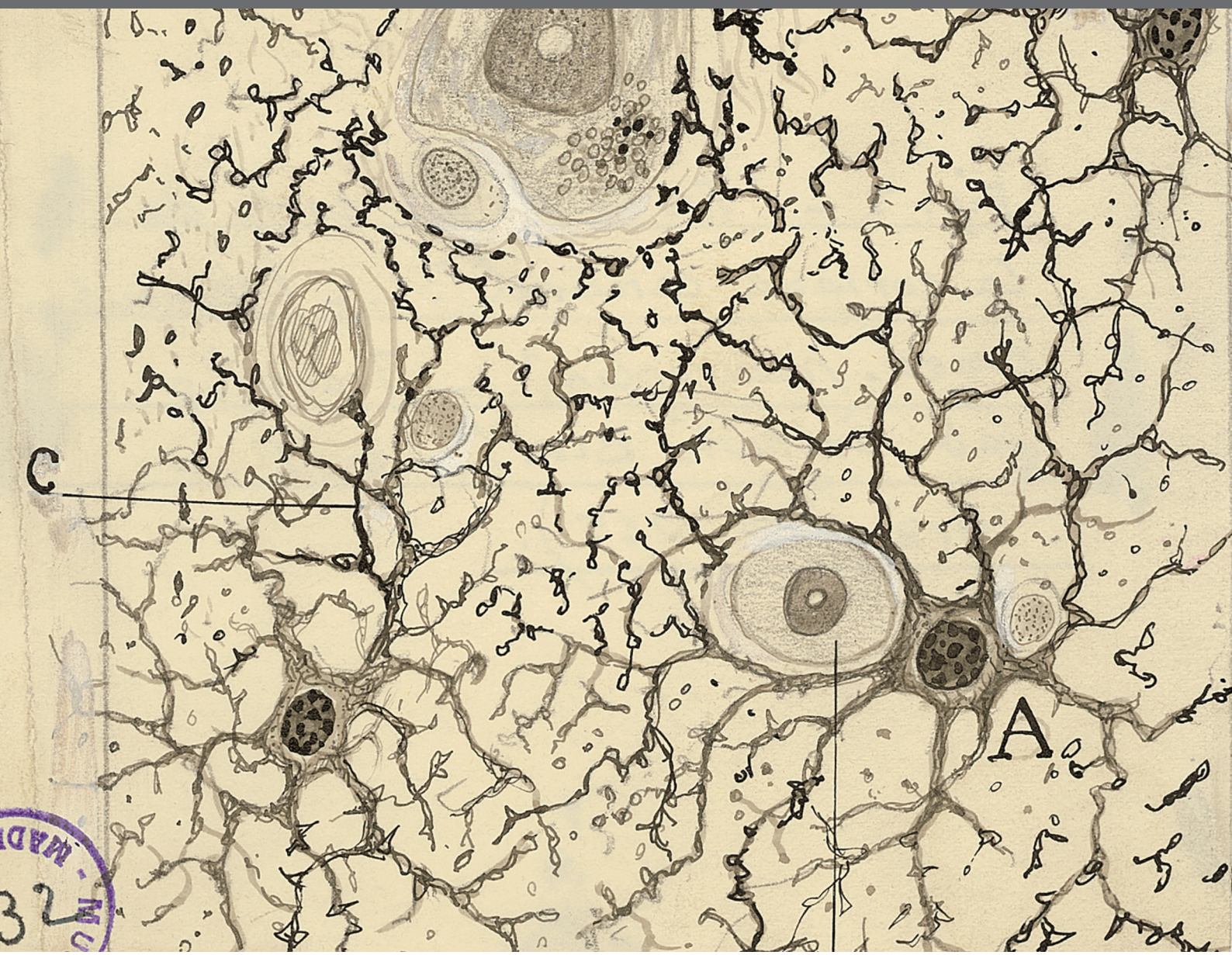
HORMONES AND NEURAL AGING: LESSONS FROM EXPERIMENTAL MODELS

EDITED BY: Isabel Varela-Nieto, Julie A. Chowen and Luis Miguel García-Segura

PUBLISHED IN: Frontiers in Aging Neuroscience



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88945-708-3
DOI 10.3389/978-2-88945-708-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

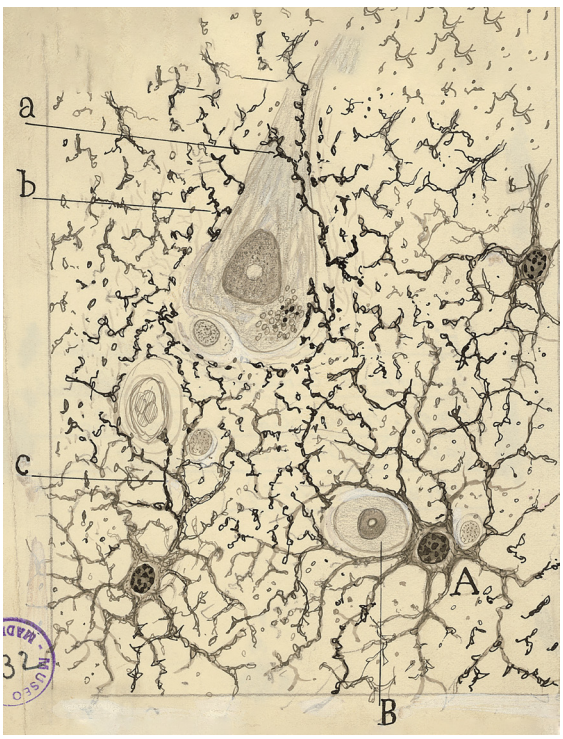
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

