal cortex of the human brain, notably at the promoter regions of several genes involved in neurotransmission [110]. Moreover, using inhibitors of HDAC activity, histone acetylation, synaptic plasticity, learning and memory have been enhanced proving that these ageing-related epigenetic modifications are involved in cognitive deficits associated to age and AD [111–115].

Focusing on SIRT2, it has been described that this deacetylase could be implicated on the etiology of AD. A recent study has shown that SIRT2 protein levels are increased in temporal cortex of AD post-mortem samples [55]. In addition, Singh and coworkers have described that SIRT2 activity is also increased in frontal and temporal cortex in postmortem tissues of AD's patients compared to control groups [90]. However, Wongchitrat et al. (2018) [62] have recently found that mRNA SIRT2 levels in plasma were

significantly higher in AD and healthy ageing patients compared to healthy young controls suggesting that it is not a biomarker of the disease but of the ageing process [62].

Moreover, a meta-analysis performed in 2013 revealed that there was an association between a polymorphism (rs10410544, C/T) in *SIRT2* gene and AD susceptibility in humans [116–118]. In this line, Cacabelos et al. (2019) [85] have recently shown an association between this rs10410544 C/T polymorphism of *SIRT2* and AD susceptibility in the *APOEε4*-negative population. Interestingly, SIRT2 variants influence the biochemical, hematological, metabolic, and cardiovascular phenotypes of AD and they can also affect the response to treatment. The authors have observed that *SIRT2-C/T* carriers are the best responders, *SIRT2-T/T* carriers show an intermediate pattern, and *SIRT2-C/C* carriers are the worst responders to a multifactorial treatment [85]. In addition, another recent study has compared blood samples from control and AD patients and has identified two different polymorphisms in 3'UTR region of *SIRT2* gene (rs2015 and rs2241703) which are associated with AD risk [119].

Regarding to SIRT2 inhibitory studies, the first research providing a proof-of-concept for therapeutic benefits of SIRT2 inhibitors in both Tau-associated frontotemporal dementia and AD came in 2012 [120]. The authors tested AK-1 SIRT2 inhibitor which was administered directly into the hippocampus of Tau transgenic rTg4510 mice and they confirmed that SIRT2 inhibition protected to neurodegeneration without affecting neurofibrillary tangles pathology [120]. Afterwards, Scuderi et al. (2014) [121] evaluated if the compound AGK-2, a SIRT2-selective inhibitor, could prevent reactive gliosis, an important hallmark of AD. They showed that AGK-2 reduced astrocyte activation as well as pro-inflammatory mediators' production in primary rat astrocytes exposed to Aβ 1-42 peptide.

Besides, Biella and coworkers tested another SIRT2 inhibitor, AK-7, which improved cognitive performance in two AD transgenic mouse models, 3xTg-AD and APP23, through the modulation of APP amyloidogenic processing and Tau stability [122]. Preliminary, *in vitro*

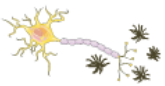
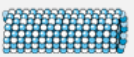
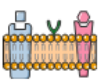
results showed that SIRT2 inhibition reduced Aβ production. Afterwards, *in vivo* data showed an improvement of cognitive performance in the novel object recognition test and an effect on APP proteolytic processing. Additionally, AK-7 increased acetylation of α-tubulin, which may have promoted microtubule stability and raised the steady-state levels of Tau, increasing total Tau protein levels in 3xTgAD mice [122].

In order to further understand the role of SIRT2 on microtubule stability, Silva et al. (2017) [55] have demonstrated that over-activation of SIRT2 results in tubulin deacetylation, Tau phosphorylation and microtubule destabilization which leads to a dysfunction in autophagy, accumulation of Aβ oligomers and neuritic dystrophy. Accordingly, SIRT2 inhibition or knock-out improved autophagy and decreased typical AD cytoskeletal pathology [55]. These results have been recently confirmed by Esteves et al. (2019) [53]. They have demonstrated that SIRT2 inhibition induced α-tubulin acetylation, a decrease in Tau phosphorylation and an increased in Tau/tubulin binding associated with microtubules dynamic improvement [53].

Finally, a more recent study has demonstrated that the administration of the compound 33i, a SIRT2-selective inhibitor, prevents cognitive decline in the senescence accelerated mouse model (SAMP8), which is considered an accurate model of sAD [123]. In this study, 7-month-old SAMP8 mice treated with 33i compound have shown marked improvement in learning and memory in Morris Water Maze test as well as an increase of protein subunits of NMDA and AMPA receptors and a decrease in anti-inflammatory modulators [123].

Overall, these findings establish a link between SIRT2 and principal AD neuropathological hallmarks (Table 2). In this sense, although more studies are needed, the positive effects observed after SIRT2 inhibition on neuronal homeostasis makes SIRT2 inhibition a desirable candidate for the treatment of age-related neurodegenerative diseases.

Table 2. Beneficial effects of SIRT2 inhibition in Alzheimer's disease

Beneficial effects of SIRT2 inhibition in AD		Reference
	Modulation of APP processing and decrease amyloid plaques	55, 122
	Increase the stability of MT favoring the autophagic flux	53, 55
	Prevention of neuroinflammation process	121, 123
	Increase memory-related proteins expression	123, 129, 130
	Decline of the degradation of AMPA receptor subunits	48, 123

SIRT2 Inhibitors

Currently, several selective SIRT2 inhibitors have been identified and 12 of them have come into preclinical studies [124]. Their principal targets are cancer and neurodegenerative diseases.

Within all these, AK-1, AK-7 and AGK-2 have extensively been used in cellular and animal models of neurodegenerative diseases including PD, HD and AD, as it has been explained in this chapter. Despite their promising therapeutic results, none have been approved. In this sense, their low selectivity for SIRT2 has been reported to be one of their main limitations.

Even though AK-1 (IC_{50} = 12.5 µM) is more potent that AK-7 (IC_{50} = 16 µM), it lacks of blood-brain barrier permeability, a crucial characteristic for the treatment of neurodegenerative diseases [124]. Furthermore, AGK-2 was until the date the most potent selective SIRT2 inhibitor (IC_{50} = 3.5 µM) [125]. In this regard, the compound 33i, a 2-anilinobenzamide derivative, exhibited potent and selective SIRT2 inhibition in enzyme assays compared to previous reported SIRT2 inhibitors. 33i showed more than 3.5-fold

greater SIRT2-inhibitory activity and more than 10-fold greater SIRT2-selectivity over SIRT1 and SIRT3 compared to AGK2 with an IC_{50} of 0.57 µM [125]. However, Sakai et al. (2015) [126] designed in 2015 a new compound called 17k which showed similar SIRT2-inhibitory activity than 33i (IC_{50} = 0.60 µM). Nevertheless, although 17k showed more than 150-fold selectivity over SIRT1 and SIRT3 isoforms, 33i did not inhibit either SIRT1 or SIRT3 at concentrations up to 300 µM showing high selectivity for SIRT2 [125]. In addition, 33i has lower molecular weight than 17k allowing better transport across the membranes (350.39g/mol compared to 403g/mol, respectively) (Table 3).

Table 3. Comparison table of the main SIRT2 inhibitors used in basic research of neurodegenerative diseases

Compound	IC_{50} (µM)			Studies (Ref.)
	SIRT2	SIRT1	SIRT3	
Name: AK-1 **Molecular Formula:** $C_{19}H_{21}N_3O_5S$ **Molecular Weight:** 403.5 g/mol **IUPAC name:** 3-(azepan-1-ylsulfonyl)-*N*-(3-nitrophenyl)benzamide	12.5	>50	>50	52, 53, 55, 60, 88, 120
Name: AK-7 **Molecular Formula:** $C_{19}H_{21}BrN_2O_3S$ **Molecular Weight:** 437.4 g/mol **IUPAC name:** 3-(1-azepanylsulfonyl)-*N*-(3-bromophenyl)benzamide	16	>50	>50	76, 78, 96, 120, 122, 126, 131, 132
Name: AGK-2 **Molecular Formula:** $C_{23}H_{13}Cl_2N_3O_2$ **Molecular Weight:** 434.3 g/mol **IUPAC name:** 2-cyano-3-[5-(2,5-dichlorophenyl)furan-2-yl]-*N*-quinolin-5-ylprop-2-enamide	3.5	30	91	48, 52, 60, 80, 83, 88, 90, 120-122, 125, 126, 133, 134
Name: 33i **Molecular Formula:** $C_{21}H_{19}FN_2O_2$ **Molecular Weight:** 350.4 g/mol **IUPAC name:** 2-[3-(3 fluorophenethyloxy) phenylamino]benzamide	0.57	>300	>300	123, 125, 129, 135, 136
Name: 17k **Molecular Formula:** $C_{22}H_{16}BrN_2O$ **Molecular Weight:** 403 g/mol **IUPAC name:** *N*-(4-Bromopyridin-3-yl)-3-(naphthalen-2-yl)benzamide	0.60	>100	>100	126
Name: γ-mangostin **Molecular Formula:** $C_{23}H_{24}O_6$ **Molecular Weight:** 396.4 g/mol **IUPAC name:** 1,3,6,7-tetrahydroxy-2,8-bis(3-methylbut-2-enyl)xanthen-9-one	3.8	22.4	26.8	127

More recently, a new SIRT2 inhibitor, called γ-mangostin, has been identified, which has been extracted from the tropical plant *Garcinia mangostana*. Although, it induces neurite outgrowth, it is less potent than 33i with an IC_{50} of 3.8 μM, being similar to AGK-2 [127].

DISCUSSION AND CONCLUSION

In the present chapter we have outlined how SIRT2 plays a role in ageing and inflammation and may act as modifier of PD, HD and AD pathology. On the whole, SIRT2 activity seems to have a detrimental effect in diseased conditions, which may have strong therapeutic value.

However, the effects of SIRT2 on different biological processes are intriguing and different studies have shown that both absence and overexpression are detrimental. For example, it has been demonstrated that a good control of SIRT2 dosage is a critical factor for successful myelin formation in the peripheral nervous system [57] and for oligodendroglial precursor differentiation [128]. Moreover it is important to note that $SIRT2^{-/-}$ mice show defective synaptic plasticity, impaired learning and memory, microglial activation, locomotor dysfunction, iron deficiency, mitochondrial modifications, tumor formation and cardiac hypertrophic among other diseases (for review, see [23]) suggesting that SIRT2 gene deletion affects several important physiological functions *in vivo*.

Remarkably, the conclusions reached by SIRT2 gene deletion do not always agree with those of the studies that have used pharmacological inhibitors. This is not surprising as, in addition to the developmental effects of SIRT2, it may be also related to the induction of compensatory mechanisms, the specificity or dosage of the SIRT2 inhibitor used, or even different phenotypes of the same diseases. Moreover, SIRT2 seems to play different roles in different cell types and during different stages of development and ageing.

In conclusion, the connection between SIRT2, ageing and neurological disorders is well established, and thus, the potential of SIRT2 as a therapeutic target deserves to be studied in greater depth. In this sense, more

complete *in vivo* studies and the development of accurate disease models and inhibitors are needed to understand the role of SIRT2 under different conditions and to decipher the underlying mechanisms. This step will be essential for optimizing translational efforts while minimizing the risk for detrimental effects.

REFERENCES

[1] United Nations, Department of Economic and Social Affairs PD. *World Population Prospects 2019: Highlights.* United Nations; 2019.

[2] Pagiatakis C, Musolino E, Gornati R, Bernardini G, Papait R. Epigenetics of aging and disease: a brief overview. *Aging Clin Exp Res.* 2019 Dec 6;(123456789): 1–9.

[3] Wu CT. Genes, Genetics, and Epigenetics: A Correspondence. *Science* (80-) [Internet]. 2001 Aug 10;293(5532):1103–5.

[4] Martín-Subero JI. How epigenomics brings phenotype into being. *Pediatr Endocrinol Rev.* 2011 Sep;9 Suppl 1:506–10.

[5] Martinez-Zamudio R, Ha HC. Histone ADP-Ribosylation Facilitates Gene Transcription by Directly Remodeling Nucleosomes. *Mol Cell Biol.* 2012 Jul 1;32(13):2490–502.

[6] Thompson PR, Fast W. Histone Citrullination by Protein Arginine Deiminase: Is Arginine Methylation a Green Light or a Roadblock? *ACS Chem Biol.* 2006 Aug 18;1(7):433–41.

[7] Lee DY, Hayes JJ, Pruss D, Wolffe AP. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell.* 1993 Jan;72(1):73–84.

[8] Kurdistani SK, Grunstein M. Histone acetylation and deacetylation in yeast. *Nat Rev Mol Cell Biol.* 2003 Apr;4(4):276–84.

[9] Sen P, Shah PP, Nativio R, Berger SL. Epigenetic Mechanisms of Longevity and Aging. *Cell.* 2016 Aug;166(4):822–39.

[10] Eisenberg T, Knauer H, Schauer A, Büttner S, Ruckenstuhl C, Carmona-Gutierrez D, et al. Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol.* 2009 Nov 4;11(11):1305–14.

[11] Barter JD, Foster TC. Aging in the Brain: New Roles of Epigenetics in Cognitive Decline. *Neurosci.* 2018 Oct 7;24(5):516–25.

[12] Wątroba M, Dudek I, Skoda M, Stangret A, Rzodkiewicz P, Szukiewicz D. Sirtuins, epigenetics and longevity. *Ageing Res Rev.* 2017 Nov;40:11–9.

[13] Lazo-Gómez R, Ramírez-Jarquín UN, Tovar-y-Romo LB, Tapia R. Histone deacetylases and their role in motor neuron degeneration. *Front Cell Neurosci.* 2013 Dec;7:243.

[14] Guarente L. Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev.* 2000 May;14(9):1021–6.

[15] Polito L, Kehoe PG, Forloni G, Albani D. The molecular genetics of sirtuins: association with human longevity and age-related diseases. *Int J Mol Epidemiol Genet.* 2010;1(3):214–25.

[16] Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J.* 2007 May 15;404(1):1–13.

[17] Jayasena T, Poljak A, Braidy N, Zhong L, Rowlands B, Muenchhoff J, et al. Application of Targeted Mass Spectrometry for the Quantification of Sirtuins in the Central Nervous System. *Sci Rep.* 2016;6(October):1–11.

[18] Ahuja N, Schwer B, Carobbio S, Waltregny D, North BJ, Castronovo V, et al. Regulation of Insulin Secretion by SIRT4, a Mitochondrial ADP-ribosyltransferase. *J Biol Chem.* 2007 Nov 16;282(46):33583–92.

[19] Liszt G, Ford E, Kurtev M, Guarente L. Mouse Sir2 Homolog SIRT6 Is a Nuclear ADP-ribosyltransferase. *J Biol Chem.* 2005 Jun 3; 280(22):21313–20.

[20] Michishita E, Park JY, Burneskis JM, Barrett JC, Horikawa I. Evolutionarily Conserved and Nonconserved Cellular Localizations and Functions of Human SIRT Proteins. *Mol Biol Cell.* 2005 Oct;16(10):4623–35.

[21] Jęśko H, Wencel P, Strosznajder RP, Strosznajder JB. Sirtuins and Their Roles in Brain Aging and Neurodegenerative Disorders. *Neurochem Res*. 2017;42(3):876–90.

[22] Satoh A, Imai SI, Guarente L. The brain, sirtuins, and ageing. *Nat Rev Neurosci.* 2017;18(6):362–74.

[23] Wang Y, Yang J, Hong T, Chen X, Cui L. SIRT2: Controversy and multiple roles in disease and physiology. *Ageing Res Rev.* 2019 Nov;55(September):100961.

[24] Maxwell MM, Tomkinson EM, Nobles J, Wizeman JW, Amore AM, Quinti L, et al. The Sirtuin 2 microtubule deacetylase is an abundant neuronal protein that accumulates in the aging CNS. *Hum Mol Genet.* 2011;20(20):3986–96.

[25] Wang F, Nguyen M, Qin FXF, Tong Q. SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction. *Aging Cell.* 2007;6(4):505–14.

[26] North BJ, Rosenberg MA, Jeganathan KB, Hafner A V, Michan S, Dai J, et al. SIRT2 induces the checkpoint kinase BubR1 to increase lifespan. *EMBO J* [Internet]. 2014 Jul 13;33(13):1438–53.

[27] Li W, Zhang B, Tang J, Cao Q, Wu Y, Wu C, et al. Sirtuin 2, a Mammalian Homolog of Yeast Silent Information Regulator-2 Longevity Regulator, Is an Oligodendroglial Protein That Decelerates Cell Differentiation through Deacetylating alfa-Tubulin. *J Neurosci*. 2007;27(10):2606–16.

[28] Southwood CM, Peppi M, Dryden S, Tainsky MA, Gow A. Microtubule deacetylases, SirT2 and HDAC6, in the nervous system. *Neurochem Res.* 2007;32(2):187–95.

[29] Zhu H, Zhao L, Wang E, Dimova N, Liu G, Feng Y, et al. The QKI-PLP pathway controls SIRT2 abundance in CNS myelin. *Glia.* 2012 Jan;60(1):69–82.

[30] Werner HB, Kuhlmann K, Shen S, Uecker M, Schardt A, Dimova K, et al. Proteolipid Protein Is Required for Transport of Sirtuin 2 into CNS Myelin. *J Neurosci.* 2007 Jul 18;27(29):7717–30.

[31] Pais TF, Szego ÉM, Marques O, Miller-Fleming L, Antas P, Guerreiro P, et al. The NAD-dependent deacetylase sirtuin 2 is a suppressor of microglial activation and brain inflammation. *EMBO J.* 2013;32(19):2603–16.

[32] North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. The Human Sir2 Ortholog, SIRT2, Is an NAD+-Dependent Tubulin Deacetylase. *Mol Cell.* 2003;11:437–44.

[33] Vaquero A. SirT2 is a histone deacetylase with preference for histone H4 Lys 16 during mitosis. *Genes Dev.* 2006 May 15;20(10):1256–61.