HORMONES AND NEURAL AGING: LESSONS FROM EXPERIMENTAL MODELS

Topic Editors:
Isabel Varela-Nieto, Alberto Sols Biomedical Research Institute, CSIC-UAM, Centro de Investigación Biomédica en Red de Enfermedades Raras, Instituto de Salud Carlos III, Hospital La Paz Institute for Health Research (IdiPAZ), Spain
Julie A. Chowen, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación la Princesa**,** Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, IMDEA Food Institute, CEI UAM + CSIC, Spain
Luis Miguel García-Segura, Instituto Cajal, CSIC, Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable, Instituto Carlos III, Spain



Neural tissue through the eyes of Santiago Ramón y Cajal: drawing of astrocytes.
Image courtesy of the Cajal Institute, Cajal Legacy, Spanish National Research Council (CSIC), Madrid.

How can we slow the signs of aging? Although aging is a natural process for all living things, doing so without dramatic alterations of health and well-being is an important aim in health care. Understanding this gradual but continuous process

is fundamental in order to avoid, or at least improve, aging associated illnesses and conditions. The reviews and studies compiled here address various aspects of the relationship between systemic and central changes during the aging process, with hormonal signals as the important liaison.

Citation: Varela-Nieto, I., Chowen, J. A., García-Segura, L. M., eds. (2019). Hormones and Neural Aging: Lessons From Experimental Models. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-708-3

Table of Contents

frontiers in Aging Neuroscience

EDITORIAL
published: 15 November 2018
doi: 10.3389/fnagi.2018.00374

Editorial: Hormones and Neural Aging: Lessons From Experimental Models

Isabel Varela-Nieto[1,2,3], *Julie A. Chowen*[4,5,6*] *and Luis Miguel García-Segura*[7]

[1] *"Alberto Sols" Biomedical Research Institute, CSIC-UAM, Madrid, Spain,* [2] *Centro de Investigación Biomédica en Red de Enfermedades Raras, Instituto de Salud Carlos III, Madrid, Spain,* [3] *Hospital La Paz Institute for Health Research (IdiPAZ), Madrid, Spain,* [4] *Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación la Princesa, Madrid, Spain,* [5] *Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain,* [6] *IMDEA Food Institute, CEI UAM + CSIC, Madrid, Spain,* [7] *Instituto Cajal, CSIC and Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable, Instituto Carlos III, Madrid, Spain*

Keywords: apoptosis, insulin-like factors, neurodegeneration, senescence, sex hormones

Editorial on the Research Topic

Hormones and Neural Aging: Lessons From Experimental Models

The relationship between the hormonal status and neural aging has been studied in different contexts, from metabolism to reproduction. Age-related modifications in circulating hormones that perform neuroprotective functions have been associated with neural aging. In addition, the sensitivity of neurons and glial cells to respond to some peripheral metabolic signals and neuroprotective substances declines with aging. In turn, neural aging affects the control exerted by the hypothalamus on peripheral endocrine glands and systemic metabolism. Therefore, the feed-back loops between the brain and the body are progressively altered during the aging process, contributing to neural dysfunction.

The study of aging by using experimental models offers an opportunity to better understand human physiopathology, with these studies complementing clinical and epidemiological studies. This collection of articles attempts to portray how animal studies have advanced our current knowledge on the interaction of hormones with neurodegeneration changes, including those in sensory systems, that take place during aging. These articles discuss the molecular pathways implicated in the decreased cell renewal, cell senescence and cell death, as well as potential strategies to promote healthy brain aging and rejuvenation.

In the review by Arroba et al., age-related neurodegenerative diseases of the retina are discussed. Neuroinflammation is one of the hallmarks of these diseases, including age-related macular degeneration, retinitis pigmentosa, and diabetic retinopathy. Insulin-like growth factor (IGF)-1 plays numerous roles in the retina and the involvement of this growth factor, as well as microglia and exosomes, in these neurodegenerative processes is highlighted. Mukai et al. present original data regarding how the complement system is involved in maintaining retinal integrity during aging. Various complement knock-out strains of mice were employed. Electroretinograms (ERG) and thicknesses of retinal layers in spectral domain optical coherence tomography were determined in young and adult wild type and $C1q^{-/-}$, $Mbl\,a/c^{-/-}$, $Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$ knock-out mice. The data presented support the hypothesis that the complement system is important in maintaining retinal integrity during aging.

OPEN ACCESS

Edited and reviewed by:
Filippo Tempia,
Università degli Studi di Torino, Italy

***Correspondence:**
Julie A. Chowen
jachowen@gmail.com

Received: *18 October 2018*
Accepted: *29 October 2018*
Published: *15 November 2018*

Citation:
Varela-Nieto I, Chowen JA and García-Segura LM (2018) Editorial: Hormones and Neural Aging: Lessons From Experimental Models. Front. Aging Neurosci. 10:374. doi: 10.3389/fnagi.2018.00374

Hearing function is also affected during aging and Balogová et al. present an original study employing the fast aging Fischer 344 rat model. They compared age-related changes in cochlear morphology, hearing thresholds, auditory brainstem responses, and distortion product otoacoustic emissions in both male and female rats, and found that the age-related changes in hearing function are more pronounced in males compared to females. A mini review by Rodríguez-de la Rosa et al. delves into the role of IGF-1 in age-related hearing loss and the mechanisms involved. This group has reported a correlation between the decline in IGF-1 availability and the reduction in hearing during aging. The primary actions of IGF-1 on audition and the mechanisms involved are discussed.

The mechanisms of neuroprotective effects of IGF-1 are reviewed in depth by Labandeira-Garcia et al. The role of this growth factor in protection against neuroinflammation and the possible explanations for some of the controversies surrounding this topic are presented. The implication of astrocytes and microglia in IGF-1 mediated neuroprotection, such as their production of local IGF-1 and the cross-talk between these glial cells and neurons, is discussed. Importantly, these authors also review the interaction between IGF-1 and estrogens in neuroprotection.

Estrogens exert important neurotrophic and neuroprotective effects and the decline in this sex steroid is suggested to be involved in some of the aging processes in women. Various articles in this collection focus on the role and mechanisms of estrogens in the aging brain. In the review by Lejri et al., the relationship between estrogens and mitochondrial function in the aging brain is discussed. Indeed, mitochondria are essential for the production of sex steroids and, in turn, these hormones can modify mitochondrial function. Here, mitochondrial dysfunction during aging is described, as well as how estrogens affect this process. Special emphasis on recent evidences in the involvement of brain-derived neurotrophic factor and sirtuin 3 (SIRT3) in the neuroprotective effects of estrogens, especially in female aging, is made. This topic is also reviewed by Gaignard et al., where they analyze in depth the intracellular mechanisms of sex steroid actions on mitochondrial function. These authors also address aspects of sexual dimorphism in mitochondria during processes of aging and how this might be involved in the sex differences in age-related neurodegenerative diseases. The mechanisms by which sex steroids promote neuroprotection have been analyzed in the review by Zárate et al. These authors discuss the effects of sex steroids on DNA repair enzymes and how a reduction in estrogens in postmenopausal women may be involved in the reduced capacity for DNA repair that occurs in aging processes.

Women's health during aging is also addressed in the review by Navarro-Pardo et al. Here, the authors concentrate on the important question of how declining circulating estrogen levels during aging affect cognitive function and psychological health in women. The debate as to whether hormone therapy is beneficial in postmenopausal women is also examined. A study analyzing the connection between adrenomedullin levels and memory preservation during aging by Larrayoz et al. show an inverse relationship between these two factors. Knock-out mice for adrenomedullin and its generated peptide, promadreullin N-terminal 20 peptide, were employed and memory retention was found to be improved in these knock-out mice compared to wild type controls, with this effect being more prominent in females compared to males. This improvement was associated with decreased phospho-Tau accumulation. They also report that adrenomedullin accumulation increases with aging in human brain suggesting that this hormone may be a target for improving age-related memory decline.

A relationship between cognitive decline during aging and the hypothalamic-pituitary-adrenal (HPA) axis has been hypothesized. Pesce et al. present an original article where they assessed the effects of memory training on cognitive performance and the HPA axis, as well as systemic inflammation, in human subjects. Young and older adults were analyzed at baseline and the young adults were also analyzed 6-months after memory training. These authors report an improvement in cognitive function, memory, and attention by memory training that was associated with a decrease in circulating levels of cortisol and inflammatory cytokines. They also raise the question as to whether the wild type p53-induced phosphatase-1 (WIP-1) is involved in this process, as its expression in mononuclear cells was positively associated to the cognitive function scores.

Together these articles indicate that, although numerous factors are involved in the aging process, the decline in endocrine function appears to play a very important role in neural aging. Aging of the central nervous system clearly impacts the physiological control of the entire being, including a decline or alteration in endocrine system function, which in turn would contribute to further neural aging. Further studies employing animal models are necessary to understand how to slow this vicious cycle.

AUTHOR CONTRIBUTIONS

IV-N, JAC, and LG-S contributed equally to this editorial, in its conception, writing, and revision.

FUNDING

The authors are funded by grants from the Spanish Ministry of Science and Innovation/FEDER with the grants BFU2017-82565-C2-1-R to JAC, BFU2017-82754-R to LG-S, and SAF2017-86107-R to IV-N.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

REVIEW
published: 05 July 2018
doi: 10.3389/fnagi.2018.00203

IGF-1, Inflammation and Retinal Degeneration: A Close Network

Ana I. Arroba[1,2,3*†], *Antonio Campos-Caro*[3], *Manuel Aguilar-Diosdado*[3,4] *and Ángela M. Valverde*[1,2*]

[1] *Alberto Sols Biomedical Research Institute (IIBm) (CSIC/UAM), Madrid, Spain,* [2] *Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders (CIBERdem), ISCIII, Madrid, Spain,* [3] *Research Unit, Instituto de Investigación e Innovación en Ciencias Biomédicas de la Provincia de Cádiz (INiBICA), University Hospital "Puerta del Mar", Cádiz, Spain,* [4] *Department of Endocrinology and Metabolism, Instituto de Investigación e Innovación en Ciencias Biomédicas de la Provincia de Cádiz (INiBICA), University Hospital "Puerta del Mar", Cádiz, Spain*

Retinal degenerative diseases are a group of heterogeneous diseases that include age-related macular degeneration (AMD), retinitis pigmentosa (RP), and diabetic retinopathy (DR). The progressive degeneration of the retinal neurons results in a severe deterioration of the visual function. Neuroinflammation is an early hallmark of many neurodegenerative disorders of the retina including AMD, RP and DR. Microglial cells, key components of the retinal immune defense system, are activated in retinal degenerative diseases. In the microglia the interplay between the proinflammatory/classically activated or antiinflammatory/alternatively activated phenotypes is a complex dynamic process that occurs during the course of disease due to the different environmental signals related to pathophysiological conditions. In this regard, an adequate transition from the proinflammatory to the anti-inflammatory response is necessary to counteract retinal neurodegeneration and its subsequent damage that leads to the loss of visual function. Insulin like-growth factor-1 (IGF-1) has been considered as a pleiotropic factor in the retina under health or disease conditions and several effects of IGF-1 in retinal immune modulation have been described. In this review, we provide recent insights of inflammation as a common feature of retinal diseases (AMD, RP and RD) highlighting the role of microglia, exosomes and IGF-1 in this process.