[34] Hoffmann G, Breitenbücher F, Schuler M, Ehrenhofer-Murray AE. A Novel Sirtuin 2 (SIRT2) Inhibitor with p53-dependent Pro-apoptotic Activity in Non-small Cell Lung Cancer. *J Biol Chem.* 2014 Feb 21;289(8):5208–16.

[35] Funato K, Hayashi T, Echizen K, Negishi L, Shimizu N, Koyama Nasu R, et al. SIRT2-mediated inactivation of p73 is required for glioblastoma tumorigenicity. *EMBO Rep.* 2018 Nov 13;19(11).

[36] Jing H, Lin H. Sirtuins in Epigenetic Regulation. *Chem Rev*. 2015 Mar 25;115(6):2350–75.

[37] Rothgiesser KM, Erener S, Waibel S, Luscher B, Hottiger MO. SIRT2 regulates NF- B-dependent gene expression through deacetylation of p65 Lys310. *J Cell Sci*. 2010 Dec 15;123(24):4251–8.

[38] Yang X, Park SH, Chang HC, Shapiro JS, Vassilopoulos A, Sawicki KT, et al. Sirtuin 2 regulates cellular iron homeostasis via deacetylation of transcription factor NRF2. *J Clin Invest.* 2017 Mar 13;127(4):1505–16.

[39] Hu F, Sun X, Li G, Wu Q, Chen Y, Yang X, et al. Inhibition of SIRT2 limits tumour angiogenesis via inactivation of the STAT3/VEGFA signalling pathway. *Cell Death Dis*. 2019 Jan 18;10(1):9.

[40] Wang F, Tong Q. SIRT2 Suppresses Adipocyte Differentiation by Deacetylating FOXO1 and Enhancing FOXO1's Repressive Interaction with PPARγ. Chernoff J, editor. *Mol Biol Cell.* 2009 Feb;20(3):801–8.

[41] Watanabe H, Inaba Y, Kimura K, Matsumoto M, Kaneko S, Kasuga M, et al. Sirt2 facilitates hepatic glucose uptake by deacetylating glucokinase regulatory protein. *Nat Commun.* 2018 Dec 2;9(1):30.

[42] Tang X, Chen XF, Wang NY, Wang XM, Liang ST, Zheng W, et al. SIRT2 Acts as a Cardioprotective Deacetylase in Pathological Cardiac Hypertrophy. *Circulation*. 2017 Nov 21;136(21):2051–67.

[43] Cha Y, Han MJ, Cha HJ, Zoldan J, Burkart A, Jung JH, et al. Metabolic control of primed human pluripotent stem cell fate and function by the miR-200c–SIRT2 axis. *Nat Cell Biol.* 2017 May 24;19(5):445–56.

[44] Zhang H, Park S-H, Pantazides BG, Karpiuk O, Warren MD, Hardy CW, et al. SIRT2 directs the replication stress response through CDK9 deacetylation. *Proc Natl Acad Sci.* 2013 Aug 13;110 (33):13546–51.

[45] Wang J, Koh H, Zhou L, Bae U, Lee H, Bang IH, et al. Sirtuin 2 aggravates postischemic liver injury by deacetylating mitogen-activated protein kinase phosphatase-1. *Hepatology.* 2017 Jan 7;65(1):225–36.

[46] Fischer A, Mühlhäuser WWD, Warscheid B, Radziwill G. Membrane localization of acetylated CNK1 mediates a positive feedback on RAF/ERK signaling. *Sci Adv.* 2017 Aug 11;3(8):e1700475.

[47] Liu G, Park SH, Imbesi M, Nathan WJ, Zou X, Zhu Y, et al. Loss of NAD-Dependent Protein Deacetylase Sirtuin-2 Alters Mitochondrial Protein Acetylation and Dysregulates Mitophagy. *Antioxid Redox Signal.* 2017 May 20;26(15):849–63.

[48] Wang G, Li S, Gilbert J, Gritton HJ, Wang Z, Li Z, et al. Crucial Roles for SIRT2 and AMPA Receptor Acetylation in Synaptic Plasticity and Memory. *Cell Rep.* 2017;20(6):1335–47.

[49] Yang P, Xu C, Reece EA, Chen X, Zhong J, Zhan M, et al. Tip60- and sirtuin 2-regulated MARCKS acetylation and phosphorylation are required for diabetic embryopathy. *Nat Commun*. 2019 Dec 17;10(1):282.

[50] de Oliveira RM, Vicente Miranda H, Francelle L, Pinho R, Szegö ÉM, Martinho R, et al. The mechanism of sirtuin 2–mediated exacerbation of alpha-synuclein toxicity in models of Parkinson disease. Bates G, editor. *PLOS Biol.* 2017 Mar 3;15(3):e2000374.

[51] Anwar T, Khosla S, Ramakrishna G. Increased expression of SIRT2 is a novel marker of cellular senescence and is dependent on wild type p53 status. *Cell Cycle.* 2016;15(14):1883–97.

[52] She DT, Wong LJ, Baik SH, Arumugam TV. SIRT2 Inhibition Confers Neuroprotection by Downregulation of FOXO3a and MAPK Signaling Pathways in Ischemic Stroke. *Mol Neurobiol.* 2018;1–16.

[53] Esteves AR, Palma AM, Gomes R, Santos D, Silva DF, Cardoso SM. Acetylation as a major determinant to microtubule-dependent autophagy: Relevance to Alzheimer's and Parkinson disease pathology. *Biochim Biophys Acta - Mol Basis Dis.* 2019 Aug; 1865(8):2008–23.

[54] Harting K, Knöll B. SIRT2-mediated protein deacetylation: An emerging key regulator in brain physiology and pathology. *Eur J Cell Biol.* 2010;89(2–3):262–9.

[55] Silva DF, Esteves AR, Oliveira CR, Cardoso SM. Mitochondrial Metabolism Power SIRT2-Dependent Deficient Traffic Causing Alzheimer's-Disease Related Pathology. *Mol Neurobiol.* 2017; 54(6):4021–40.

[56] Tang BL, Chua CEL. SIRT2, tubulin deacetylation, and oligodendroglia differentiation. *Cell Motil Cytoskeleton.* 2008; 65(3):179–82.

[57] Beirowski B, Gustin J, Armour SM, Yamamoto H, Viader A, North BJ, et al. Sir-two-homolog 2 (Sirt2) modulates peripheral myelination through polarity protein Par-3/atypical protein kinase C (aPKC) signaling. *Proc Natl Acad Sci.* 2011 Oct 25;108(43):E952–61.

[58] Gal J, Bang Y, Choi HJ. SIRT2 interferes with autophagy-mediated degradation of protein aggregates in neuronal cells under proteasome inhibition. *Neurochem Int*. 2012 Dec;61(7):992–1000.

[59] Inoue T, Nakayama Y, Li Y, Matsumori H, Takahashi H, Kojima H, et al. SIRT2 knockdown increases basal autophagy and prevents postslippage death by abnormally prolonging the mitotic arrest that is induced by microtubule inhibitors. *FEBS J.* 2014;281(11):2623–37.

[60] Luthi-Carter R, Taylor DM, Pallos J, Lambert E, Amore A, Parker A, et al. SIRT2 inhibition achieves neuroprotection by decreasing sterol biosynthesis. *Proc Natl Acad Sci*. 2010;107(17):7927–32.

[61] Mendes KL, Lelis D de F, Santos SHS. Nuclear sirtuins and inflammatory signaling pathways. *Cytokine Growth Factor Rev*. 2017 Dec;38(November):98–105.

[62] Wongchitrat P, Pakpian N, Kitidee K, Phopin K, Dharmasaroja PA, Govitrapong P. Alterations in the Expression of Amyloid Precursor Protein Cleaving Enzymes mRNA in Alzheimer Peripheral Blood. *Curr Alzheimer Res*. 2018 Dec 31;16(1):29–38.

[63] Braidy N, Poljak A, Grant R, Jayasena T, Mansour H, Chan-Ling T, et al. Differential expression of sirtuins in the aging rat brain. *Front Cell Neurosci*. 2015;9(May):1–16.

[64] Kireev RA, Vara E, Tresguerres JAF. Growth hormone and melatonin prevent age-related alteration in apoptosis processes in the dentate gyrus of male rats. *Biogerontology*. 2013 Aug 13;14(4):431–42.

[65] Luo H, Mu WC, Karki R, Chiang HH, Mohrin M, Shin JJ, et al. Mitochondrial Stress-Initiated Aberrant Activation of the NLRP3 Inflammasome Regulates the Functional Deterioration of Hematopoietic Stem Cell Aging. *Cell Rep*. 2019 Jan;26(4):945–954.e4.

[66] Yudoh K, Karasawa R, Ishikawa J. Age-related Decrease of Sirtuin 2 Protein in Human Peripheral Blood Mononuclear Cells. *Curr Aging Sci*. 2015 Oct 27;8(3):256–8.

[67] Fourcade S, Morató L, Parameswaran J, Ruiz M, Ruiz-Cortés T, Jové M, et al. Loss of SIRT2 leads to axonal degeneration and locomotor disability associated with redox and energy imbalance. *Aging Cell*. 2017;16(August):1404–13.

[68] Crocco P, Montesanto A, Passarino G, Rose G. Polymorphisms Falling Within Putative miRNA Target Sites in the 3′UTR Region of SIRT2 and DRD2 Genes Are Correlated With Human Longevity. *Journals Gerontol Ser A Biol Sci Med Sci*. 2016 May;71(5):586–92.

[69] Martin B, Mattson MP, Maudsley S. Caloric restriction and intermittent fasting: Two potential diets for successful brain aging. *Ageing Res Rev.* 2006 Aug;5(3):332–53.

[70] Park S, Mori R, Shimokawa I. Do sirtuins promote mammalian longevity?: A Critical review on its relevance to the longevity effect induced by calorie restriction. *Mol Cells.* 2013 Jun 8;35(6):474–80.

[71] Morrisette-Thomas V, Cohen AA, Fülöp T, Riesco É, Legault V, Li Q, et al. Inflamm-aging does not simply reflect increases in pro-inflammatory markers. *Mech Ageing Dev.* 2014 Jul;139:49–57.

[72] Bektas A, Schurman SH, Sen R, Ferrucci L. Aging, inflammation and the environment. *Exp Gerontol.* 2018 May;105:10–8.

[73] Gray S, Kinghorn K, Woodling N. Shifting equilibriums in Alzheimer's disease: the complex roles of microglia in neuroinflammation, neuronal survival and neurogenesis. *Neural Regen Res*. 2020;15(7):1208.

[74] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta - Mol Cell Res.* 2011 May;1813(5):878–88.

[75] Lin J, Sun B, Jiang C, Hong H, Zheng Y. Sirt2 suppresses inflammatory responses in collagen-induced arthritis. *Biochem Biophys Res Commun.* 2013 Nov;441(4):897–903.

[76] Yuan F, Xu ZM, Lu LY, Nie H, Ding J, Ying WH, et al. SIRT2 inhibition exacerbates neuroinflammation and blood-brain barrier disruption in experimental traumatic brain injury by enhancing NF-κB p65 acetylation and activation. *J Neurochem.* 2016;136(3):581–93.

[77] Lo Sasso G, Menzies KJ, Mottis A, Piersigilli A, Perino A, Yamamoto H, et al. SIRT2 Deficiency Modulates Macrophage Polarization and Susceptibility to Experimental Colitis. Heimesaat MM, editor. *PLoS One.* 2014 Jul 29;9(7):e103573.

[78] Zhang Y, Chi D. Overexpression of SIRT2 Alleviates Neuropathic Pain and Neuroinflammation Through Deacetylation of Transcription Factor Nuclear Factor-Kappa B. *Inflammation.* 2018;41(2):569–78.

[79] Chen H, Wu D, Ding X, Ying W. SIRT2 is required for lipopolysaccharide-induced activation of BV2 microglia. *Neuroreport.* 2015;26:88–93.

[80] Jiao F, Wang Y, Zhang W, Zhang H, Chen Q, Wang L, et al. AGK2 Alleviates Lipopolysaccharide Induced Neuroinflammation through Regulation of Mitogen-Activated Protein Kinase Phosphatase-1. *J Neuroimmune Pharmacol.* 2019 Nov 30;1–13.

[81] Lee AS, Jung YJ, Kim D, Nguyen-Thanh T, Kang KP, Lee S, et al. SIRT2 ameliorates lipopolysaccharide-induced inflammation in macrophages. *Biochem Biophys Res Commun.* 2014;450(4):1363–9.

[82] Zhao T, Alam H, Liu B, Bronson R, Nikolian V, Wu E, et al. Selective Inhibition of SIRT2 Improves Outcomes in a Lethal Septic Model. *Curr Mol Med.* 2015;15:634–41.

[83] Wang B, Zhang Y, Cao W, Wei X, Chen J, Ying W. SIRT2 Plays Significant Roles in Lipopolysaccharides-Induced Neuroinflammation and Brain Injury in Mice. *Neurochem Res.* 2016;41(9):2490–500.

[84] Krey L, Lühder F, Kusch K, Czech-Zechmeister B, Könnecke B, Outeiro TF, et al. Knockout of silent information regulator 2 (SIRT2) preserves neurological function after experimental stroke in mice. *J Cereb Blood Flow Metab.* 2015;35(12):2080–8.

[85] Cacabelos R, Carril J, Cacabelos N, Kazantsev A, Vostrov A, Corzo L, et al. Sirtuins in Alzheimer's Disease: SIRT2-Related GenoPhenotypes and Implications for PharmacoEpiGenetics. *Int J Mol Sci.* 2019 Mar 12;20(5):1249.

[86] Pfister JA, Ma C, Morrison BE, D'Mello SR. Opposing effects of sirtuins on neuronal survival: SIRT1-mediated neuroprotection is independent of its deacetylase activity. *PLoS One.* 2008;3(12):1–8.

[87] Suzuki K, Koike T. Mammalian Sir2-related protein (SIRT) 2-mediated modulation of resistance to axonal degeneration in slow Wallerian degeneration mice: A crucial role of tubulin deacetylation. *Neuroscience.* 2007;147(3):599–612.

[88] Outeiro TF, Kontopoulos E, Altmann SM, Kufareva I, Strathearn KE, Amore AM, et al. Sirtuin 2 Inhibitors Rescue a-Synuclein–Mediated

Toxicity in Models of Parkinson's Disease. *Science* (80-). 2007; 317:516–9.

[89] Di Fruscia P, Zacharioudakis E, Liu C, Moniot S, Laohasinnarong S, Khongkow M, et al. The Discovery of a Highly Selective 5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3- d]pyrimidin-4(3 H)-one SIRT2 Inhibitor that is Neuroprotective in an in vitro Parkinson's Disease Model. *Chem Med Chem.* 2015 Jan;10(1):69–82.

[90] Singh P, Hanson PS, Morris CM. Sirtuin-2 Protects Neural Cells from Oxidative Stress and Is Elevated in Neurodegeneration. *Parkinsons Dis.* 2017;2017:1–17.

[91] Esteves AR, Arduíno DM, Silva DF, Viana SD, Pereira FC, Cardoso SM. Mitochondrial Metabolism Regulates Microtubule Acetylome and Autophagy Trough Sirtuin-2: Impact for Parkinson's Disease. *Mol Neurobiol.* 2018 Feb 6;55(2):1440–62.

[92] Sun S, Han X, Li X, Song Q, Lu M, Jia M, et al. MicroRNA-212-5p Prevents Dopaminergic Neuron Death by Inhibiting SIRT2 in MPTP-Induced Mouse Model of Parkinson's Disease. *Front Mol Neurosci.* 2018;11(October):1–14.

[93] Chen X, Mai H, Chen X, Cai Y, Cheng Q, Chen X, et al. Rs2015 Polymorphism in miRNA Target Site of Sirtuin2 Gene Is Associated with the Risk of Parkinson's Disease in Chinese Han Population. *Biomed Res Int.* 2019 May 12;2019:1–8.

[94] Aylward EH, Nopoulos PC, Ross CA, Langbehn DR, Pierson RK, Mills JA, et al. Longitudinal change in regional brain volumes in prodromal Huntington disease. *J Neurol Neurosurg Psychiatry*. 2011 Apr 1;82(4):405–10.

[95] Baldo B, Gabery S, Soylu-Kucharz R, Cheong RY, Henningsen JB, Englund E, et al. SIRT1 is increased in affected brain regions and hypothalamic metabolic pathways are altered in Huntington disease. *Neuropathol Appl Neurobiol.* 2019 Jun 29;45(4):361–79.

[96] Chopra V, Quinti L, Kim J, Vollor L, Narayanan KL, Edgerly C, et al. The Sirtuin 2 Inhibitor AK-7 Is Neuroprotective in Huntington's Disease Mouse Models. *Cell Rep.* 2012 Dec;2(6):1492–7.

[97] Pallos J, Bodai L, Lukacsovich T, Purcell JM, Steffan JS, Thompson LM, et al. Inhibition of specific HDACs and sirtuins suppresses pathogenesis in a Drosophila model of Huntington's disease. *Hum Mol Genet*. 2008 Dec 1;17(23):3767–75.

[98] Quinti L, Casale M, Moniot S, Pais TF, Van Kanegan MJ, Kaltenbach LS, et al. SIRT2- and NRF2-Targeting Thiazole-Containing Compound with Therapeutic Activity in Huntington's Disease Models. *Cell Chem Biol.* 2016 Jul;23(7):849–61.

[99] Bobrowska A, Donmez G, Weiss A, Guarente L, Bates G. SIRT2 Ablation Has No Effect on Tubulin Acetylation in Brain, Cholesterol Biosynthesis or the Progression of Huntington's Disease Phenotypes In Vivo. Okazawa H, editor. *PLoS One*. 2012 Apr 12;7(4):e34805.

[100] Guzman-Martinez L, Maccioni RB, Farías GA, Fuentes P, Navarrete LP. Biomarkers for Alzheimer's Disease. *Curr Alzheimer Res.* 2019 Jul 23;16(6):518–28.

[101] Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* 1991;82:239–59.