Keywords: retina, inflammation, neurodegeneration, IGF-1, microglia and exosomes

OPEN ACCESS

Edited by:
Luis Miguel Garcia-Segura, Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
Thomas Langmann, Lehrstuhl für Experimentelle Immunologie des Auges, Medizinische Fakultät, Universität zu Köln, Germany
Jose L. Labandeira-Garcia, Universidade de Santiago de Compostela, Spain

***Correspondence:**
Ana I. Arroba
anaarroba@gmail.com
Ángela M. Valverde
avalverde@iib.uam.es

†Present Address:
Ana I. Arroba, Research Unit, Instituto Investigación e Innovación Biomédica de Cádiz (INIBICA), University Hospital "Puerta del Mar", Cádiz, Spain

Received: *17 April 2018*
Accepted: *14 June 2018*
Published: *05 July 2018*

Citation:
Arroba AI, Campos-Caro A, Aguilar-Diosdado M and Valverde ÁM (2018) IGF-1, Inflammation and Retinal Degeneration: A Close Network. Front. Aging Neurosci. 10:203. doi: 10.3389/fnagi.2018.00203

ROLE OF IGF-1 IN RETINAL INFLAMMATION AND DEGENERATION

Neuroinflammation is currently considered as an early event in the pathophysiology of many neurodegenerative disorders because despite its essential role in protecting tissues during the early steps of disease, the continuous presence of proinflammatory stimuli induces cellular damage (Glass et al., 2010; Arroba et al., 2016a; Arroba and Valverde, 2017). It is widely accepted that in the central nervous system (CNS) astrocytes and microglia are the cells that play a critical role in neuroinflammation that precedes the neurodegenerative diseases (Cherry et al., 2014; Arroba et al., 2016a). In this scenario, activated microglia and reactive astrocytes participate into the release of different inflammatory mediators including cytokines, chemokines, reactive oxygen species (ROS), and nitric oxide (NO), all of them contributing to the maintenance of a chronic neuroinflammatory milieu that ultimately may be responsible of neurotoxic damage in the CNS (Cuenca et al., 2014).

Insulin-like growth factor-I (IGF-1) is the ligand of the IGF-1 receptor (IGF-1R) which belongs to the tyrosine kinase receptor superfamily and regulates normal developmental growth through endocrine and autocrine/paracrine-mediated mechanisms (Bates et al., 1995). IGF-1 is also a potent survival factor for many tissues (Heemskerk et al., 1999). Particularly, IGF-1 is a neurotrophic peptide in the CNS where it promotes synaptic plasticity, enhances nerve growth and triggers antiapoptotic-mediated signaling cascades (Carro et al., 2003). All these IGF-1 functions are critical for the protection of nerve cells against neurodegenerative processes (Varela-Nieto et al., 2013; Yamamoto et al., 2014). Deficiency in the *IGF1* gene in humans is related with neuronal disorders such as microcephaly, mental retardation, and bilateral sensorineural deafness (Woods et al., 1996; Walenkamp et al., 2005; Netchine et al., 2009).

In several pathologies such as type 1 diabetes mellitus (T1DM) antiinflammatory properties have been attributed to the IGF-1/IGF-IR system in the CNS by counteracting the inflammatory milieu triggered by microglial activation in the hypothalamus (Zhang et al., 2016). However, controversial effects of this peptide have been described regarding its proinflammatory effect in other pathological contexts. In a recent study, IGF-1 was overexpressed in hepatic stellate cells of mice deficient in the *Abcb4* gene, a preclinical model for chronic cholangiopathy. The authors of this work found a higher stimulation of the fibrogenic processes in these double mutant mice which was accompanied by the increased expression of proinflammatory markers together with the presence of infiltrating macrophages in the liver (Sokolovic et al., 2013). Regarding this duality of IGF-1 effects in inflammation, a recent study in zebrafish has evidenced that growth factors including IGF-1 and insulin together with cytokines activate common signaling pathways that are necessary for the reprogramming of Müller glial cells and retinal regeneration upon injury (Wan et al., 2014).

The retina has been considered as a projection of the CNS and, in this tissue, neuroinflammatory processes occur in a similar way as in the brain. In fact, retina and brain share similarities due to their common neuroectodermal origin and derivation from the anterior neural tube and, therefore, both tissues respond similarly to the proinflammatory insults. Based on that, it is conceivable to integrate the retina as a part of the brain (MacCormick et al., 2015).

Retinal degenerative diseases are heterogeneous pathologies; among them, age-related macular degeneration (AMD), retinitis pigmentosa (RP), and diabetic retinopathy (DR) have a high incidence and prevalence in humans (Hernandez et al., 2016; Narayan et al., 2016; Shaw et al., 2017). In fact, a plethora of factors including genetic alterations, aging, vascular defects, chemical insults, oxidative stress, or light-induced damage are responsible of the development of retinal degeneration (Semeraro et al., 2015; van Norren and Vos, 2016). In the pathologies associated to retinal degenerative diseases, progressive degeneration of the retinal neurons, predominantly in photoreceptors, retinal ganglion cells (RGCs), and cells of the retinal pigment epithelium (RPE), results in a severe deterioration of the visual function that in many cases leads to a complete blindness (Nazari et al., 2015).

Inflammatory responses contribute to the pathophysiology of numerous ocular diseases (Dick, 2017). In this regard, RPE cells play a critical role in mediating immune responses to stressing agents such as bacterial endotoxins or proinflammatory cytokines. In fact, persistent inflammation can induce a severe damage in the RPE, thereby contributing to the activation of choroidal neovascularization (CNV), which is observed in more advanced forms of AMD (Chen et al., 2017). In RP, anti-retinal antibodies are associated with the development of cystoid macular edema (Nishikawa et al., 2017) and there are several studies suggesting that the systemic inflammatory profile is altered and may contribute to disease progression (Li et al., 2015; Mori et al., 2017). In addition, recent work supports the involvement of two key factors linking type 2 diabetes mellitus (T2DM) with neurodegeneration; the elevation of proinflammatory cytokines and the onset of insulin/IGF-1 resistance. Moreover, in T2DM, proinflammatory cytokines could be responsible of the disruption in the insulin/IGF-1 signaling pathways in peripheral tissues and the pancreas (Feve and Bastard, 2009). Likewise, accumulation of peripheral proinflammatory mediators, some of which can cross the blood-brain barrier, likely triggers insulin/IGF-1 resistance in the CNS that results in the attenuation of their neuroprotective signaling pathways, thus contributing to the onset of neurodegenerative diseases (Arroba et al., 2011).

Many studies have shown that insulin and IGFs play a relevant role in modulating the balance between growth and survival of the retinal cells (Hernandez-Sanchez et al., 1995; Frade et al., 1996; Alarcon et al., 1998). In the retina, IGF-1 is a potent proangiogenic factor that is present in the neovascular membranes from AMD patients (Lambooij et al., 2003). Moreover, in those patients, elevated concentrations of IGF-1 in plasma have been detected (Machalinska et al., 2011). Likewise, in the majority of experimental models of RP the loss of visual function parallels photoreceptor cell death (Chang et al., 1993; Sancho-Pelluz et al., 2008). In this regard, in the *rd10* experimental mouse model of RP proinsulin delays the death of photoreceptors and prolongs visual function (Corrochano et al., 2008) and, importantly, IGF-1 also decreases apoptosis of photoreceptors in both genetic and experimentally induced RP models (Arroba et al., 2009).

During aging, bioactive IGF-1 circulating levels are reduced, a trend that has been associated with human frailty and cognitive decline (Vestergaard et al., 2014). In the retina, controversies on the beneficial or deleterious effects IGF-1/IGF-1R levels on aging-related degenerative diseases have been reported. Regarding deleterious effects, elderly onset patients with diabetes have lower prevalence of proliferative DR (PDR) than those younger onset patients for similar diabetes duration, which may be related with lower serum IGF-1 levels detected in the older patients (Zhang et al., 2017). Thus, less stringent glycemic control in older onset patients with diabetes may not increase the prevalence of PDR. On the other hand, the evaluation of mice lacking the *Igf1* gene by electroretinography and selective labeling of retinal cells has evidenced an age-related accelerated loss in visual function accompanied by a significant loss of cell contacts between photoreceptors and their postsynaptic cells that may be

related to the physiopathology of human IGF-1 deficiency and resistance (Rodriguez-de la Rosa et al., 2012).

EFFECTS OF IGF-1 ON MICROGLIA IN RETINAL DISEASES ASSOCIATED TO NEURODEGENERATION

The neural retina contains a barrier system, the blood-retinal barrier (BRB), with two components: the inner BRB formed by tight junctions responsible of sealing neighboring capillary endothelial cells, and the outer BRB with tight junctions that restrict the paracellular trafficking between the retinal pigment epithelial cells (Spadoni et al., 2017). Thus, the increase in the permeability of the BRB results in the leak of plasma components into the retina (Eshaq et al., 2017). Importantly, both layers ensure an immune-privileged status of the eye and are also essential for regulating retinal homeostasis and visual function. In this regard, the BRB creates an immunosuppressive milieu that inhibits the activation of immune cells as they cross the barrier and promotes immune privilege (Forrester and Xu, 2012). The outer BRB also expresses immunoregulatory molecules that inhibit lymphocyte activation while the RPE of the BRB secretes immunomodulatory mediators into the aqueous humor that restrain the immune and inflammatory responses within the eye (Sohn et al., 2000). Therefore, under physiological conditions, immune cells of the circulation are not able to enter into the retina to combat with endogenous insults. Instead, the retina has an unique immune defense system consisting of innate immune cells (microglia, perivascular macrophages, and dendritic cells) and the complement system (Ramirez et al., 2017). The function of these cells suggests that they are "gatekeepers" of the retina. Thus, the importance of the research aimed to dissect the function of the different inflammatory processes in the retina and, especially the contribution of microglial-mediated neuroinflammation that antecedes neurodegeneration, could provide useful knowledge for the implement of challenging therapies.

IGF-1 has been associated with the pathogenesis of BRB breakdown. In mice, high intraocular IGF-1 due to its overexpression in the retina increased IGF-1R-mediated signaling resulting in the accumulation of VEGF, up-regulation of vascular intercellular adhesion molecule I and retinal infiltration by bone marrow-derived microglial cells. Altogether these alterations increased vessel paracellular permeability to both low and high molecular weight compounds and correlated with the loss of vascular tight junction integrity. In contrast, mice with chronically elevated serum IGF-1 did not show alterations in the retinal vasculature structure and permeability, indicating that circulating IGF-1 cannot initiate BRB breakdown. Importantly, in human retinas of patients with marked gliosis a strong up-regulation of the IGF-1R was detected, suggesting that therapeutic interventions aimed to counteract local IGF-1 effects may prove successful to prevent BRB disruption (Haurigot et al., 2009).