[102] Armstrong RA. The molecular biology of senile plaques and neurofibrillary tangles in Alzheimer's disease. *Folia Neuropathol.* 2009;47(4):289–99.

[103] Avila J. Tau phosphorylation and aggregation in Alzheimer's disease pathology. *FEBS Lett.* 2006;580(12):2922–7.

[104] Querfurth HW, LaFerla FM. Alzheimer's Disease. *N Engl J Med.* 2010 Jan 28;362(4):329–44.

[105] Heppner FL, Ransohoff RM, Becher B. Immune attack: The role of inflammation in Alzheimer disease. *Nat Rev Neurosci.* 2015; 16(6):358–72.

[106] Hwang JY, Aromolaran KA, Zukin RS. The emerging field of epigenetics in neurodegeneration and neuroprotection. *Nat Rev Neurosci.* 2017 Jun 18;18(6):347–61.

[107] Kleefstra T, Schenck A, Kramer JM, van Bokhoven H. The genetics of cognitive epigenetics. *Neuropharmacology*. 2014 May;80:83–94.

[108] Gräff J, Tsai LH. The Potential of HDAC Inhibitors as Cognitive Enhancers. *Annu Rev Pharmacol Toxicol.* 2013 Jan 6;53(1):311–30.

[109] Sharma M, Shivarama Shetty M, Arumugam TV, Sajikumar S. Histone deacetylase 3 inhibition re-establishes synaptic tagging and capture in aging through the activation of nuclear factor kappa B. *Sci Rep*. 2015 Dec 18;5(1):16616.

[110] Tang B, Dean B, Thomas EA. Disease- and age-related changes in histone acetylation at gene promoters in psychiatric disorders. *Transl Psychiatry* [Internet]. 2011 Dec 20;1(12):e64–e64.

[111] Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, et al. HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature*. 2009;459(7243):55–60.

[112] McQuown SC, Wood MA. HDAC3 and the molecular brake pad hypothesis. *Neurobiol Learn Mem*. 2011;96:27–34.

[113] Cuadrado-Tejedor M, Oyarzabal J, Lucas MP, Franco R, García-Osta A. Epigenetic drugs in Alzheimer's disease. *Biomol Concepts.* 2013 Oct 1;4(5):433–45.

[114] Fonseca R. The aging memory: Modulating epigenetic modifications to improve cognitive function. *Neurobiol Learn Mem.* 2016 Sep;133:182–4.

[115] Krishna K, Behnisch T, Sajikumar S. Inhibition of Histone Deacetylase 3 Restores Amyloid-β Oligomer-Induced Plasticity Deficit in Hippocampal CA1 Pyramidal Neurons. Arancio O, editor. *J Alzheimer's Dis.* 2016 Mar 30;51(3):783–91.

[116] Wei W, Xu X, Li H, Zhang Y, Han D, Wang Y, et al. The SIRT2 polymorphism rs10410544 and risk of Alzheimer's disease: A meta-analysis. *Neuro Molecular Med.* 2014;16(2):448–56.

[117] Polito L, Kehoe PG, Davin A, Benussi L, Ghidoni R, Binetti G, et al. The SIRT2 polymorphism rs10410544 and risk of Alzheimer's disease in two Caucasian case-control cohorts. *Alzheimer's Dement.* 2013 Jul;9(4):392–9.

[118] Xia M, Yu JT, Miao D, Lu RC, Zheng XP, Tan L. SIRT2 polymorphism rs10410544 is associated with Alzheimer's disease in a Han Chinese population. *J Neurol Sci.* 2014 Jan;336(1–2):48–51.

[119] Shen Y, Chen L, Zhang S, Xie L. Correlation Between SIRT2 3'UTR Gene Polymorphism and the Susceptibility to Alzheimer's Disease. *J Mol Neurosci*. 2020 Mar 2;1–9.

[120] Spires-Jones TL, Fox LM, Rozkalne A, Pitstick R, Carlson GA, Kazantsev AG. Inhibition of sirtuin 2 with sulfobenzoic acid derivative AK1 is non-toxic and potentially neuroprotective in a mouse model of frontotemporal dementia. *Front Pharmacol.* 2012;3(42):1–7.

[121] Scuderi C, Stecca C, Bronzuoli R. MR, Rotili D, Valente S, Mai A, et al. Sirtuin modulators control reactive gliosis in an in vitro model of alzheimer's disease. *Front Pharmacol.* 2014;5(89):1–8.

[122] Biella G, Fusco F, Nardo E, Bernocchi O, Colombo A, Lichtenthaler SF, et al. Sirtuin 2 Inhibition Improves Cognitive Performance and Acts on Amyloid-β Protein Precursor Processing in Two Alzheimer's Disease Mouse Models. *J Alzheimer's Dis.* 2016;53(3):1193–207.

[123] Diaz-Perdigon T, Belloch FB, Ricobaraza A, Elboray EE, Suzuki T, Tordera RM, et al. Early sirtuin 2 inhibition prevents age-related cognitive decline in a senescence-accelerated mouse model. *Neuropsychopharmacology.* 2020;45(2):347–57.

[124] Zhou Z, Ma T, Zhu Q, Xu Y, Zha X. Recent advances in inhibitors of sirtuin1/2: an update and perspective. *Future Med Chem*. 2018 Apr;10(8):907–34.

[125] Suzuki T, Khan MNA, Sawada H, Imai E, Itoh Y, Yamatsuta K, et al. Design, synthesis, and biological activity of a novel series of human sirtuin-2-selective inhibitors. *J Med Chem.* 2012;55(12):5760–73.

[126] Sakai T, Matsumoto Y, Ishikawa M, Sugita K, Hashimoto Y, Wakai N, et al. Design, synthesis and structure–activity relationship studies of novel sirtuin 2 (SIRT2) inhibitors with a benzamide skeleton. *Bioorg Med Chem.* 2015 Jan;23(2):328–39.

[127] Yeong KY, Khaw KY, Takahashi Y, Itoh Y, Murugaiyah V, Suzuki T. Discovery of gamma-mangostin from Garcinia mangostana as a potent and selective natural SIRT2 inhibitor. *Bioorg Chem.* 2020 Jan;94(June 2019):103403.

[128] Thangaraj MP, Furber KL, Gan JK, Ji S, Sobchishin L, Doucette JR, et al. RNA-binding Protein Quaking Stabilizes Sirt2 mRNA during Oligodendroglial Differentiation. *J Biol Chem.* 2017 Mar 31; 292(13):5166–82.

[129] Erburu M, Muñoz-Cobo I, Diaz-Perdigon T, Mellini P, Suzuki T, Puerta E, et al. SIRT2 inhibition modulate glutamate and serotonin systems in the prefrontal cortex and induces antidepressant-like action. *Neuropharmacology.* 2017;117:195–208.

[130] Muñoz-Cobo I, Erburu MM, Zwergel C, Cirilli R, Mai A, Valente S, et al. Nucleocytoplasmic export of HDAC5 and SIRT2 downregulation: two epigenetic mechanisms by which antidepressants enhance synaptic plasticity markers. *Psychopharmacology* (Berl). 2018 Oct 8;235(10):2831–46.

[131] Taylor DM, Balabadra U, Xiang Z, Woodman B, Meade S, Amore A, et al. A Brain-Permeable Small Molecule Reduces Neuronal Cholesterol by Inhibiting Activity of Sirtuin 2 Deacetylase. *ACS Chem Biol.* 2011 Jun 17;6(6):540–6.

[132] Jung HY, Yoo DY, Kim JW, Kim DW, Choi JH, Chung JY, et al. Sirtuin-2 inhibition affects hippocampal functions and sodium butyrate ameliorates the reduction in novel object memory, cell proliferation, and neuroblast differentiation. *Lab Anim Res.* 2016;32(4):224–30.

[133] Li Y, Nie H, Wu D, Zhang J, Wei X, Ying W. Poly(ADP-ribose) polymerase mediates both cell death and ATP decreases in SIRT2 inhibitor AGK2-treated microglial BV2 cells. *Neurosci Lett.* 2013 Jun;544:36–40.

[134] Skoge RH, Ziegler M. SIRT2 inactivation reveals a subset of hyperacetylated perinuclear microtubules inaccessible to HDAC6. *J Cell Sci.* 2016;129:2972–82.

[135] Erburu M, Muñoz-Cobo I, Domínguez-Andrés J, Beltran E, Suzuki T, Mai A, et al. Chronic stress and antidepressant induced changes in Hdac5 and Sirt2 affect synaptic plasticity. *Eur Neuropsychopharmacol.* 2015;25:2036–48.

[136] Muñoz-Cobo I, Belloch FB, Díaz-Perdigón T, Puerta E, Tordera RM. SIRT2 inhibition reverses anhedonia in the VGLUT1+/− depression model. *Behav Brain Res*. 2017;335(July):128–31.

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Chapter 4

BIOLOGICAL MOTION PERCEPTION AND THE THEORY OF MIND IN NEURODEVELOPMENTAL DISORDERS

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ABSTRACT

Biological motion perception is the unique ability to perceive movement of the human body. This perception requires attention to global configurations and is an essential ability in relation to theory of mind and social cognition. Weak central coherence of global-ignoring and local-focusing styles in visuospatial perception is characteristic of neurodevelopmental disorders. This weakness causes deficits in face processing and biological motion perception, which in turn influences development of the theory of mind, or emotional intelligence. Emotion is

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an essential component of social cognition. This chapter examines the relationship between biological motion, emotions, and theory of mind in people with neurodevelopmental disorders. Specifically, this chapter uses bottom-up and top-down investigations to systematically uncover behavioral and neurological patterns of biological motion perception (with and without emotions) in people with Williams syndrome, autism spectrum disorder, and Down syndrome. The results show that neurodevelopmental disorders cause changes to distinct genotypes in the early stage of life, and these changes have devastating effects on later development of phenotypes.

Keywords: biological motion, emotion, theory of mind, social cognition, Williams syndrome, autism spectrum disorder, Down syndrome

INTRODUCTION

Contextual integration is an essential technique for acquiring meaningful information in humans. It is an important component of social abilities, such as face processing, sensation of emotions, and perception of biological motion. Previous studies have shown that social abilities start to develop in infancy (Grossman and Johnson 2007). Certain neural indexes indicate early development of social awareness, such as the N290 shift in the brainwaves of infants and the N170 component in adult face processing. A fundamental factor of this social awareness is the ability to sense global information. Healthy adults perceive global information automatically in verbal (e.g., language) and nonverbal (e.g., facial recognition) domains.

In the verbal domain, adults integrate propositions (meaningful segments in sentences) with discretely embedded propositions in sentences (Bransford and Franks 1971, 1972; Bransford, Barclay, and Franks 1972; Franks and Bransford 1972, 1974a, 1974b). Healthy adults interpret novel information by integrating the new information with the old. This mixing of old and new can lead to false positives, however, wherein individuals believe that they have seen information before when in fact they have not. The high rate of such false positives suggests gist-oriented contextual integration.

In the nonverbal domain, contextual integration is essential for achieving meaningful coherence. Impaired coherence may weaken the

ability to perceive global information and thus limit focus to local elements only. For example, weak coherence is a major characteristic of social atypicality in people with Williams syndrome (WS), a rare disorder involving missing genes on chromosome 7q11.23 (Hsu and Tzeng 2011; Hsu 2013a, 2013b, 2013c, 2014a, 2014b; Hsu and Chen 2014c), and in people with autism spectrum disorder (ASD), which affects communication and social behaviors (Frith 1989; Happé 1995; Happé and Frith 2006). Interestingly, these two clinical populations display distinct patterns of social ability: people with WS tend to be hyper-social, and people with ASD tend to be social-avoidant. In contrast, people with Down syndrome (DS), which is a condition involving an extra copy of chromosome 21, are better at global perception but still have difficulties in interpersonal interaction.

Biological motion perception is a kind of social and visuospatial information processing of human movement. Social cognition includes the perception of biological motion paired with emotions. This perception is atypically developed in people with neurodevelopmental disorders. In people with WS, for example, it is characterized by a local-focused but global-ignoring processing style (Korenberg et al. 2000; Mervis and John 2010; Semel and Rosner 2003), which has been documented in verbal and non-verbal domains. People with ASD also display this perceptual processing style (Sha and Frith 1983, 1993). In contrast, people with DS pay more attention to global than local information in both verbal and nonverbal domains (Hsu 2019a; Karmiloff-Smith et al. 2016). However, this seemingly better perception of global information still shows atypical processing of socially related information.

Face processing is related to biological motion perception and has specific visuospatial cognitive processing styles and patterns. People with WS tend to focus intently on human faces (Bellugi et al. 2000), but people with ASD have difficulty making eye contact with people (Happé and Frith 2006). Although children with DS often have unintelligible verbal production, they are relatively sociable and able to approach people starting at around two years old (Fidler, Hepburn, and Rogers 2006). Impairment of face processing is related to biological motion perception in other visuospatial disorders, such as people with prosopagnosia (Lange et al.

2009; Pavlova 2012). These atypical sensitivities to faces may originate from a weak perception of biological motion among people with neurodevelopmental disorders. A weak perception of biological motion thus may help predict social cognition ability (Pavlova et al. 2018).

Neurodevelopmental disorders display distinct patterns of sociability: people with WS are hyper-social, people with ASD are social-avoidant, and people with DS are socially friendly. These distinct sociability traits yield different emotional processing styles, leading to atypical theory of mind and social cognition. Thus, atypical visuospatial perception may cause deficits in biological motion perception (with and without emotions), resulting in distinct theory of mind outcomes and atypical social development in people with neurodevelopmental disorders.

This chapter focuses on biological motion processing by examining social abilities in people with WS, ASD, and DS at the behavioral and neurological levels. It also explores further linkages between emotions embedded in biological motion and the theory of mind in people with these disabilities. Future possible research directions in the field of neurodevelopmental disorders also are suggested. Unlike traditional testing that uses false beliefs to probe the social-cognitive aspect of the theory of mind, this chapter contributes to the understanding of this theory using a social-perceptual domain to systematically survey this ability in people with neurodevelopmental disorders using component analyses of the theory of mind (Tager-Flusberg and Sullivan 2000).

BIOLOGICAL MOTION

The perception of biological motion relies on configural, not local, information that conveys visuospatial awareness and social information about human movement. Physical movement contains signals about a person's intentions, desires, and beliefs, which are important cues in social interactions. The processing of biological motion with or without emotion involves distinct neural networks. In a study using positron emission tomography to assess perception of meaningful hand movements (e.g.,

reaching for and opening a bottle) and meaningless hand movements (Grèzes, Costers, and Decety 1998), the meaningful movements activated the left inferior frontal gyrus, left fusiform gyrus, and right inferior temporal gyrus, and the meaningless movements activated the bilateral inferior parietal lobe, right superior parietal lobe, and right cerebellum. These findings suggest that specific brain areas are involved in visuospatial perceptions of human body movements.

Sensitivity to biological motion starts early in life. Infants show lateralized N290 activation in response to biological motion, compared with bilateral activation in response to faces, suggesting a distinct adult-like pattern in recognizing human movement (Grossman and Johnson 2007). These findings further suggest that humans have unique visuospatial perceptions of human body movement.

Hadad, Maurer, and Lewis (2011) compared developmental trajectories in detection of biological motion and global motion in children aged 6–14 years and in adults. In the global motion task, participants observed two dots, one moving at a slow speed and one moving at a fast speed, and were asked to determine whether the dots moved upward or downward by responding verbally or pointing by hand. The results revealed that sensitivity to global motion was a function of age for both faster- and slower-moving dots. Higher sensitivity was observed at the faster rate (main effect of speed), and adults had a lower threshold than children for detecting global motion. To assess biological motion perception, participants completed two tasks (a yes-no judgment and a forced-choice judgment). In the yes-no task, participants watched a biological motion image and responded *yes* if there was a person in the image and *no* if no person was observed. In the forced-choice judgment, participants watched a sequence of noise-dot images and reported the order (first or second) in which biological motion images with a person appeared. The results of the yes-no judgment for biological motion detection showed children aged 6–8 years showed significantly different sensitivity in detection, compared with the adults, whereas children aged 9–14 years showed similar sensitivity to adults. The forced-choice task also revealed an age effect. Adults correctly identified more noise-dot images with human figures, compared with children aged 6–11 years, and children aged 12–14

performed similarly to adults. These results indicate that biological motion detection develops over time.

People with WS have delayed sensitivity to noise-dot representations of biological movement. In a study by Jordan et al. (2002), people with WS and neurotypical controls consisting of mental-age- and chronological-age-matched adults were assessed for their ability to identify walking figures in point-light images containing low and high signal-to-noise ratios. Three noise types (static, random, and yoked) were used. For static noise, the point-light images of figures walking left or right were fixed. For random noise, the point-light images were presented with noise signals at the same velocity but in random trajectories. For yoked noise, the point-light images were paired with all noise signals moving at the same trajectory, except for one that moved at a different velocity. Trials with low signal-to-noise combined the same number of noise lights as the point-light figure and trials with high signal-to-noise used three times more noise lights than those for the figure.

In the low signal-to-noise trial, people with WS identified the moving figure most accurately during static noise (no difference was observed for random and yoked noises). They also performed similarly to the chronological-age-matched controls across all conditions, whereas mental-age-matched participants had the lowest percentage of correct responses. In the high signal-to-noise trial, people with WS showed marginally significantly worse, compared with the chronological-age-matched controls, and performed no differently than the mental-age-matched controls in their responses for random and yoked noises. These findings suggest that individuals with WS are sensitive to contextual information portraying biological movement.

People with ASD demonstrate atypical sensitivity to biological movement. For example, they look longer at scrambled, spinning-top, object motion but not at biological motion (Annaz et al. 2012). Blake et al. (2003) reported that people with ASD often inaccurately identify human actions due to difficulties in perceiving social information. They showed people with autism pictures of biological motion and pictures of globally incoherent configurations and asked participants to verbally identify them. The results revealed that people with ASD were in the normal range for detecting

incoherent configurations but below average at recognizing biological motion, perhaps due to atypical processing in the superior temporal sulcus leading to impaired social skills.