In 1932 Pio del Rio-Hortega characterized microglial cells, a component of the retinal immune defense system that constitutes approximately 5–12% of the total cells of the CNS, as an exclusive cell type in the brain with phagocytic functions that differs from glial and neuronal cells in morphology (Ginhoux et al., 2013). In the retina, microglial cells are distributed in the inner plexiform and outer plexiform layers (IPL and OPL, respectively), ganglion cell layer (GCL), and nerve fiber layer (NFL), showing highly motile protrusions that survey the surrounding environment (Cheng et al., 2002). Activated microglia has the capacity to move in all directions, but this movement is not accompanied by soma migration. Moreover, under specific circumstances microglia may be translocated to the different retinal layers where the injury is located (Lee et al., 2008; Arroba et al., 2016a; Arroba and Valverde, 2017).

Under physiological conditions, microglial cells are maintained in a resting state characterized by a small cell body and long thin dendrites (Ginhoux et al., 2013) and immunolabelling for Cd-11b. However, microglial cells are chronically activated in retinal diseases such as AMD, DR, and RP (Penfold et al., 2001; Arroba et al., 2011, 2016a) and, as occurs in with the immune cells of the periphery, this activation contributes to boost retinal damage and to accelerate disease progression. Classically, microglial cells coexist in two states, resting and activated (Nayak et al., 2014). The traditional states or definitions of microglia are currently being modified based on new knowledge about their role in pathogenesis. Therefore, microglia can develop a number of different phenotypes and functions aimed to preserve retinal homeostasis in health and disease, mainly depending on their specific environment (Herrera et al., 2015; Du et al., 2017).

Results from *in vitro* experiments have shown that, as occurs with macrophages, activated microglia cells coexist in two distinct phenotypes depending on the stimuli; proinflammatory/classically activated (M1) immunolabelling Iba-1$^+$/iNOS$^+$ or antiinflammatory/alternatively activated (M2) immunolabelling Iba-1$^+$/Arginase-1$^+$. M1/proinflammatory microglia releases neurotoxic and/or inflammatory mediators including TNF-α, interleukin-1β (IL-1β), IL-6, and glutamate and increases the expression of inducible nitric oxide synthase (iNOS), all of them exacerbating the death of retinal neurons (Varnum and Ikezu, 2012; Gonzalez et al., 2014). By contrast, M2/immunoregulatory microglia induces retinal repair and regeneration, as well as secretes growth factors and antiinflammatory cytokines aimed to resolve inflammation and ensure the survival of the retinal neurons (Arroba et al., 2011, 2016a,b; Li et al., 2015; Arroba and Valverde, 2017). Under the M2 phenotype, microglia releases the typical antiinflammatory cytokines such as IL-4, IL-13, IL-10, transforming growth factor (TGF)-β and neurotrophic factors such as IGF-1 (Jung and Suh, 2014). To add more complexity to the diversity of microglia polarization stages, several subclasses of M2/immnunoregulatory activation have been identified. The M2a participates in attenuation of inflammation whereas the M2c is involved in restoring or repairing the tissue once the M2a response has effectively completed its participation (Gordon et al., 2003; Chang et al., 2009; Sica and Mantovani, 2012). Finally, a subtype of microglia named M2b is referred to a component involved in the memory immune response which is also able to elicit

both pro- and responses (Edwards et al., 2007; Barilli et al., 2014). However, as stated above, this concept initially came from *in vitro* experiments with defined ligands and, therefore, it is difficult to translate to the *in vivo* context where many overlapping phenotypes in inflamed tissues coexist.

As recently reviewed (Labandeira-Garcia et al., 2017), IGF-1 is a mitogenic factor for microglia which also modulates the neuroinflammatory responses from microglial cells by promoting a switch toward the microglial phenotype. Furthermore, the decrease in IGF-1 that occurs with aging has been proposed to contribute to the loss of the capacity of microglia. Therefore, a tight modulation of IGF-1 levels is necessary for the regulation of the neuroinflammatory responses of the microglia since, as stated above, IGF-1 is likely be involved in inflammation in a context-dependent manner (Hotamisligil et al., 1993; Bluthe et al., 2006). Several studies in the retina have found a direct relationship between microglial activation and increased neuronal injury in experimental models of RP, glaucoma, light-induced photoreceptor degeneration, DR, and AMD (Zeng et al., 2005; Glybina et al., 2010). Regarding this issue, recent work of our laboratory and others has provided new insights on the effect of *Igf1* deficiency during aging in the blockade of autophagic flux in the retina which is closely related to neuroinflammation. By using an experimental mouse model of *Igf1* deficiency we demonstrated for the first time that in these mice aging concurs with retinal neuroinflammation. Importantly, in retinas from aged *Igf1*-deficient mice, the inflammatory process concurred with the blockade of the autophagic flux (Arroba et al., 2016c). At the molecular level, we identified elevations in the phosphorylation of mTORC1, as well as in the levels of the autophagy substrate p62, in whole retinal extracts. The corroboration of these results was evidenced by transmission electronic microscopy analysis in which we detected an accumulation of autophagosomes in the INL and OPL layers of the retina in $Igf1^{-/-}$ mice at the age of 12 months. Since it was previously reported that in these layers the neural synapsis were disrupted (Rodriguez-de la Rosa et al., 2012), we hypothesized that autophagy might be a necessary process in the neuronal cells of the INL and OPL layers of the retina to preserve photoreceptor connectivity (Shen et al., 2016), a process which is likely negatively affected by the proinflammatory milieu. Remarkably, in these layers the accumulation of autophagosomes concurred with the presence of activated amoeboid microglia (Iba-1^{+}) (Arroba et al., 2016a). As stated above, these are the critical layers where the cells responsible for the amplification of the synaptic transmission, which are highly sensitive to the inflammatory environment, are located (Noailles et al., 2016). Altogether, the results of our work in *Igf1*-deficient mouse model have identified for the first time autophagy as an adaptive response against the chronic activation of the inflammasome in microglial cells of the retina during aging. In this regard, we and others have proposed that autophagy can be considered as a relevant defense against deterioration of the retinal synapses and visual function (Noailles et al., 2014; Arroba et al., 2016c). In this concept, in *Igf1* deficient mice, the malfunction of microglia during aging can lead to the establishment of a chronic low-grade inflammatory environment, favoring the onset and further progression of retinal degeneration.

Aged-Related Macular Edema

AMD is the progressive damage in the macular region of the retina. Although the exact mechanisms involved in its pathogenesis have not been completely elucidated, chronic inflammation together with oxidative stress play a major role (Kauppinen et al., 2016). The infiltration of macrophages from the peripheral circulation is a unique and interesting component of the inflammatory component of AMD (Funk et al., 2009; Lavalette et al., 2011). In this regard, one of the characteristics of atrophic or "dry" AMD is the accumulation of microglia/macrophages in the outer retina and subretinal space (Penfold et al., 1987, 2001). Previous investigations have reported that eyes of patients with AMD have elevated concentrations of proinflammatory cytokines that modify the activity of CNV (Hageman et al., 2001). Histological studies have revealed that chronic inflammation occurs at the retinal pigment epithelial/choroidal interface in eyes with early signs of AMD such as drusen (Hernandez-Zimbron et al., 2018). Furthermore, in exudative AMD a higher up regulation of inflammatory cytokines/chemokines from RPE cells and macrophages/monocytes positively and negatively control CNV activity (Kauppinen et al., 2016). Regarding the involvement of the IGF-1 system in AMD, a case-control study involving 962 subjects showed that in non-diabetic individuals from the Age-Related Eye Disease Study (AREDS) Genetic Repository the SNP rs2872060 in the IGF-1R was significantly associated with the risk for advanced AMD and this association remained significant after patient stratification by the two types of the disease: neovascularization and geographic atrophy (Chiu et al., 2011). Interestingly, the risk allele (G) showed an additive effect and a significant interaction with BMI on the risk for neovascularization, but not for geographic atrophy. On the other hand, another study has reported increased levels of insulin-like growth factor binding protein 2 (IGFBP-2) and IGF-1 in exudative AMD eyes, indicating that defects in the expression of IGF-related molecules may be involved in the disease pathogenesis for exudative AMD (Cha et al., 2013).

Retinitis Pigmentosa

RP belongs to a retinal degenerative group of inherited diseases that affects about 2.5 million people worldwide and is characterized by a progressive photoreceptor cell death that ultimately leads to a severe vision loss (Dias et al., 2018). Initially, the cell death affects only to photoreceptor cells; however, during the progression of the disease abnormalities in the RPE and the cones are detected (Campochiaro and Mir, 2018). In addition to mutations in 70 different genes, a proinflammatory component is also a hallmark of the pathogenesis of RP [http://www.retnet.org (latest entry 2017)] (Gupta et al., 2003; Whitcup et al., 2013; Yoshida et al., 2013; Eandi et al., 2016). In fact, the levels of proinflammatory cytokines have been found markedly elevated in both the vitreous and aqueous humor from RP patients (Yoshida et al., 2013), supporting the interaction between photoreceptor cell death and intraocular inflammation.

In this context, hyperactivation of microglial cells has been demonstrated to play an important role in the photoreceptor neurodegeneration in animal models of RP (Peng et al., 2014). A recent study using on live-cell imaging in the *rd10* mouse model of RP has identified that during the early stages of the disease microglia is able to migrate, interact with, and phagocyte non-apoptotic photoreceptors, after which it becomes hyperactivated and promotes the loss of non- and apoptotic photoreceptors (Zhao et al., 2015). The authors of this study propose that primary microglial phagocytosis could be a potential cellular target for therapy. Remarkably, microglial activation mediates photoreceptor loss not only in RP, but also in AMD and DR in both preclinical animal models as well as in human patients (Zhao et al., 2015). In this regard, the emerging therapeutic strategies to combat degenerative diseases of the retina are focused in the attenuation of microglial activation (Karlstetter et al., 2015). However, to achieve this purpose more research is needed to decipher new molecular mechanisms involved in microglial activation during retinal degenerative diseases.

The effect of IGF-1 as a neuroprotective factor has been also demonstrated during RP progression. In this regard, our previous work has found a beneficial effect of IGF-1 since it attenuated reactive gliosis and apoptosis in *ex vivo* retinal explants from *rd10* mice (Arroba et al., 2011). Importantly, the elimination of retinal microglia by using of clodronate-filled liposomes reduced the efficacy of IGF-1 on photoreceptor viability. Likewise, IGF-1 was not able to inhibit the reactive Müller gliosis in absence of microglial cells. Altogether, these results indicate that the beneficial effects of IGF-1 are mediated, at least in part, by the microglia (Arroba et al., 2011, 2014). These findings suggest first a critical role of the crosstalk between microglia and Müller glial cells during neuroprotection (Arroba et al., 2014) and, second, that microglia is necessary for the neuroprotective effects of IGF-1 in the dystrophic retina (Arroba et al., 2011).