Riddell et al. (2017) examined deficits in biological motion detection in people with DS by asking them to identify human figures in images of point-lights linked by purple lines. They found impairments in global perception of biological motion but not of local motion, suggesting that people with DS have deficient biological perception. Virji-Babul et al. (2006) reported similar deficits in people with DS, who were less able than healthy controls to discern human movement from object movement. Pavlova et al. (2018) compared the ability to recognize actual faces and facial shapes composed of fruits and vegetables in people with DS and revealed significantly lower sensitivity to actual faces in people with DS, compared with healthy controls. This finding confirms a relationship between impaired face processing and recognition of biological motion in people with DS, as previously identified in people affected by prosopagnosia (Lange et al. 2009; Pavlova 2012).

BIOLOGICAL MOTION WITH EMOTION

Emotion is a response to environmental stimuli. Emotions are essential to survival, such as the fear response, and they help regulate behavioral, social, and internal functions. Emotion also is important for social, cognitive, personality, and motor development (Barrett 1998). Concepts, lexical labels, and emotions are processed via several perception modalities. Biological motion is a type of visuospatial perception embedded with social information that requires local and global information processing. This processing can vary in people with neurodevelopmental disorders. For example, people with WS and people with ASD both tend to focus on local elements and ignore global configurations, but these disorders affect sensitivities to social information differently (Bernardino et al. 2002; Bihrle et al. 1989). People with WS are hyper-social, and people with ASD are social-avoidant, which can manifest in differences in awareness of emotions.

Thus, it is important to understand the unique effects of these disorders on perceptions of biological motion with and without emotion.

Processing of biological motion with emotion is contextual and influenced by several factors. Clarke et al. (2005) investigated emotion recognition by having participants view clips of professional actors expressing anger, fear, disgust, sadness, joy, or love. The actors had point-lights attached to their body, and the point-light images were displayed in either upright or inverted orientations. Participants were asked to rate the strength of the actor's emotion from 0 to 100, using a slider on the screen. They identified upright portrayals of target emotions with over 70 percent accuracy and portrayals of non-target emotions with 19 percent accuracy. They rated all target emotions higher than any non-target emotions (except for disgust, which seemed to be confusing to participants). No difference was observed in participants' recognition of upright sadness, anger, and fear. Strength ratings were weaker for inverted orientations of sadness, anger, fear, and disgust (joy and love were not affected). They further found that the number of actors displaying the emotions affected participants' detection of biological motion. Recognition of joy and love was low when acted out by only one actor; on the contrary, recognition of sadness and fear was high when acted out by only one actor. Recognition was lowest for inverted orientations of fear, sadness, and love when acted out by only one actor. Anger was not influenced by the number of actors. Overall, the findings suggest that emotion is context-sensitive and represented distinctly in human cognition. It remains unclear whether certain neurodevelopmental disorders, such as WS, alter context perceptions when processing biological motion with emotions. So far, no study has investigated this issue.

In ASD, typicality in autistic traits has been shown to influence the accuracy of emotion recognition in biological motion. Actis-Grosso, Bossi, and Ricciardelli (2015) examined sensitivities to emotions in people with low autistic traits and high autistic traits by presenting static faces and dynamic biological motion embedded with emotions. The results revealed that people with low autistic traits were least accurate in recognizing fear on faces and that people with high autistic traits showed no difference in recognition of any facial expression. The low autistic traits group was least

sensitive to sadness in biological motion, and the high autistic traits group was least sensitive to fear in biological motion. These findings suggest distinct sensitivities in emotion perceptions among people with different levels of autistic traits.

Hubert et al. (2007) examined whether people with ASD could differentiate among simple human actions (e.g., lifting, hopping), subjective feelings (e.g., itchy, tired), emotional states (e.g., surprised, angry), and motions involved with objects (e.g., dustpan and brush sweeping). Participants viewed clips and were asked to verbally describe them. Compared with healthy controls, participants in the autistic group showed no difference in their ability to detect simple human actions and motions involving objects; however, the autistic participants were worse at describing emotional states and subjective feelings. The findings indicate that people with ASD have deficits in detecting biological motion with emotion.

In Virji-Babul et al. (2006), people with DS viewed point-light images of biological motion (i.e., walking) coupled with emotions of happiness, sadness, madness, and anger. Participants were asked to describe or point to the cartoon face depicting the emotion in each image. The responses were no better than chance when identifying sadness and similar to the healthy control group when recognizing the other three emotions. The findings suggest that people with DS are deficient in perceiving biological motion with emotion.

THEORY OF MIND

Emotion is an important part of theory of mind, a term coined to describe the ability to make inferences about, or "mentalize," others' mental states. This mentalizing ability was first observed in chimpanzees (Premack and Woodruff 1978) and later in humans (Wimmer and Perner 1983). Mentalizing includes inferences about others' beliefs, desires, intentions, imagination, and emotions (Blake et al. 2003). Mental representations form based on these inference conditions and the consequences of changes in

those inferences. When the representations do not match, it can lead to the development of false beliefs. The two standard protocols for measuring false beliefs include the location-changing (i.e., Sally–Anne story) and content-changing (i.e., Smarties) tests. In the location-changing test (Baron-Cohen et al., 1985), participants observe two puppet protagonists, Sally and Anne, who are playing with marbles in a room. After a while, Sally puts her marbles in a basket and leaves the room. Anne then removes the marbles from the basket and puts them in a box instead. Participants are asked where they think Sally will look for her marbles when she returns to the room. Children who predict that Sally will look in the basket demonstrate an understanding of Sally's false belief (or mental states), whereas children who predict that Sally will look in the box demonstrate that they cannot predict someone else's false belief. This ability to recognize others' false beliefs typically starts to develop in infancy and becomes firmly established by 4 years old (Frith 1989; Happé 1995).

Theory of mind, also known as social intelligence or social knowledge, involves integrating social information with reason, understanding intentions, and inferring desires and beliefs. This processing is an essential part of social development (Brothers 1990). Tager-Flusberg and Sullivan (2000) proposed a componential view of theory of mind, which includes social-perceptual and social-cognitive components based on how the component relates to other cognitive abilities, such as language, development time, neurobiological substrates of each component, and selective impairment in special populations. The social-perceptual component is closely related to the affective system, which includes emotions. Emotions can be recognized in faces and voices, inferred from body movements, and used to differentiate between humans and objects.

Emotion recognition begins in infancy. For example, newborns are face-oriented and reflectively imitate facial expressions (Atkinson, Tunstall, and Dittrich 2007). They also use eye gazes to make inferences. The neurobiological substrates of the social-perceptual theory of mind include the amygdala, medial temporal cortex, and superior temporal gyrus. The social-cognitive component relates to language and concept expression, such as false beliefs. The brain areas related to activation of the social-

cognitive theory of mind are the prefrontal, orbitofrontal, and medial-frontal cortexes; left medial temporal gyrus; and left temporal lobe. People with ASD are impaired in both social-perceptual and social-cognitive components of theory of mind. In contrast, people with Asperger syndrome are deficient in the social-perceptual component but not the social-cognitive component, as they usually have better language abilities than those with ASD.

Unlike traditional investigations into the theory of mind, which examine false beliefs, this chapter investigates the social-perceptual theory of mind in terms of biological motion (with and without emotion) and social-cognitive ability and comparing theory of mind in those with typical and those with atypical neurological development. Such comparison reveals the distinct social-perceptual theory of mind for processing biological motion with and without emotion in atypical neurological development.

In studies of brain and behavioral asymmetry in people with WS (Hsu et al. 2007; Hsu and Chen 2014c), people with WS are less able to recognize emotions in the eyes, suggesting weaker awareness of others' mental states (Tager-Flusberg, Boshart, and Baron-Cohen 1998). In Tager-Flusberg et al.'s (1998) study, young children with WS show deficiency in recognizing emotions on faces: they can identify fear most often, followed by happiness, anger, and then sadness. However, no similar correlations were found in the false belief tests when comparing cohorts based on age, intelligence, or verbal ability. Adults with WS show low accuracy in recognizing emotions in people's eyes (Baron-Cohen et al. 1997; Baron-Cohen 2000). Rose et al. (2007) found that compared with healthy controls, people with WS demonstrate similar abilities to recognize happiness, sadness, anger, surprise, fear, and neutrality on upright faces but less accuracy when identifying inverted neutral faces. Moreover, participants with ASD performed significantly worse than those with WS and healthy controls when asked to identify emotions on upright faces.

Happé et al. (1996) conducted a theory-of-mind study using positron emission tomography and found reverse activation of brain areas in participants with Asperger syndrome when presented with mentalizing stories (theory of mind), non-mentalizing stories (physical stories), and

unstructured sentences. While processing theory-of-mind stories, the left medial prefrontal cortex (Brodmann area 8/9) was not activated, but adjacent areas were activated. In healthy controls, Brodmann area 9/10 was activated to a lesser extent than Brodmann area 8/9. The findings suggest that people with Asperger syndrome process other people's mental states differently than healthy controls and that the left prefrontal cortex is a crucial part in understanding other people's minds.

People with DS show worse false belief ability than people with non-specific intellectual disabilities and healthy controls (Giaouri, Alevriadou, and Tsakiridou 2010). In location-change, content-change, and physical-appearance-reality-change (images differ in color, size, and identity sequentially) tests, people with DS display distinct theory of mind, compared with others with delayed developments.

BRAIN AREAS ASSOCIATED WITH BIOLOGICAL MOTION (WITH AND WITHOUT EMOTION) AND THEORY OF MIND

Biological motion is associated with action observation, which is reflected through mirror neurons in the brain. The linkage conveying information related to action observation travels from the posterior superior temporal sulcus (pSTS) through the inferior parietal lobe to the inferior frontal gyrus, which then bounces information back to the inferior parietal lobe and pSTS (Yang et al. 2016a). Grossman et al. (2000) reported that biological human movements are processed in the bilateral ventral pSTS, cerebellum, middle occipital gyrus, and extrastriate areas of the brain in typical adults. The superior temporal sulcus is responsive to hand actions, lip reading, gaze direction, and movements of mouth and eyes. The right superior temporal sulcus is responsive to social cues and makes inferences about facial emotion, behavior, mental state, and trustworthiness of visual cues, as well as integrating information from the dorsal and ventral pathways (Gallagher and Frith 2003). The left orbital frontal cortex, right cingulate gyrus, and left frontal pole are responsive to upright and inverted point-light images of human walking (Grèzes et al. 2001).

The brain areas that process biological motion with emotion include the amygdala, orbital frontal cortex, anterior cingulate cortex, anterior insula, nucleus accumbens, and subcortical structures (superior colliculus, pulvinar, and caudate nucleus). Hadjikhani and de Gelder (2003) demonstrated that the fusiform gyrus and right amygdala are activated while observing fearful faces but not happy or neutral faces, suggesting similarities in the brain's processing of biological motion and facial emotion. This finding of similarity suggests a co-occurrence of motor movements and facial expressions due to muscle changes that occur while engaging in biological motion with emotion. Recognition of facial emotions thus largely depends on accuracy in interpreting body language. Healthy people can detect any incongruence between emotional body language and facial expression within 100 ms (de Gelder 2006).

In a study using virtual reality, people with ASD showed improved performance in recognizing biological motion with emotion in the left pSTS, superior temporal gyrus, middle temporal gyrus, right insula, orbital frontal cortex, and inferior frontal gyrus, suggesting a strong relationship between emotion perception and extraction of forms from body movements (Yang et al. 2016b). In another study of people with ASD, atypical performance in processing biological motion was correlated with their symptom severity (Koldewyn et al. 2011). Herrington et al. (2007) and Freitag et al. (2008) identified hypo-activation of the pSTS in the processing of biological motion in people with ASD. Pelphrey and Carter (2008) identified failures in differentiating biological motions from non-biological motions in the superior temporal sulcus in people with ASD. Future studies are needed to assess neural correlates of biological motion in people with WS and people with DS.

The amygdala and orbital frontal cortex are involved in the development of theory of mind (Meyer-Lindenberg, Mervis, and Berman 2006). For example, the amygdala is activated while observing untrustworthy faces in a judgment task, and impairment of the amygdala leads to dysfunctional theory of mind. The orbital frontal cortex is engaged in regulation of social behavior, so impairment can result in deficits in theory of mind. The orbital frontal cortex reflects task difficulty in typically developing controls, but

people with WS do not have this linkage. People with WS have an atypical amygdala and deficiencies in the orbital frontal cortex, the latter of which might account for their social disinhibition (Bellugi et al. 2000).

In their review, Gallagher and Frith (2003) conclude that inferring other people's intentions, desires, and beliefs through actions is important for social interaction. They cite neuroimaging studies revealing that these inferences involve the anterior paracingulate cortex, superior temporal sulcus, and bilateral temporal poles, which also are important areas of the brain for social skills. In particular, the anterior paracingulate cortex is part of the anterior cingulate cortex, which has evolved to decouple beliefs from realities. The anterior cingulate cortex supports intention-, cooperation-, and attention-related tasks. The anterior paracingulate cortex of the anterior cingulate cortex specializes in understanding mental states and remains active during rest and self-monitoring tasks. The superior temporal sulcus, a region that may aid in mentalizing, is involved in causality and intentionality.

In a study by Kana et al. (2014) contrasting physical causality and intentional causality, people with ASD showed lower accuracy than healthy controls in recognizing causally intentional attributions depicted in passive viewing of comic strips. They further found that people with ASD have weaker connectivity in the neural network of theory of mind between the ventral premotor cortex and the temporoparietal junction, and they demonstrate lower activation of the inferior frontal gyrus and inferior parietal lobe when processing intentional causality. The results confirm atypical patterns in the processing theory of mind in people with ASD. It is unknown how people with WS and people with DS process theory of mind neurologically; hence, future studies are needed to fill the gaps.

Correlations between perception of biological motion and theory of mind have been observed. For example, Rice et al. (2016) took direct measurements of theory of mind in healthy children aged 7 to 12 years using Baron-Cohen et al.'s (2001) test of "reading the mind in the eyes" and White et al.'s (2009) test using mental-state inference stories. Biological motion tests included images of figures walking forward or backward and facing left or right. The results revealed that children's performance improved with age.

Theory of mind scores correlated with full-scale IQ, nonverbal IQ, and verbal IQ, but biological motion perception did not show any correlations with IQ. Rice et al. (2016) found significant correlations between walking direction and face orientation with theory of mind scores, but biological motion perception correlated only with face orientation. No correlation was observed between scores for theory of mind scores and for recognizing biological motion with physical inferences. These findings indicate that social perception of biological motion and social cognition of theory of mind might originate from the same mechanism. More studies are needed to investigate correlations between biological motion with and without emotion and theory of mind in neurodevelopmental disorders. As more neural correlates are identified, the atypical social brain can be better understood.

CONCLUSION

This chapter reviewed the literature exploring how people with certain neurodevelopmental disorders (i.e., those involving impairments in visuospatial perception) process biological motion with and without emotion from the perspective of the social-perceptual aspect of theory of mind. Distinct patterns in the theory of mind and processing of social information in atypical populations were reviewed. The chapter also reviewed studies focusing on people with WS and people with ASD, revealing their extreme sensitivities to social information and similar visuospatial characteristics of global ignorance and local focusing. The chapter also covered studies of people with DS showing their sensitivity to global information and weak sociability.

This chapter contributes to the literature by offering a systematic review of how people with neurodevelopmental disorders process biological motion with and without emotions and the relationship with theory of mind. Neural correlates of biological motion with and without emotion remain unknown in people with WS and people with DS. Thus, future studies should focus on neural correlates of social cognition in these populations using perspectives

of biological motion with and without emotion, theory of mind, and the relationship between the two. Further comparisons will help identify the developmental trajectories of social cognition in neurodevelopmental disorders and thus may reveal useful interventions.

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REFERENCES

Actis-Grosso, R., Bossi, F., & Ricciardelli, P. (2015). Emotion recognition through static faces and moving bodies: A comparison between typically developed adults and individuals with high level of autistic traits. *Frontiers in Psychology*, *6*, 1570–1584.

Annaz, D., Campbell, R., Coleman, M., Milne, E., & Swettenham, J. (2012). Young children with autism spectrum disorder do not preferentially attend to biological motion. *Journal of Autism and Developmental Disorders*, *42*(3), 401–408.

Atkinson, A. P., Tunstall. M. L., & Dittrich, W. H. (2007). Evidence for distinct contributions of form and motion information to the recognition of emotions from body gestures. *Cognition*, *104*(1), 59–72.

Baron-Cohen, S. (2000). Theory of mind and Autism: A review. *International Review of Research in Mental Retardation*, *23*, 169–184.

Baron-Cohen, S., Wheelwright, S., Spong, A., Scahill, V., & Lawson, J. (2001). Are intuitive physics and intuitive psychology independent? A test with children with Asperger syndrome. *Journal of Developmental and Learning Disorders*, *5*, 47–78.

Baron-Cohen, S., Jolliffe, T., Mortimore, C., & Robertson, M. (1997). Another advanced test of theory of mind: Evidence from very high

functioning adults with autism or Asperger syndrome. *Journal of Child Psychology and Psychiatry*, *38*(7), 813–822.

Barrett, K. C. (1998). A functionalist perspective to the development of emotions. In: Mascolo M. F., Griffin, S. (Eds). *What Develops in Emotional Development?* (pp. 105–133). New York: Plenum Press.

Bellugi, U., Lichtenberger, L., Jones, W., Lai, Z., & George, M. (2000). The neurocognitive profile of Williams syndrome: A complex pattern of strengths and weaknesses. *Journal of Cognitive Neuroscience*, *12*(1), 7–29.

Bernardino, I., Mouga, S., Almeida, J., van Asselen, M., Oliveira, G., & Castelo-Branco, M. (2002). A direct comparison of local–global integration in autism and other developmental disorders: Implications for the central coherence hypothesis. *PLoS One*, *7*(6), 1–11.

Bihrle, A. M., Bellugi, U., Delis, D., & Marks, S. (1989). Seeing either the forest or the trees: Dissociation in visuospatial processing. *Brain and Cognition*, *11*, 37–49.

Blake, R., Turner, L. M., Smoski, M. J., Pozdol, S. L., & Stone, W. L. (2003). Visual recognition of biological motion is impaired in children with autism. *Psychological Science*, *14*(2), 151–157.

Bransford, J., & Franks, J. (1971). The abstraction of linguistic ideas. *Cognitive Psychology*, *2*, 331–350.

Bransford, J., & Franks, J. (1972). The abstraction of linguistic ideas: A review. *Cognition*, *1*, 213–248.

Bransford, J., Barclay, J., & Franks, J. (1972). Sentence memory: A constructive versus interpretive approach. *Cognitive Psychology*, *3*, 193–209.

Brothers, L. (1990). The neural basis of primate social communication. *Motivation and Emotion*, *14*(2), 81–91.

Clarke, T. J., Bradshaw, M. F., Field, D. T., Hampson, S. E., & Rose, D. (2005). The perception of emotion from body movement in point-light displays of interpersonal dialogue. *Perception*, *34*, 1171–1180.

de Gelder, B. (2006). Towards the neurobiology of emotional body language. *Nature Reviews Neuroscience*, *7*(3), 242–249.

Fidler, D. J., Hepburn, S., & Rogers, S. (2006). Early learning and adaptive behavior in toddlers with Down syndrome: Evidence for an emerging behavioral phenotype? *Down Syndrome Research and Practice*, *9*(3), 37–44.

Franks, J., & Bransford, J. (1972). The acquisition of abstract ideas. *Journal of Verbal Learning and Verbal Behavior*, *11*, 311–315.

Franks, J., & Bransford, J. (1974a). A brief note on linguistic integration. *Journal of Verbal Learning and Verbal Behavior*, *13*(2), 217–219.

Franks, J., & Bransford, J. (1974b). Memory for syntactic form as a function of semantic context. *Journal of Experimental Psychology*, *103*(5), 1037–1039.

Freitag, C. M., Konrad, C., Haberlen, M., Kleser, C., von Gontard, A., Reith, W., Troje, N. F., & Krick, C. (2008). Perception of biological motion in autism spectrum disorders. *Neuropsychologia*, *46*(5), 1480–1494.

Frith, U. (1989). *Autism: Explaining the Enigma*. Oxford: Blackwell Publishing.

Gallagher, H. L., & Frith, C. D. (2003). Functional imaging of 'theory of mind'. *Trends in Cognitive Sciences*, *7*(2), 77–83.

Giaouri, S., Alevriadou, A., & Tsakiridou, E. (2010). Theory of mind abilities in children with Down syndrome and non-specific intellectual disabilities: An empirical study with some educational implications. *Procedia Social and Behavioral Sciences*, *2*, 3883–3887.

Grèzes, J., Costers, N., & Decety, J. (1998). Top-down effect of strategy on the perception of human biological motion: A PET investigation. *Cognitive Neuropsychology*, *15*(6/7/8), 553–582.

Grèzes, J., Fonlupt, P., Bertenthal, B., Delon-Martin, C., Segebarth, C., & Decety, J. (2001). Does perception of biological motion rely on specific brain regions? *NeuroImage*, *13*, 775–785.

Grossman, E., Donnelly, M., Price, R., Pickens, D., Morgan, V., Neighbor, G., & Blake, R. (2000). Brain areas involved in perception of biological motion. *Journal of Cognitive Neuroscience*, *12*(5), 711–720.

Grossman, T., & Johnson, M. (2007). The development of the social brain in human infancy. *European Journal of Neuroscience*, *25*, 909–919.

Hadad, B. S., Maurer, D., & Lewis, T. L. (2011). Long trajectory for the development of sensitivity to global and biological motion. *Developmental Science*, 1330–1339.

Hadjikhani, N., & de Gelder, B. (2003). Seeing fearful body expressions activates the fusiform cortex and amygdala. *Current Biology*, *13*, 2201–2205.

Halit, H., Grice, S. J., Bolton, P., & Johnson, M. H. (2008). Face and gaze processing in Prader-Willi syndrome. *Journal of Neuropsychology*, *2*, 65–77.

Happé, F. (1995). The role of age and verbal ability in the TOM performances of subjects with autism. *Child Development*, *66*, 843-855.

Happé, F., Ehlers, S., Fletcher, P., Frith, U., Johansson, M., Gillberg, C., Dolan, R., Frackowiak, R., & Frith, C. (1996). 'Theory of mind' in the brain. Evidence from a PET scan study of Asperger syndrome. *NeuroReport*, *8*(1), 197–201.

Happé, F., & Frith, U. (2006). The weak coherence account: Detailed-focus cognitive style in autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *36*(1), 5–25.

Herrington, J. D., Baron-Cohen, S., Wheelwright, S. J., Singh, K. D., Bullmore, E. T., Brammer, M., & Williams, S. C. R. (2007). The role of MT+/V5 during biological motion in individuals with autistic spectrum disorders. *Journal of Autism and Developmental Disorders*, *37*(7), 1386–1392.

Hsu, C. F. (2013a). Cross-modal Contextual Coherence of Semantic Integration in People with Williams Syndrome. *Research of Developmental Disability*, *34*(12), 4319–4327.

Hsu, C. F. (2013b). Contextual Integration of Causal Coherence in People with Williams Syndrome. *Research of Developmental Disability*, *34*(10), 3332–3342.

Hsu, C. F. (2013c). Is the Contextual Effect Weak in People with Williams Syndrome? An Investigation of Information Integration Ability Using Pictures. *Research of Developmental Disability*, *34*(3), 932–939.

Hsu, C. F. (2014a). Cross-domain investigation of weak central coherence in people with Williams syndrome: Asymmetrical brain and behavioral

performances in verbal and nonverbal domains. In Olivia H. Porter (Ed.), *Semantic Memory: Neurobiology, Disorders and Therapeutic Strategies for Improvement* (pp.71–82). New York: Nova Science Publishers.

Hsu, C. F. (2014b). Modality Effect of Contextual Integration in People with Williams Syndrome. *Research of Developmental Disability*, *35*(7), 1571–1578.

Hsu, C. F. (2019a). Contextual Effects on Semantic Grouping in People with Down Syndrome. *International Journal of Developmental Disabilities, 65*(2), 65–72.

Hsu, C. F. (2019b). Neuroimaging Causal Inferences and Semantic Priming in People with Williams Syndrome: An fMRI Study. *Proceedings of the 15th Annual Meeting of Chinese Developmental Psychology*, 191–193.

Hsu, C. F., & Chen, J. Y. (2014c). Deviant Neural Correlates of Configural Detection in Facial Processing of People with Williams Syndrome. *Bulletin of Special Education, 39*(1), 61–84.

Hsu, C. F., Karmiloff-Smith, A., Tzeng, O., Chin, R. T., & Wang, H. C. (2007). Semantic Knowledge in Williams Syndrome: Insights from Comparing Behavioral and Brain Processes in False Memory Tasks. *Proceedings of the 6th IEEE International Conference on Development and Learning, 6*, 48–52.

Hsu, C. F., & Tzeng, O. J. L. (2011). Contextual Effect in People with Williams Syndrome. *Research in Developmental Disabilities, 32*(2), 781–787.

Hubert, B., Wicker, B., Moore, D. G., Monfardini, E., Duverger, H., Fonséca, D. D., & Deruelle, C. (2007). Brief report: Recognition of emotional and non-emotional biological motion in individuals with autistic spectrum disorders. *Journal of Autism Developmental Disorders*, *37*, 1386–1392.

Jordan, H., Reiss, J. E., Hoffman, E., & Landau, B. (2002). Intact perception of biological motion in the face of profound spatial deficits: Williams syndrome. *Psychological Science*, *13*(2), 162–167.

Kana, R. K., Liberto, L. E., Hu, C. P., Deshpande, D. D., & Colburn, J. S. (2014). Functional brain networks and white matter underlying theory-

of-mind in autism. *Social Cognitive and Affective Neuroscience*, *9*, 98–105.

Karmiloff-Smith, A., Al-Janabi, T., D'Souza, H., Groet, J., Massand, E., & Mok, K., et al. (2016). The importance of understanding individual differences in Down syndrome. *F1000Research*, *5*, 389–397.

Koldewyn, K., Whitney, D., & Rivera, S. M. (2011). Neural correlates of coherent and biological motion perception in autism. *Developmental Science*, *14*(5), 1075–1088.

Korenberg, J. R., Chen, X. N., Hirota, H., Lai, Z., Bellugi, U., Burian, D., Roe, B., & Matsuoka, R. (2000). Genome structure and cognitive map of Williams syndrome. In U. Bellugi & M. St. George (Eds.), *Journey from cognition to brain to gene: Perspectives from Williams syndrome* (pp. 147–178). Cambridge, MA: MIT Press.

Lange, J., de Lussanet, M., Kuhlmann, S., Zimmermann, A., Lappe, M., Zwitserlood, P., & Dobel, C. (2009). Impairments of biological motion perception in congenital prosopagnosia. *PLoS One*, *4*, e7414.

Mervis, C. B., & John, A. E. (2010). Cognitive and behavioral characteristics of children with Williams syndrome: Implications for intervention approaches. *American Journal of Medical Genetics* (Part C, Seminars in Medical Genetics), *154C*(2), 229–248.

Meyer-Lindenberg, A., Mervis, C. B., & Berman, K. F. (2006). Neural mechanism in Williams syndrome: A unique window to genetic influences on cognition and behavior. *Nature*, *7*, 380–393.

Pavlova, M. (2012). Biological motion processing as a hallmark of social cognition. *Cerebral Cortex*, *22*, 981–995.

Pavlova, M., Galli, J., Pagani, F., Micheletti, S., Guerreschi, M., Sokolov, A. N., Fallgatter, A. J., & Fzaai, E. M. (2018). Social cognition in Down syndrome: Face tuning in face-like non-face images. *Frontiers in Psychology*, *9*, 2583–2592.

Pelphrey, K. A., & Carter, E. J. (2008). Brain mechanisms for social perception: Lessons from autism and typical development. *Annals of the New York Academy of Sciences*, *1145*, 283–299.

Premack, D., & Woodruff, G. (1978). Does the chimpanzee have a theory of mind? *Behavioral and Brain Sciences*, *1*(4), 515–526.

Riddell, H., Tolentino-Castro, J. W., Wagner, H., & Lappe, M. (2017). Impairments in the visual processing of global biological motion cues in Down syndrome. *Perception*, *46*(11), 1283–1297.

Rice, K., Anderson, L. C., Velnoskey, K., Thompson, J. C., & Redcay, E. (2016). Biological motion perception links diverse facets of theory of mind during middle childhood. *Journal of Experimental Psychology*, *146*, 238–246.

Rose, F. E., Lincoln, A. J., Lai, Z., Ene, M., Searcy, Y. M., & Bellugi, U. (2007). Orientation and affective expression effects on face recognition in Williams syndrome and autism. *Journal of Autism and Developmental Disabilities*, *37*, 513–522.

Semel, E., & Rosner, S. R. (2003). *Understanding Williams syndrome: Behavioral patterns and interventions*. Mahwah: NJ Lawrence Erlbaum and Associates.

Sha, A., & Frith, U. (1983). An islet of ability in autistic children: A research note. *Journal of Child Psychology and Psychiatry*, *24*, 613–620.

Sha, A., & Frith, U. (1993). Why do autistic individuals show superior performance on the block design task? *Journal of Child Psychology and Psychiatry*, *34*, 1351–1364.

Tager-Flusberg, H., Boshart, J., & Baron-Cohen, S. (1998). Reading the windows to the soul: Evidence of domain-specific sparing in Williams syndrome. *Journal of Cognitive Neuroscience*, *10*(5), 631–639.

Tager-Flusberg, H., & Sullivan, K. (2000). A componential view of theory of mind: Evidence from Williams syndrome. *Cognition*, *76*, 59–89.

Virji-Babul, N., Kerns, K., Zhou, E., Kapur, A., & Shiffrar, M. (2006). Perceptual-motor deficits in children with Down syndrome: Implications for intervention. *Down Syndrome Research and Practice*, *10*, 74–82.

White, S., Hill, E., Happé, F., & Frith, U. (2009). Revisiting the strange stories: Revealing mentalizing impairments in autism. *Child Development*, *80*, 1097–1117.

Wimmer, H., & Perner, J. (1983). Beliefs about beliefs: Representation and constraining function of wrong beliefs in young children's understanding of deception. *Cognition*, *13*(1), 103–128.

Yang, D. Y. J., Rosenblau, G., Keifer, C., & Pelphrey, K. A. (2016a). An integrative neural model of social perception, action observation, and theory of mind. *Neuroscience and Behavioral Reviews, 51*, 263–275.

Yang, Y. B., Lueken, U., Wittmann, A., Holtz, K., Kleint, N. I., & Herrmann, M. J., et al. (2016b). Neural correlates of individual differences in anxiety sensitivity: An fMRI study using semantic priming. *Social Cognitive and Affective Neuroscience*, 1245–1254.

BIOGRAPHICAL SKETCH

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Latest publication list

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Hsu, C.F. (2019). Neuroimaging Causal Inferences and Semantic Priming in People with Williams Syndrome: An fMRI Study. *Proceedings of the 15th Annual Meeting of Chinese Developmental Psychology*, 191–193.

Hsu, C.F. (2017). Linking Counterfactuality with Theory of Mind: Evidence from Developmental Studies with Yaobushi in Chinese. *Lingua Sinica*, 3, 14–33.

Hsu, C.F. (2017). Semantic Priming and Associative Priming in People with Williams Syndrome. In Lowell T. Duncan (ed.), *Advances in Health and Disease*, Vol.2., pp.171–196. Nova Science Publishers. ISBN: 978-1-53612-695-2 (Hardcover), ISBN: 978-1-53612-696-9 (ebook).

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Chapter 5

THE EMERGENCE OF CONSCIOUSNESS: THE CONTRIBUTION OF THE CINGULATE CORTEX

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ABSTRACT

Among all the cognitive abilities of the human brain, the one that has most deeply interested neuroscientists is *consciousness*, which at its simplest refers to *"sentience or awareness of internal or external existence."* Several theories have been proposed to explain this phenomenon. Stuss, Picton, and Alexander (2001) and Stuss and Anderson (2004) argued that there are different types of consciousness, hierarchically organized, which need to be differentiated. The different types of consciousness are associated with distinct neural substrates, which remain the subject of intense investigation. Someone suggested that it could be a "real function" localized in a precise region of the brain, which would deal precisely with collecting and synthesizing stimuli deriving from other areas. For others, it would depend on the synchronization between sensory

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and mnemonic areas: critical groups of neurons, in different areas of the brain, would discharge simultaneously, thus giving rise to that integration of stimuli which is consciousness. Some neurobiological models of consciousness assume that the contents of consciousness are widely distributed in the brain.

In "The Astonishing Hypothesis" (1994), Francis Crick identifies the anterior cingulate, as a likely candidate for the center of free will in humans. The anterior cingulate cortex acts as an important interface between emotion and cognition, and more specifically in the conversion of feelings into intentions and actions. It has been implicated in 1) emotion, motivation, and attention; 2) facial self-recognition, interceptive and emotional awareness; 3) integration of conscious experience; 4) error detection, conflict-monitoring, and self-related information monitoring (Palermo, 2017). Given the above, the ACC would play an important role in both "core" and introspective self-awareness (Philippi et al., 2012). Damasio and Mayer (2008) has previously suggested that "core consciousness" occurs when an organism becomes consciously aware of feelings associated with changes occurring to its internal bodily state; it is able to recognize that its thoughts are his own, and that they are formulated in its own perspective.

Modern neuroscience suggests that the brain's intrinsic activity may be an important process underlying consciousness. The Salience Network (SN) is an intrinsically connected large-scale network anchored in the anterior insula and dorsal anterior cingulate cortex. Together with its interconnected resting state networks, it contributes to a variety of complex brain functions. The SN has been implicated in modulating the switch between the externally directed cognition of the Central Executive Network (CES) and the internally directed cognition of the Default Mode Network (DMN). Moreover, the SN has been implicated in the detection and integration of emotional and sensory stimuli, coming for this considered responsible for self-awareness (Craig, 2009; Gogolla, et al., 2014; Menon & Uddin, 2010).

The chapter will deal with the definition of consciousness, the description of the neural substrates that have been associated with it, and the examination of the main interpretative models. Particular attention will be given to the role played by the cingulate cortex as a hub in functional networks involved in the emergence of consciousness.

Keywords: consciousness, self-awareness, anterior cingulate cortex, salience network, central executive network, default mode network

INTRODUCTION

Consciousness is the most pervasive and complex phenomenon to study. The word “consciousness” refers to those states of sensitivity and awareness that characteristically begin when we wake up from a dreamless sleep and continue until we go to sleep again, or fall into a coma or die, or somehow become “unconscious” (Searle, 1997). Thus defined, consciousness is an all-or-nothing phenomenon. But there are "states of intensity" of consciousness that range from drowsiness to complete awareness. Defined in this way, consciousness is an interior, first-person and qualitative phenomenon. higher level humans and animals are conscious, but we do not know to what extent the phylogenetic scale extends (Searle, 1997).

There are many questions to ask when dealing with consciousness. What really is consciousness? What does consciousness do? Could we have evolved without it? Where does it come from? Where is it? How do the neurobiological processes that take place in the brain cause consciousness? Although there is no definitive answer for any of these questions, there is nevertheless much to be said about it. Researchers have long since abandoned the level of mere speculation for progressively more empirical foundations of solving these and other questions about consciousness. The scientific discussion on consciousness is the modern version of the famous mind-body problem. The enormous variety of stimuli that affects a human being triggers a sequence of processes that produce unified, ordered, coherent and internal subjective states of consciousness. The problem of how this occurs concerns not only perception, but also the experience of voluntary action and inner mental processes.

It is an amazing fact that anything in conscious life is attributable to brain processes. We know that the brain and consciousness are intimately linked, because changes in one cause it in the other. Substances that alter brain functions also influence subjective experiences; stimulation of certain brain areas can induce hallucinations, physical or emotional sensations; some brain injuries can profoundly afflict consciousness (Purves et al., 2013). It remains a mystery why we are conscious. The brain does not seem designed to give rise to the type of consciousness that distinguishes the

human being (Blackmore, 2005). Our central nervous system acts largely according to a parallel and distributed pattern. It resembles a large set of interconnected functional networks. Nothing is centralized. Nonetheless, the impression is that consciousness is a unitary block (Blackmore, 2005). There are three different descriptions of this alleged “unity”:

- The unity of consciousnes would consist in the convergence of all that I feel right now: the contents of consciousness constitute my actual experiences;
- It would consist of a temporal unity based on the apparent continuity that binds one instant to the next, or that crosses a whole life of conscious experiences.
- It would consist of the "I" that experiences the contents of consciousness: that is, there is a single subject for a single flow of experiences.

A theory of consciousness must therefore explain the contents of consciousness, its continuity and the existence of a conscious self in order to be valid (Blackmore, 2005). It must also do so starting from a central nervous system that is not centralized, but organized according to a parallel and multiple-track process (Blackmore, 2005).

There are a number of special features that make neuroscience problems difficult to solve (Searle, 1997). Some are of a practical nature: the human brain is the most complex organ in the universe, with more than one hundred billion neurons, each of which has countless synaptic connections with other neurons (Searle, 1997). It is also difficult to work with brain microelements without risking damage them or kill the organism (Searle, 1997). In addition to the difficulties of a practical nature, there are numerous philosophical and theoretical obstacles and confusions which make it difficult to ask questions and answer them correctly (Searle, 1997). We may approach consciousness through internal subjective states and modern neuroimaging techniques. But how to do it with scientific rigor? How to subject the most volatile and multifaceted experience - all in the first person - to the most subtle criteria of science, which are all in the third person? This question grips the scientists

who placed consciousness at the center of their research, with a convergence similar to that which had been given, a few decades ago, on the atomic structure of matter.

THE GREAT MYSTERY OF NEUROSCIENCE

Conscience, Consciousness or Self-Awareness? The Role of the Anterior Cingulate Cortex

The first requirement for any sensible discussion about consciousness is to establish clear definitions of the terms involved. First, the meanings of the two terms "conscience" and "consciousness" are often confused and are misunderstood by many people (Vithoulkas & Muresanu, 2014). The concept of conscience is the inherent ability of every healthy human being to perceive what is right and what is wrong and, on the strength of this perception, to control, monitor, evaluate and execute their actions (Vithoulkas & Muresanu, 2014). The word "consciousness" is derived from Latin, having its roots *in conscio* formed by the coalescence of *cum* meaning 'with' and *scio* meaning 'know'. In its original sense, to be conscious of something was to share the knowledge of it with someone else or with oneself (Zeman, 2001; Koch, 2012).

There is no generally accepted definition of consciousness, but the following definitions illustrate what is commonly meant by this term (Blackmore, 2005):

- *"What is it like to be ...."*: if I feel something about being an animal (or a computer, or a baby), then the animal, the computer or the baby is conscious. Otherwise no.
- *Subjectivity and phenomenality*: consciousness means subjective or phenomenal experience. It is the way things present themselves to me, as opposed to how they are objectively.

- *Qualia*: the ineffable subjective qualities of the experience, such as the sensation of the red of a tulip or the difficult to describe smell of turpentine.
- *The hard problem*: it is the problem of explaining why there is "something it is like" for a subject in conscious experience, why conscious mental states "light up" and directly appear to the subject.

Most neuroscientific definitions of consciousness refer to three different aspects of this phenomenon (Blackmore, 2005):

- consciousness as a physiological state (being awake)
- consciousness intended as awareness of the world and self (being aware)
- consciousness intended as self-awareness (being aware of yourself as agents in the world)

Wakefulness and awareness seem to represent the two main components of consciousness (Vithoulkas & Muresanu, 2014). *Arousal* is defined by the level of consciousness, *awareness* is defined by the content of consciousness. Awareness contains *self-awareness*, which perceives the internal world of thoughts, reflection, imagination, emotions, and daydreaming, as well as external awareness, which perceives the outside world with the help of the five senses (Vithoulkas & Muresanu, 2014). Another aspect of consciousness to be considered concerns its temporal aspect. Consciousness occupies the time between the past and the future and thus defines our sense of the present. This raises the question of how consciousness, in the sense of a constantly changing present moment, differs from the continually moving attentional focus (Purves et al., 2013). Our conscious sense of the present moment, or "right now," is defined by neural processing. In rough terms, the object of conscious attention represents another way of defining the present moment. Being physiologically awake and potentially reactive to information in the present time are necessary conditions for consciousness understood in its further meanings: being aware of the world and of oneself in the world (Purves et al., 2013).

Stuss, Picton, and Alexander (2001) and Stuss and Anderson (2004) argued that there are different types of consciousness, hierarchically organized, which need to be differentiated. They proposed different elements in a four-levels hierarchical framework of awareness, where the processing in turn operating in a modular manner at any level of the hierarchy, so that damage in one functional domain may result in a different kind of reduced self-awareness. The different types of consciousness are associated with distinct neural substrates, with the higher levels related to self-awareness and theory of mind, with an emphasis on the role of the frontal lobes (Palermo, 2017; p. 148-149).

At the lowest level, the brainstem reticular system mediates the arousal aspects of consciousness. The second level of awareness involves the sensory and motor regions of the neocortex, which in turn, sustains the analysis of incoming sensory information and the construction of complex motor activity. This level leads to the simple awareness of the sensory world and one's bodily responses. At the highest level of conscious processing, the frontal lobes are considered particularly important for self-awareness a multifaceted phenomenon that has been proposed central to human consciousness (Philippi et al., 2012). The third level mediates the executive functions that integrate the information provided by the sensory systems in the posterior part of the brain and organizes goal-directed responses to this input. A final level relates to self-awareness and theory of mind is associated with the medial prefrontal cortex [MPFC] (Palermo, 2017; p. 148-149). Inrestengly, the MPFC is referred to a functional network including the anterior cingulate cortex [ACC] (Amodio & Frith, 2006) and other heteromodal association areas that respond to multiple types of affective and cognitive events (Shackman et al., 2011).

The ACC contribute to behavior by modifying responses in reaction to challenging cognitive and physical states requiring additional effortful cognitive control. The ACC monitors and modulates cognitive activity in dorsolateral frontal cortex, supervises the emotional salience (avereness/pleasant) of stimuli in conjunction with orbitofrontal cortex, exerts control over the autonomic nervous system and subjective feelings

with insular cortex (Gasquoine, 2013). The ACC is therefore a neural hub which expresses itself as a monitoring-attentional system.

In "The Astonishing Hypothesis" (1994), Francis Crick identifies the anterior cingulate sulcus, as a likely candidate for the center of free will in humans. Crick bases this suggestion on scans of patients with specific lesions that seem to interfere with their sense of independent will, such as alien hand syndrome. Moreover, anterior cingulate is more active when the brain is engaged in internal monitoring and in processing information related to self (Cavanna & Trimble, 2006; Cavanna, 2007). In particular, the ACC has been implicated in interoceptive and emotional awareness (Damasio, 1999; Chritchley et al., 2004; Lane et al., 1998; Critchley, 2005), facial self-recognition (Kircher et al., 2001), and more generally in the integration of our conscious experience (Damasio, 1999; Dehaene, Kerszberg, and Changeux, 1998). Moreover, it is closely linked to conscious conflict monitoring (Botvinick, Cohen, and Carter, 2004; Botvinick et al., 2001; Dehaene et al., 2003; Mayr, 2004, Palermo, Stanziano and Morese, 2018), and to the monitoring of self-related information underlying introspection. Research on patients with bilateral ACC damage provide evidence for the role of the ACC in emotion, motivation, and attention (Barris & Schuman, 1953; Cohen et al., 1999a, 1999b, Damasio & Van Hoesen, 1983; Shackman et al., 2011). When ACC damage is combined with damage to the adjacent supplementary motor area, patients can manifest a profound state of akinetic mutism (Damasio & Van Hoesen, 1983; Devinsky, Morrell, and Vogt, 1995). Finally, selective right anterior cingulate damage has been previously associated with impaired self-awareness (Palermo et al., 2014). Given the above, the ACC would play an important role in both "core" and introspective self-awareness (Philippi et al., 2012), defined as follows:

- Core self-awareness is grounded on the protoself, which includes ''primordial feelings'' of the living body and a preattentive, elementary form of self-consciousness. On a moment to moment basis, core self-awareness generates a sense of personal agency and ownership over behavioral actions and sensory representations (Philippi et al., 2012).

- Introspective self-awareness relies on higher-order executive, attentional and metacognitive functions, which enable introspection, the ability to perform a controlled reflection on one's own mental states, behaviors, and their consequences (Philippi et al., 2012).

Philippi's (2012) theory is based on the model of consciousness previously proposed by Damasio and Mayer (2008), who has previously suggested a three-layered theory based on a hierarchy of stages, with each stage building upon the last. The most basic representation of the organism is referred to as the protoself, next is core consciousness, and finally, extended consciousness. Core consciousness occurs when an organism becomes consciously aware of feelings associated with changes occurring to its internal bodily state; it can recognize that his thoughts are his own, and that they are formulated in his own perspective (Damasio & Mayer, 2008). Self-awareness should therefore be considered a pivotal component of conscious experience (Lou, Changeux, and Rosenstand, 2017). Despite applying different methodologies on the different aspects of self-awareness, neuroscientific research showed converging evidence for medial prefrontal/anterior cingulate and parietal/posterior cingulate paralimbic regions being correlated with self- awareness (Lou, Changeux, and Rosenstand, 2017). Are these data enough to define the neural substrates of consciousness? Is this level of investigation enough or should we go further?

NEUROSCIENCE AND CONSCIOUSNESS: THE NEW FRONTIERS OF RESEARCH

The Mystery of Consciousness? It Is All a Question of "Relationships"

Nobody has any idea what exactly consciousness is and where it resides. Also, because it should not be sought from a morphological or anatomical point of view, but from a functional one: consciousness would not be the result of the action of a single brain area, but of the relationship established

between neurons of different brain/mental compartments. One of the most ambitious goals of contemporary neuroscience is the explanation of consciousness. Many scientists emphasize the necessary role of the prefrontal and frontal cortex for consciousness, while others argue that the brain stem and other structures of the midbrain may be sufficient conditions for consciousness. The brain stem is the sorting center for nerve impulses: the fibers that innervate the spinal cord, brain and cerebellum pass through here. The brain stem regulates fundamental actions such as breathing, sleep-wake rhythm, blood circulation, pressure in the vessels. It would be closely connected with the functioning of consciousness. The latter has always been thought to reside in an unspecified point of the cerebral cortex, the outermost layer of the brain, linked to thought, speech and concentration.

Nonetheless, neurologists from Harvard Medical School and Beth Israel Deaconess Medical Center have identified a connection between the region of the brain stem involved in excitement and regions that concern awareness, key prerequisites for explaining consciousness in terms of relationship (Fischer et al., 2016). The neuroimaging study was performed on 36 patients with brain stem lesions (12 of whom were in coma), using a new brain tissue analysis technique, the voxel-based lesion-symptom mapping. Fischer and colleagues (2016) have found that a small region in the rostral dorsolateral pontine tegmentum was significantly associated with coma-causing lesions. In healthy adults, this brainstem site was functionally connected to the ventral anterior insula [AI] and pregenual anterior cingulate cortex [pACC]. The first resides in the cerebral cortex and expresses an individual's cognitive and emotional development; the second is fundamental for the elaboration of experiences and dangers. These cortical areas aligned poorly with previously defined resting-state networks, better matching the distribution of von Economo neurons. Connectivity between the AI and pACC was disrupted in patients with disorders of consciousness, and to a greater degree than other brain networks (Fischer et al., 2016). The functional network composed of pontine tegmentum AI and pACC may have a role in the maintenance of human consciousness.

These findings help to understand brain connectivity at the base of consciousness and to explain how a localized lesion ends up affecting the

entire neuronal system. It is no coincidence that scholars have begun to speak of a "connectome" to indicate the map of connections between all neurons in the brain. The thesis is also ridden by Stuart Hameroff and by mathematician Roger Penrose, author of the famous "The Emperor's New Mind" (1989). The two speak of "*quantum vibrations*" by asserting that many anesthetics act on cellular structures of a protein nature, the microtubules. They reside in the nerve cells and would explain anomalous electroencephalographic rhythms, but completely similar to a conscientious flow (Penrose, 1994). Consciousness, therefore, may not be a human prerogative and nestle innately in microstructures assigned to the transport of substances and cellular stability. Penrose is convinced of this and thus justifies "the mouse who evades a trap and takes away a chocolate"; but also, the hypothesis that, being a product of a quantum nature, it can survive the individual.

The Orch-OR Model and the Implications for Consciousness

The Penrose-Hameroff hypothesis on quantum effects in neurobiology, also called Orchestrated Objective Reduction [Orch-OR], is a conjecture on the possibility that some phenomena typical of quantum mechanics (especially the collapse of the wave function and the entanglement) affect the neurochemical processes that contribute to defining consciousness.

Penrose (1989) hypothesized, with a controversial analogy on Gödel's incompleteness theorems, that the human brain has non-algorithmic functions and that, therefore, its processes cannot be formalized-computable and cannot be reproduced on a computer level. Subsequently, after discussing with Hameroff about the physical processes inherent in the functioning of neurons, Penrose (1994) suggested that quantum effects play a role in neurochemical processes, since the superimposed states between electrons (according to his vision of quantum gravity) are associated with a relative curvature of spacetime, and if they exceed the Planck length (1.616 × 10−35m) in mutual distance, they begin to collapse.

Hameroff proposed that microtubules are suitable candidates to support quantum processing. Quantum entanglement is a state in which particles can alter their properties instantaneously and at a distance, in a way that would not be possible, if they were large-scale extended objects (which would obey the laws of classical mechanics and not of quantum physics). The microtubules condensed in a single neuron can be links with condensates of microtubules in other neurons and glial cells via gap junctions. Gap junctions are connections where the gap between cells is small enough to make it possible for quantum objects to cross it by means of a process known as a quantum tunnel. This tunnel would allow a quantum object to pass into other neurons, and therefore to spread over a large area of the brain, generating a unique quantum object. It is also hypothesized that this large-scale quantum feature is the source of the synchronization gamma waves observed in the brain, and sometimes considered to be related to the phenomenon of consciousness.

The Orch-OR theory combines Penrose's hypothesis regarding Gödel's theorem with the Hameroff's hypothesis about microtubules: when condensations occur in the brain subjected to an objective reduction of the wave function, collapse is connected to decisions of a non-computational nature and incorporated in the space-time geometry (Penrose, 1994).

According to biocentrism, space and time are simply the tools our mind uses to weave information together into a coherent experience — they are the language of consciousness (Lanza & Berman, 2009, 2016). At death there is a break in our linear stream of consciousness, and thus a break in the linear connection of times and places. Indeed, biocentrism suggests it is a manifold that leads to all physical possibilities. More and more physicists are beginning to accept the "many-worlds" interpretation of quantum physics, which states that there are an infinite number of universes. Everything that can possibly happen occurs in some universe. Death does not exist in these scenarios, since all of them exist simultaneously regardless of what happens in any of them. It is possible to assume that, if consciousness is released from the evolution of species, it can be a prerogative of the universe that completely transcends our existence (Lanza & Berman, 2009).

The "me" feeling is just energy operating in the brain. But energy never dies; it cannot be destroyed. In short, you die, but in a sense you exist forever. Lanza (2016) knows how to express it with more poetry: "with death, our life becomes a perennial flower that returns to live in the multiuniverse."

LOOKING FOR THE NEURAL UNDERPINNINGS OF CONSCIOUSNESS

The concept of «localization of function» was an important milestone for behavioural neuroscience. Today we know that the contemporaneous functional modulation of different cerebral area varies in a predictable way depending on what a subject is doing. Thanks to modern neuroimaging and a more carefully validated understanding of human cognition, a detailed view of the brain organization is now emerging. Modular systems are outdated, the network approach is the current one (Kandel et al., 2012). One of the main topics of discussion in the 20th century was whether *mental activities* - such as thought, emotions, self-awareness and will - are functions different from *brain activities* - such as the movement of a limb, the perception of a color, etc. - or if they also represent functional expressions of the brain neurons (Palermo & Morese, 2019).

In recent decades, several researchers have used modern neuroimaging techniques to explicitly identify the neural substrates of consciousness. The dilemma is called "the hard problem of consciousness," an expression coined in 1995 by David Chalmers. The challenge arises because it does not seem that the qualitative and subjective aspects of conscious experience - how consciousness "feels" and the fact that it is directly "for me" - fit into a physicalist ontology, one consisting of just the basic elements of physics plus structural, dynamical, and functional combinations of those basic elements (Chalmers, 1995).

Starting a few years earlier, researchers have made significant progress toward identifying which neurobiological events occur concurrently to the experience of subjective consciousness (Koch et al., 2016). The expression

"neural correlates of consciousness" had first been used to describe the neural models that tried to explain consciousness. It has been defined as the minimum neuronal mechanisms jointly sufficient for any one specific conscious experience (Crick & Koch, 1990). Importantly. a hot topic is to investigate the difference between neural activities that are associated with awareness and those that are not.

The Neural Substrates of Consciousness

When we are trying to associate consciousness with structures in the brain, we must clearly define what level of consciousness we are talking about (Damasio, & Mayer, 2008; Stuss, Picton, and Alexander, 2001; Stuss & Anderson, 2004).

A very primitive form of consciousness (the so-called protoself in Damasio's three-layered theory) - which function is to constantly detect and record, moment by moment, the internal physical changes that affect the homeostasis of the organism - is associated with activity of such brain structures as the reticular formation, the hypothalamus, and the somatosensory cortex (Damasio & Mayer, 2008). The reticular formation is also associated with consciousness in the minimal sense of wakefulness. Other structures involved in simply maintaining wakefulness include the pons, the raphe nuclei and the locus coeruleus (Purves et al., 2013). Core consciousness depends chiefly on the cingulate cortex and on the intralaminar nuclei of the thalamus (Damasio & Mayer, 2008). When consciousness moves beyond the here and now, Damasio's third and final layer emerges as extended consciousness. This level could not exist without its predecessors, and, unlike them, requires a vast use of conventional memory. This autobiographic dimension of consciousness implies that a subject can form mental representations of conscious experiences in the past or the future, and therefore requires the support of memory and the higher functions that make abstract conceptualization and planning possible. Not only frontal and parietal lobes would be actively engaged, but also the angular gyrus, the precuneus, and the anterior cingulate cortex, which are

often very active in a conscious state of rest, are part of a functional network that makes self-consciousness possible.

How Are Different Neural Networks Related to Consciousness?

Modern neuroscience suggests that the brain's intrinsic activity may be an important process underlying consciousness. The level of vigilance can be modulated by the dynamics of resting state and task-engaged networks (Nani et al., 2019).

The Salience Network (SN) is an intrinsically connected large-scale network anchored in the anterior insula and dorsal anterior cingulate cortex. The SN functions to segregate the most relevant among internal and extrapersonal stimuli in order to guide behavior (Menon & Uddin, 2010). Together with its interconnected resting state networks, the SN contributes to a variety of complex brain functions. The SN is important for detection and mapping of external salient inputs and task control (Dosenbach et al., 2007; Seeley et al., 2007; Menon and Uddin, 2010; Uddin, 2015). Moreover, the SN features extensive connectivity with subcortical and limbic structures involved in reward and motivation (Menon & Uddin, 2010).

The SN - and its two major cortical nodes, the insula and the anterior cingulate cortex - influence other core networks that have a different intrinsic organization (Menon & Uddin, 2010). It has been implicated in modulating the switch between the externally directed cognition of the Central Executive Network (CES) and the internally directed cognition of the Default Mode Network (DMN). The CES is engaged in higher-order cognitive and attentional control and is supposed to be related to externally guided awareness (Boveroux et al., 2010). Central executive network links the dorsolateral frontal and parietal neocortices, with subcortical coupling that is distinct from that of the salience network (Menon & Uddin, 2010). The DMN is characterized by a high degree of self-referential thinking, but not without exceptions (Lou, Changeux, and Rosenstand, 2017). Its major hubs are medial prefrontal/anterior cingulate and medial parietal/posterior

cingulate cortices like pure examples of self- awareness (Lou, Changeux, and Rosenstand, 2017).

Moreover, the SN has been implicated in the detection and integration of emotional and sensory stimuli, coming for this considered responsible for self-awareness (Craig, 2009; Gogolla, et al., 2014; Menon & Uddin, 2010). Damasio and Mayer (2008) have previously suggested that core consciousness is an emergent process that occurs when an organism becomes consciously aware of feelings associated with changes occurring to its internal bodily state; it is able to recognize that its thoughts are its own, and that they are formulated in its own perspective. The insula is the brain structure implicated in disparate cognitive, affective, and regulatory functions, including interoceptive awareness, emotional responses, and empathic processes (Menon & Uddin, 2010). Indeed, the insular cortex has been associated with the emotional modulation of conscious experience (Craig, 2010; Seth, Suzuki, and Critchley, 2011). Still, it is debated whether the insula can contribute by processing an essential ingredient of consciousness or just an attribution, albeit important, of an emotional and salient flavor to the contents of experience. Rather than being involved in phenomenal consciousness, the insula might be fundamental for creating self-awareness (Modinos, Ormel, and Aleman, 2009; Manuello et al., 2018). The other major evolutionary modification in the insula is a type of neuron that is found only in the great apes and in humans. These large, elongated, cigar-shaped nerve cells are known as von Economo neurons (VENs). VENs occur only in the insula and in the anterior cingulate cortex. The ACC acts as an important interface between emotion and cognition, and more specifically in the conversion of feelings into intentions and actions. It has been implicated in emotion, motivation, and attention; facial self-recognition, interceptive and emotional awareness; integration of conscious experience; error detection, conflict-monitoring, and self-related information monitoring (Palermo, 2017).

The fundamental role of the SN in the emergence of consciousness was confirmed by a study by Quin and collaborators (2015), aimed to investigate the roles of different resting-state networks in predicting both the actual level of consciousness and its recovery in brain injury patients. Authors found that

functional connectivity strength in SN, especially connectivity between the supragenual anterior cingulate cortex and left anterior insula, was reduced in the unconscious state compared to the conscious state. The findings show that SN (supragenual anterior cingulate cortex and left anterior insula) connectivity correlates with behavioral signs of consciousness, whereas DMN (posterior cingulate cortex and left lateral parietal cortex) connectivity instead predicts recovery of consciousness (Quin et al., 2015).

Human consciousness is supported by dynamic complex patterns of brain signal coordination (Demertzi et al., 2019). Authors examined 159 people who underwent functional magnetic resonance imaging. Among them were 47 volunteers who were examined awake and under general anesthesia, and 112 patients with severe brain injury, who were divided into two groups: state of minimal consciousness and vegetative state. Demertzi and colleagues (2019) - adopting the framework of brain dynamics as a cornerstone of human consciousness - determined whether dynamic signal coordination provides specific and generalizable patterns pertaining to conscious and unconscious states after brain damage. They found four patterns. The most complex one represents the intricate neural interactions between 42 different brain areas with important roles in consciousness (among which core "hubs" of the SN and DMN such as the dorsal anterior cingulate, midcingulate cortex and posterior cingulate): this pattern was observed especially in healthy awake patients and sometimes in those with minimal states of consciousness. Vegetative patients showed this neural pattern only in cases where they had responded positively to simple mental imagination tests. The most basic brain activation pattern - which reflected the simple physical connections between brain regions - was observed mainly in less reactive patients; while the two intermediate patterns were found in all the people examined. Interestingly, the most complex pattern disappeared during general anesthesia - evidence that the scheme really reflected the state of consciousness and not, for example, the result of brain damage.

CONCLUSION

Is the human mind intelligent enough to understand its own existence? Is the brain the "place of consciousness"? Today it is possible to analyze the biological basis of consciousness, using the best theories available and all the research apparatus for the brain, from electroencephalographic psycho-physiological techniques to advanced functional neuroimaging. Consciousness is supported by a complex interplay of different networks, including the ascending reticular activating system in the brainstem, the non-specific nuclei of the thalamus, and the widespread thalamocortical projections to anterior cingulate, posteromedial cortex and fronto-parietal association cortices (Nani et al., 2019). The anterior cingulate cortex appears to be involved in the more complex levels of consciousness and self-awareness. Nevertheless, little is known about the interaction between complex areas and the processes taking place at the level of individual neurons.

The fact is that very little is known about consciousness and that much is still to be discovered. The challenge involves overcoming numerous theoretical, philosophical, religious and technological difficulties. It is no coincidence that the Nobel prize for physics, Neville Mott, said: «Neither physical science nor psychologu can ever "explain" human consciousness. To me then, human consciousness lies outside science, and it is here that I seek the relationship between God and man» (Mott, 1991).

REFERENCES

Barris, R. W., & Schuman, H. R. (1953). Bilateral anterior cingulate gyrus lesions; syndrome of the anterior cingulate gyri. *Neurology,* 3:44-52.

Blackmore, S. J. (2005). *Consciousness: A Very Short Introduction.* Oxford: Oxford University Press.

Botvinick, M. M., Braver, T. S., Barch, D. M., Carter, C. S., Cohen, J. D. (2001). Conflict monitoring and cognitive control. *Psychological Review 108*:624-652.

Botvinick, M. M., Cohen, J. D., Carter, C. S. (2004), Conflict monitoring and anterior cingulate cortex: an update. *Trends Cogn Sci,* 8:539-546.

Boveroux, P., Vanhaudenhuyse, A., Bruno, M. A., et al., (2010). Breakdown of within- and between-network resting state functional magnetic resonance imaging connectivity during propofol-induced loss of consciousness. *Anesthesiology,* 113:1038-1053.

Cavanna A. E., Trimble M. R. (2006). The precuneus: a review of its functional anatomy and behavioural correlates. *Brain*, 129(Pt 3):564-583.

Cavanna, A. E. (2007). *The precuneus and consciousness*. CNS Spectr., 12:545-552.

Chalmers, D. (1995). Facing up to the problem of consciousness. *Journal of Consciousness Studies*, 2(3):200-219.

Cohen, R. A., Kaplan, R. F., Zuffante, P., Moser, D. J., Jenkins, M. A., et al., (1999a). Alteration of Intention and Self-Initiated Action Associated With Bilateral Anterior Cingulotomy. *J Neuropsychiatry Clin Neurosci,* 11:444-453.

Cohen, R. A., Kaplan, R. F., Moser, D. J., Jenkins, M. A., Wilkinson, H. (1999). Impairments of attention after cingulotomy. *Neurology,* 53:819.

Craig, A. D. (2009). How do you feel--now? The anterior insula and human awareness. *Nat Rev Neurosci*, 10(1):59-70.

Craig, A. D. (2010). The sentient self. *Brain Struct. Funct.,* 214:563-577.

Crick, F., & Koch, C. (1990). Towards a neurobiological theory of consciousness. *Sem Neurosci*., 2:263-75.

Crick, F. (1994). *Astonishing Hypothesis: The Scientific Search for the Soul*. New York: Simon and Schuster.

Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., Dolan, R. J. (2004), Neural systems supporting interoceptive awareness. *Nat Neurosci,* 7:189-195.

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *The Journal of Comparative Neurology*, 493:154-166.

Damasio, A., Van Hoesen, G. (1983). Emotional disturbances associated with focal lesions of the limbic frontal lobe. In: *Neuropsychology of Human Emotion*. K. Heilman, P. Satz (Eds.). New York: Guilford.

Damasio, A. (1999). *The Feeling of What Happens: Body and Emotion in the Making of Consciousness*. New York: Harvest Books.

Damasio, A., & Meyer, K. (2008). Consciousness: An overview of the phenomenon and of its possible neural basis. In: *The Neurology of Consciousness: Cognitive Neuroscience and Neuropathology*, S. Laureys & G. Tononi (Eds.). London: Academic Press.

Dehaene, S., Kerszberg, M., Changeux, J. P. (1998). A Neuronal Model of a Global Workspace in Effortful Cognitive Tasks. *Proc Natl Acad Sci USA*, 100:8520- 8525.

Dehaene, S., Artiges, E., Naccache, L., Martelli, C., Viard, A., et al., (2003). Conscious and subliminal conflicts in normal subjects and patients with schizophrenia: The role of the anterior cingulate. *Proceedings of the National Academy of Sciences of the United States of America*, 100:13722-13727.

Devinsky, O., Morrell, M. J., Vogt, B. A. (1995), Contributions of anterior cingulate cortex to behaviour. *Brain*, 118(Pt 1):279-306.

Dosenbach, N. U., Fair, D. A., Miezin, F. M., Cohen, A. L., Wenger, K. K., Dosenbach, R. A., et al., (2007). Distinct brain networks for adaptive and stable task control in humans. *Proc. Natl. Acad. Sci.* U.S.A., 104:11073-11078.

Fischer, D. B., Boes, A. D., Demertzi, A., Evrard, H. C., Laureys, S., Edlow, B. L., Geerling, J. C. (2016). A human brain network derived from coma-causing brainstem lesions. *Neurology*, 87(23):2427-2434.

Gasquoine, P. G. (2013). Localization of function in anterior cingulate cortex: from psychosurgery to functional neuroimaging. *Neurosci Biobehav Rev*, 37(3): 340-348.

Gogolla, N., Takesian, A. E., Feng, G., Fagiolini, M., and Hensch, T. K. (2014). Sensory integration in mouse insular cortex reflects GABA circuit maturation. *Neuron*, 83:894-905.

Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A., Hudspeth, A. J. (2012). *Principles of Neural Science*, 5th ed. McGraw-Hill, New York.

Kircher, T. T., Senior, C., Phillips, M. L., Rabe-Hesketh, S., Benson, P.J, et al., (2001). Recognizing one's own face. *Cognition*, 78:B1-B15.

Koch, C. (2012). *Consciousness: Confessions of a Romantic Reductionist*. Cambridge: MIT Press.

Koch, C., Massimini, M., Boly, M., Tononi, G. (2016). Neural correlates of consciousness: Progress and problems. *Nature Reviews Neuroscience,* 17(5): 307–321.

Lane, R. D., Reiman, E. M., Axelrod, B., Yun, L. S., Holmes, A., et al., (1998). Neural Correlates of Levels of Emotional Awareness: Evidence of an Interaction between Emotion and Attention in the Anterior Cingulate Cortex. *Journal of Cognitive Neuroscience*, 10:525-535.

Lanza, R., & Berman, B. (2009). *Biocentrism: How Life and Consciousness are the Keys to Understanding the Universe. Dallas:* Benbella Books.

Lanza, R., & Berman, B. (2016). *Beyond Biocentrism: Rethinking Time, Space, Consciousness, and the Illusion of Death*. Dallas: Benbella Books.

Lou, H. C., Changeux, J. P., Rosenstand, A. (2017). Towards a cognitive neuroscience of self-awareness, *Neuroscience & Biobehavioral Reviews*, 83: 765-773.

Manuello, J., Nani, A., Cauda, F. (2018). Attention, Salience, and self-awareness: The role of insula in meditation" In: *Island of Reil (Insula) in the Human Brain*. M. Turgut, C. Yurttas, T. R. Shane (Eds.). Berlin: Springer Verlag.

Mayr, U. (2004). Conflict, consciousness, and control. *Trends in Cognitive Sciences*, 8:145-148.

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Struct Funct*, 214(5-6):655-667.

Modinos, G., Ormel, J., Aleman, A. (2009). Activation of anterior insula during self-reflection. *PLoS One*, 4:e4618.

Mott, N. (1991). *Can Scientists Believe?* London: James & James Science Publishers Ltd.

Nani, A., Manuello, J., Mancuso, L., Liloia, D., Costa, T., & Cauda, F. (2019). The Neural Correlates of Consciousness and Attention: Two Sister Processes of the Brain. *Frontiers in neuroscience*, 13:1169.

Palermo, S., Leotta, D., Bongioanni, M. R., Amanzio, M. (2014). Unawareness of deficits in ischemic injury: role of the cingulate cortex. *Neurocase*, 20(5):540-555.

Palermo, S. (2017) Reduced self-awareness across pathologies: Involvement of the Anterior Cingulate Cortex. Eds Nova Science Publishers, Hauppauge, NY. In: AA. VV. *Horizons in Neuroscience Research* (Vol. 28). A. Costa & E. Villalba (Eds.), Hauppauge, New York: Nova Science Publishers.

Palermo, S., Stanziano, M., Morese, R. (2018). Commentary: Anterior Cingulate Cortex and Response Conflict: Effects of Frequency, Inhibition and Errors. *Front. Behav. Neurosci*., 12:171.

Palermo, S., & Morese, R. (2019). Introductory Chapter: Neuroscience Wants Behavior. In: AA. VV. *Behavioral Neuroscience*. S. Palermo & R. Morese (Eds.), London: IntechOpen.

Penrose, R. (1989). *The Emperor's New Mind*. Oxford: Oxford University Press.

Penrose, R. (1994). *Shadows of the Mind*. Oxford: Oxford University Press.

Philippi, C. L., Feinstein, J. S., Khalsa, S. S., Damasio, A., Tranel, D., Landini, G., Williford, K., Rudrauf, D. (2012). Preserved self-awareness following extensive bilateral brain damage to the insula, anterior cingulate, and medial prefrontal cortices. *PLoS One*, 7(8):e38413.

Purves, D., Cabeza, R., Huettel, S. A., LaBar, K. S., Platt, M. L., Woldorff, M. G. (2013). *Principles of Cognitive Neuroscience.* Second Edition. Chapter 7. Oxford: Oxford University Press.

Searle, J. R. (1997). *The Mystery of Consciousness*. New York: New York Review of Book.

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna. H., et al., (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci*., 27:2349-2356.

Seth, A. K., Suzuki, K., Critchley, H. D. (2011). An interoceptive predictive coding model of conscious presence. *Front. Psychol.,* 2:395.

Shackman, A., Salomans, T., Slagter, H., Fox, A., Winter, J., et al., (2011). The integration of negative affect, pain and cognitive control in the cingulate cortex. *Nature Reviews Neuroscience,* 12:154-167.

Stuss, D. T., Picton, T., & Alexander, M. (2001). Counsciouness, self-awareness and the frontal lobe. In S. Salloway, P. Malloy & M. Alexander (Eds.), *The Frontal lobes and Neuropsychiatric Illness* (pp. 101-109): American Psychiatric Press.

Stuss, D. T., & Anderson, V. (2004). The frontal lobes and theory of mind: developmental concepts from adult focal lesion research. *Brain Cogn*, 55(1): 69-83.

Uddin, L. Q. (2015). Salience processing and insular cortical function and dysfunction. *Nat. Rev. Neurosci*., 16:55-61.

Vithoulkas, G., & Muresanu, D. F. (2014). Conscience and consciousness: a definition. *Journal of medicine and life*, 7(1):104-108.

Zeman, A. (2001). Consciousness. *Brain*, 124(7):1263-89.

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Chapter 6

A THEORY OF MIND AS AN IMPLICATION OF EMPOWERMENT

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ABSTRACT

A Theory of Mind (ToM) reflects humankind's evolution as social beings. That is, ToM implies potential energy, motivation, and empowerment. Empowerment is something that gives people hopes and dreams, brings them courage, and prompts them to be filled with the strength to live. Human beings are born with splendid abilities, and throughout their lives, they can continue to demonstrate magnificent strengths. Empowerment draws out that magnificent power and allows the vital force and potential that lie hidden deep within us to flow.

This chapter explores theory of mind as an implication of empowerment, using empirical data on social competence development among children.

Keywords: theory of mind, empowerment, inclusion

1. HUMAN EVOLUTION AND DEVELOPMENT: LINKING EMPOWERMENT BETWEEN INDIVIDUALS, PEERS, AND THE COMMUNITY

Human beings never survive alone. Evolutionary findings suggest that our strength lies in understanding and living harmoniously with others. This means ToM is an essential skill for survival. Moreover, the link between the three kinds of empowerment - self, peer, and community empowerment - is indispensable.

There are three criteria to ensure the sustainable development of human society[1].

1. A sense of pride in oneself
2. The ability to enjoy differences

3. Believing in the capacities of groups

(1) A Sense of Pride in Oneself

First, it is necessary to believe in and have pride in oneself, as it is impossible to have pride without first believing in oneself. Only by having a sense of self-pride does it become possible to recognize others.

According to human development theory, babies become aware of their body and physical sensations during their exploratory activities. They feel their existence via the sense of comfort and discomfort, which is affected by the environment around them, such as emotional warmth of adults and a safe and comfortable atmosphere. A sense of pride in oneself is nurtured through positive acceptance by others and the society. It becomes a core of vital energy, reflecting positive interaction with the environment.

(2) The Ability to Enjoy Differences

The next requirement is the ability to enjoy differences with other people. Diversity is the basis for the development of society. In the theory of evolution, evolution is reported not as being rational but rather as the building of rationality over the irrational. It is from diversity and ambiguity that new values and things with meaning are birthed. Therefore, it is necessary to cherish an unorganized and ambiguous world and objects that may appear at a glance to be meaningless. Diversity is also interrelated with a sense of playfulness and room for growth.

ToM is a basic skill to recognize others and understand differences. The ability to enjoy differences is an extension of the skill of ToM, which not only helps in noticing differences, but also facilitates positive acceptance of those differences.

How do we promote the ability to enjoy differences? Many studies have shown that nurturing curiosity within a safe environment is a good starting

point. If the difference brings fear, nobody would accept that difference. Safety, stability, and mutual respect are essential to cherish differences.

(3) Believing in the Capacities of Groups

Third prerequisite is to believe in the capacities of peers and groups. A sense of trust, which is necessary among peers and communities, does not stop at trust between individuals. It is the ability to recognize various characteristics and to believe in the capacities of a group or community with such diversity.

One example is inclusive education where children with special needs spend time together with others who do not have disabilities. Each child is able to gain confidence in themselves and accept their differences as things that are simply natural while they enjoy growing together. Regarding adults, recognition from peers will lead to reciprocal recognition for others, which will then tie into teamwork.

In practice, the magnificent potential that each individual initially has is awakened, prompted to manifest, and is leveraged through activities for the good of people's lives and for the development of society [3]. In groups of people such as in the context of a business enterprise, the potential vigor and capabilities of each employee can be drawn out skillfully and leveraged as energy to be linked to staff development and corporate growth. These are the things that empowerment - needed by organizations, groups, and people - are all about.

2. Evidence of ToM and Social Competence Development

Here, we explore the evidence of ToM and social competence development. The purpose of the study was to examine the relationship between children's social competence and initial index of theory of mind at 30 months of age.

The participants were 322 toddlers and parents/caregivers who were registered with the Japan Science and Technology Agency (JST) project. They completed a five-minute interaction session, which was coded using the Interaction Rating Scale (IRS, Appendix 1) as an evidence-based practical index of children's social competence [4, 5, 7].

The IRS is used to measure children's social skills and caregivers' child rearing skills through observations of caregiver-child interactions. It is appropriate for the assessment of interactions between caregivers and children from birth to age eight years. This rating scale comprises 70 items for behavioral score and 11 items for impression score, grouped into 10 subscales. Five subscales focus on children's social skills: Autonomy, Responsiveness, Empathy, Motor regulation, and Emotional regulation. Another five items assess caregivers' parenting skills: Respect for autonomy development, Respect for responsiveness development, Respect for empathy development, Respect for cognitive development, and Respect for social-emotional development. Additionally, one item assesses overall impression of synchronous relationships. The items were selected from the HOME (Home Observation for Measurement of the Environment) [9], SSRS (Social Skills Rating Systems) [10], and NCAST (Nursing Child Assessment Satellite Training) teaching scales [11]. The IRS can be used in various settings (home, laboratory, etc.), and takes less than five minutes.

Two different sets of variables are scored: behavior items and impression items of the subscale. It specifies the features of interaction for both subscale and total scores. Each subscale assesses the presence of behavior (1 = Yes, 0 = No), and the sum of all items in the subscale provides the overall behavior score. Scores on the impression items and the overall impression item are on a five-point scale, (where 1 = not evident at all, 2 = not evident, 3 = neutral, 4 = evident, 5 = evident at high level), and they measure caregiver-child interaction. The observer completes the checklist composed of 25 items focusing on children's behavior toward caregivers (e.g., child looks at caregiver's face as social referencing) and 45 items focusing on the caregiver behavior. The observer then provides an impression on a 5-point scale for each subscale and for an overall impression.

The Interaction Rating Scale (IRS) was tested on randomly selected normal children in Japan, and it showed highly significant correlations with NCAST teaching scales (child items r = .70; caregiver items r = .98; total r = .89). The IRS was also tested on children with developmental disorders (ADHD/PDD), mental retardation, and an abuse or maltreatment history. The IRS scores were significantly related to the children's behavior and environmental problems, confirming the reliability and validity of the scale. The Cronbach's alpha of the IRS subscales ranged from 0.62 to 0.80 [6].

Additionally, the children were asked to complete a diverse-desire task as a ToM (theory of mind) index.

The results showed that the ToM index was related to the total score and subscales of the IRS, such as Empathy and Emotional regulation [8]. These findings show that the IRS score was related to ToM task performance at 30 months of age.

3. Creating Inclusive Communities with the "Dynamic Synergy Model"

Recognizing others, which is a ToM skill, may sometimes increase the risk of bias. The concept of *Dynamic Synergy Model (DSM)* is an effective way to prevent this. The components of the DSM are complementary, interconnected, and interdependent in the world. Additionally, they give rise to each other, as they are interrelated. The DSM offers a framework for practice and research, using perspectives of a dual focus on lifespan development (Figure 1). It views all personal traits as valuable, indispensable, and changeable, thus moving beyond the traditional dualistic model of typical vs. atypical human development.

The DSM can be illustrated by an ellipse, which encompasses each *developmental trait* of an individual, with a *dual focus* on both *"typical"* and *"unique"* traits. A developmental trait is not static but fluctuates in both directions of typical and unique within a *dynamic equilibrium*, reflecting the dynamic interplay between personal and environmental factors.

In other words, the model presents for every individual each developmental trait along a continuum, suggesting that it can be synergistically mixed from the "typical" to the "unique" and reflects the interplay between individual and environmental factors. Depending on circumstances, during the course of an individual's life, a specific trait (or characteristic) may vary in its place on that continuum, moving toward the "typical" side from the "unique" and back again.

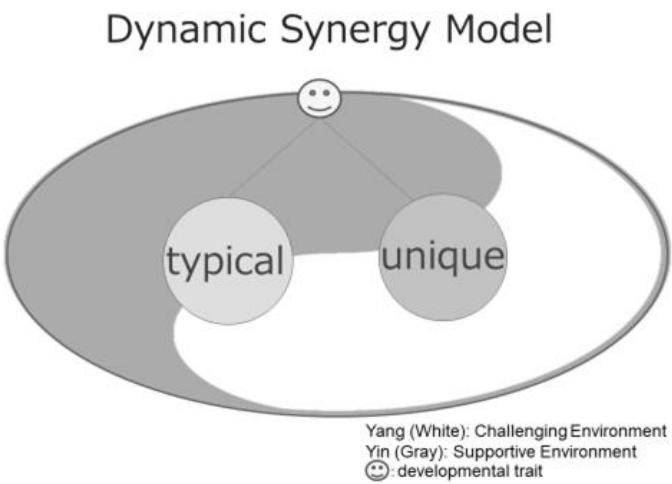

Figure 1. Dynamic Synergy Model

Any point on the ellipse represents the sum of an individual's typical and unique characteristics. It can be a metaphor for the fact that each individual has *equal dignity* wherever one is. The background represents environmental factors, with Yang (White) as the "Supportive Environment (which promotes order)" and Yin (Gray) as the "Challenging Environment (which promotes change)."

In practice, this model illustrates the importance of seeing each person with equal dignity, no matter the environment they are in, and no matter what combination of unique traits they hold. Thus, people cannot be seen as fixed in any one way, but as dynamically functioning and changing. This can lead directly to the reduction of prejudice and stereotyping, if all are seen as unique, worthy of dignity, and ever developing and changing throughout

life and circumstances. This supports a view of an interdependent, symbiotic society, where everyone is making important contributions, as well as relying on others for the talents they bring.

Practitioners can also utilize this model to examine and create supporting/challenging environments in a manner adapted to the specific needs of the parties for their well-being.

We will explore examples of DSM-based practice and research to empower people in communities with inclusive membership, values, and practices.

CONCLUSION: TOM, SOCIAL COMPETENCE, AND EMPOWERMENT

Empowerment is one method for realizing a society in which everyone is the hero of his or her life, where they can enjoy the differences between themselves and others and are able to embrace the joys of living alongside one another.

It also links self, peer, and community empowerment, which are necessary for its promotion.

Anme, 2008.

Figure 2. Synergy model for empowerment.

Self-empowerment means bringing out one's own capacities. Examples of this might include the use of a certain method for building motivation or to absorb oneself in a favorite pastime to relieve stress. Peer empowerment entails drawing out individual capacities through peers, such as by dining together or talking with one another. Community empowerment is the leveraging of communities, organizations, the workplace, or their systems to invigorate such groups. Examples of community empowerment include activities undertaken by the entire community to organize something together such as an event or a local festival [5].

The combined use and leveraging of these different types of empowerment are essential in order to realize something that is both sustainable and effective, and are called synergy model for empowerment (Figure 2).

The science of empowerment is the product of plasticity, diversity, and holistic attributes. The reason for this is that plasticity is the power to change oneself and the environment, which is accelerated within diversity, and then integrated as a holistic action. ToM has a key role in ensuring and promoting empowerment in a suitable way - to make "*A world of possibilities!*" (Figure 3).

Figure 3. Mascot Character of Empowerment: Rainbow colored cloth and flower hair ornaments mean including diversity with Dynamic Synergy Model.

APPENDIX 1. INTERACTION RATING SCALE (IRS)

Child Items

I. Autonomy

Child initiates interaction with caregiver.

1. Child vocalizes while looking at task materials.
2. Child smiles or laughs during the episode.
3. Child attempts to engage caregiver in eye contact.
4. Child initiates interaction with caregiver spontaneously.
5. Child attempts to elicit caregiver's response.

II. Responsiveness to Caregiver

Child is responsive to caregiver's behavioral cues.

1. Child displays strong reaction during the interaction.
2. Child gazes at caregiver's face or task materials after caregiver's non-verbal behaviors.
3. Child looks at caregiver's face or eyes when caregiver attempts eye contact.
4. Child vocalizes or babbles within five seconds of caregiver's verbalization.
5. Child vocalizes or babbles within five seconds of caregiver's gestures, touch, or changes in facial expression.

III. Empathy

Child behaves consistently with caregiver's affective expression.

1. Child gives, shows, or points to task material to share emotion with caregiver.
2. Child looks at caregiver's face for social referencing.
3. Child vocalizes or adjusts own behavior within five seconds of caregiver's verbalization.

4. Child smiles at caregiver within five seconds of caregiver's verbalization.
5. Child adjusts behavior within five seconds in response to caregiver's gestures, touch, or changes in expression.

IV. Motor Self Regulation

Child's behavior directed toward the task; not overactive.

1. Child widens eyes and/or shows postural attention to task situation.
2. Child becomes appropriately active in response to task situation.
3. Child's movements are clearly directed toward/away from the task or task material.
4. Child makes clearly recognizable hand motions during the episode (clapping, reaching, waving, pounding, pointing, pushing away).
5. Child is neither restless or overactive.

V. Emotional Self Regulation

Child adjusts his/her emotional state to a comfortable level.

1. Child stops displaying distress without caregiver's response.
2. Child stops displaying distress without caregiver's soothing attempts.
3. Child stops displaying distress within 15 seconds of caregiver's soothing attempts.
4. Child asks caregiver for help or consolation.
5. Child is not startled by caregiver's movements or changes in facial expression.

Caregiver Items

VI. Sensitivity to Child

Caregiver accurately interprets child's cues.

1. Caregiver positions child to safely support it.
2. Caregiver provides an environment free of distractions.
3. Caregiver positions child so it can reach and manipulate materials.
4. Caregiver seeks the child's attention before beginning the task, at the outset of the teaching interaction.
5. Caregiver gives instruction only when the child is attentive (90%).
6. Caregiver positions child so eye contact is possible during the teaching period (60%).
7. Caregiver changes position of child and/or material after the unsuccessful attempts of child to do task.
8. Caregiver keeps child in visual range.
9. Caregiver stays close to child and pays good attention.

VII. Responsiveness to Child

Caregiver responds to child's behavior.

1. Caregiver praises child's efforts at least once during the episode.
2. Caregiver emits positive, sympathetic, or soothing verbalizations.
3. Caregiver smiles, or touches child within five seconds after the child's smile or vocalization.
4. Caregiver emits soothing non-verbal response (i.e., pat, touch, rock, caress, kiss)
5. Caregiver diverts the child's distress by playing games, introducing new toy.
6. Caregiver does not vocalize to the child while the child is vocalizing.
7. Caregiver verbally praises child during the episode.
8. Caregiver smiles and/or nods at the child
9. Caregiver responds to child's vocalizations with affectionate verbal response.

VIII. Respect for Child's Autonomy

Caregiver respects for child's autonomy.

1. Caregiver allows child to explore task material for at least five seconds before providing first task related instruction.
2. Caregiver pauses when child initiates behaviors during episode.
3. Caregiver asks for no more than three repetitions when child is successful at completing the task.
4. Caregiver does not physically force child to complete task.
5. Caregiver halts the episode when child is distressed.
6. After giving instructions, caregiver allows at least five seconds for child to attempt task before intervening.
7. Caregiver allows non-task manipulation of task materials after the original presentation.
8. Caregiver does not make critical or negative comments about child's task performance.
9. Caregiver encourages and/or allows child to perform task at least once before intervening.

IX. Social-Emotional Growth Fostering

Caregiver encourages child's social-emotional development.

1. Caregiver does not make negative comments to child.
2. Caregiver does not shout at child.
3. Caregiver does not use abrupt movements or rough handling.
4. Caregiver does not slap, hit, or spank.
5. Caregiver does not make negative comments to observer about child.
6. Caregiver's body posture is relaxed during the episode (50%).
7. Caregiver faces the child while talking (50%).
8. Caregiver behaves affectionately during the episode.
9. Caregiver makes constructive or encouraging statements during the episode.

X. Cognitive Growth Fostering

Caregiver encourages child's cognitive development.

1. Caregiver focuses own attention and child's attention on task during most of the period (60%).
2. Caregiver describes perceptual qualities of task materials to child.
3. Caregiver uses at least two different sentences or phrases to describe task to child.
4. Caregiver uses an explanatory verbal style more often than a directive style during the episode.
5. Caregiver's instructions are clear and unambiguous.
6. Caregiver uses both verbal description and non-verbal instruction.
7. Caregiver uses teaching loops (alerting, instruction, performance, and feedback) in instructing child.
8. Caregiver signals completion of task to child verbally or non-verbally.
9. Length of caregiver instruction to child is age appropriate (generally 1-5 minutes).

* Overall Impression: A Synchronous Relationship

REFERENCES

[1] Anme, Tokie. "*Creating Empowerment in Communities*," NOVA publications.

[2] Anme, Tokie, McCall, Mery. *Culture, Care and Community Empowerment: International Applications of Theory and Methods*. Tokyo: Kawashima Press, 2008.

[3] Anme T. Intergenerational Activities and Aging Well: Plasticity as a Fundamental Strength of Lifespan Development, *Systems Sciences for Health-Social Services*, 21(2), 2015.

[4] Anme T. *Manual of Interaction Rating Scale*. Japan Pediatric Press. 2009.

[5] Anme T. *Bridge between Mind and Education: Evidence from Longitudinal Research on Child Care Environment and Child*. Asia Pacific Conference on Mind, Brain and Education 1. 2008; 14-18.

[6] Anme Tokie, Yato Yuko., Shinohara Ryoji, and Sugisawa Yuka. "The Validity and Reliability of Interaction Rating Scale (IRS): Characteristics for Children with Behavioral or Environmental Difficulties." *Japanese Journal of Health Care and Welfare* 14, (2007): 23-31.

[7] Anme T, Trajectories of social competence by using Interaction Rating Scale (IRS) as an evidence-based practical index of children's social skills and parenting, *Journal of Epidemiology*, 20, 419-426, 2010.

[8] Tanaka Emiko, Etsuko Tomisaki, Shinohara Ryoji, ..., and Anme Tokie. "Implications of Social Competence among Thirty-Month-Old Toddlers: A Theory of Mind Perspective." *Journal of Epidemiology* 20, (2010): 447-451.

[9] Caldwell, Bettye M, and Bradley, Robert H. "*Home Observation for Measurement of the Environment*," Arkansas: University of Arkansas at Little book, (1974), 1-164.

[10] Gresham FM. & Elliot SN. *Social skills rating system-Secondary*. Circle Pines, MN: American Guidance Service.1990; 1-223.

[11] Sumner, A, Georgina, Spietz Anita. *NCAST Caregiver/Parent-child interaction on Teaching Manual*. Seattle: NCAST Publications, 1994.

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2. New Empowerment Model on practical strategies for wellbeing, In: "*Empowerment: Cross-Cultural Perspectives, Strategies and Psychological Benefits*," Nova Science Publishers, 2016.
3. Web Application for Social Skill Development in Children with Autism, In: "*Social Skills: Perceptions, Role in Autistic Children and Assistive Technology*," Nova Science Publishers, 2016.

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