Diabetic Retinopathy

Among the microvascular complications of diabetes, DR is the most undesirable one. DR is a progressive retinal disease and a leading cause of blindness in diabetic patients due to degeneration of both the retinal vasculature and retinal neurons (Hernandez et al., 2016). DR is broadly classified into two stages: non-proliferative DR (NPDR) and proliferative DR (PDR). This classification is determined by the presence of neovascularization in the retina (Nentwich and Ulbig, 2015). NPDR typically precedes PDR and is divided into the following stages: mild, moderate, severe, and very severe based on the probability of disease progression to PDR. PDR is defined by the presence of neovascularization and is divided into the following stages: early, high risk, and severe neovascularization. Growing evidence suggests that neuroinflammation plays an early role in mediating neuronal and vascular pathology in DR (Oellers and Mahmoud, 2016).

During DR progression, the retina is affected by both external signals such as high glucose, advanced glycation-end products (AGEs) and circulating proinflammatoty cytokines (Dong et al., 2014), as well as by intrinsic signals (Sappington et al., 2006; Legacy et al., 2013). In the context of neuroinflammation, high levels of proinflammatory cytokines are detected in the retina in different animal models of DR (Li and Puro, 2002) and in retinas from diabetic patients (Arroba et al., 2016a; Hernandez et al., 2016). We have recently reported that during DR progression in diabetic *db/db* mice there is a switch from antiinflammatory to proinflammatory polarized microglia in parallel to the deterioration of visual function (Arroba et al., 2016a). In addition, in this mouse model, retinal gliosis was also detected suggesting that in addition to microglia, macroglia likely contributes to this switch boosting inflammation and promoting reactive gliosis and this scenario concurs with the death of retinal cells by apoptosis (Bogdanov et al., 2014). Whether IGF-1 is able to modulate the dynamics of microglia polarization in the setting of DR is still unknown. This is a controversial issue due to the opposite effects of IGF-1 reported in the retina with deleterious effects on the retinal vasculature (Hellstrom et al., 2002), but positive effects in the survival of retinal neurons (Kermer et al., 2000). Villacampa and co-workers have generated a transgenic mice overexpressing *Igf1* in photoreceptors to evaluate the deleterious effects of the persistent elevation of intraocular IGF-1 on retinal functionality (Villacampa et al., 2013). They showed a progressive decline in the electroretinogram amplitudes in *Igf1* transgenic animals, leading to a complete loss of response with aging. Importantly, markers of retinal stress, gliosis, and microgliosis were already present at early stages of the disease before the detection of major vascular alterations. Despite these interesting findings, the translation of this study to the human retinal diseases deserves further research.

IGF-1 AND INTERCELLULAR COMMUNICATIONS IN RETINAL DISEASES ASSOCIATED TO NEUROINFLAMMATION: INVOLVEMENT OF EXTRACELLULAR VESICLES

Extracellular vesicles (EVs) have been related to some pathologies since they are involved in a variety of immune activities having protective or detrimental properties. It is also well known that EVs have a heterogeneous molecular composition including DNA, mRNAs, micro RNAs (miRNAs), integrins, cytokines, bioactive lipids, and organelles, being these molecules similar to those of their parental cells (Colombo et al., 2014). EVs can be detected in biological fluids such as plasma (Sharma et al., 2018) and the cerebral spinal fluid (McKeever et al., 2018). Of relevance, microglial EVs retain many features of the original cells where they come from and, consequently, they are considered as a "liquid biopsy" that provide relevant information about the status of activation of their microglial parental cells during the course of the neurodegenerative processes (Kalani et al., 2014; Nigro et al., 2016). Furthermore, the EVs cargo is modulated by the pathophysiological environment in a way that they become vehicles loaded with pathogenic cargo such as aggregating proteins in neurodegenerative diseases (Schneider and Simons, 2013), oncoproteins in cancer (Nakano et al., 2015), or inflammatory cytokines in neuroinflammatory diseases (Verderio et al., 2012; Prada C. E. et al., 2013).

EVs present a small size (<1,000 nm for microvesices and <100 nm for exosomes) which favors the migration from the site of discharge and allows the communication between distant cells (Mulcahy et al., 2014). In the brain, EVs released from the surface of reactive microglia provoke glial activation due to the induction of an inflammatory reaction in target glial cells, both microglia and astrocytes in an autocrine and paracrine manner, respectively (Antonucci et al., 2012). The inflammatory reaction induced by microglial cells-derived EVs is related to their ability of transferring mRNAs encoding inflammatory cytokines such as IL-1β (Prada I. et al., 2013). However, little or no uptake of EVs or exosomes occurs in astrocytes, and there are no evidences for microglia to astrocyte transfer of nucleic acids through EVs. It should be noted that EVs production is not exclusively restricted to microglial cells because, as it will be detailed below, the RPE is proactive secreting EVs.

Recent investigations have described that EVs from the aqueous humor of patients with AMD present specific proteins suggesting that EVs could be used as predictor biomarkers of this retinal disease (Biasutto et al., 2013; Kang et al., 2014; Tong et al., 2016). In another study, the molecular processes associated to aged RPE have revealed increased in autophagy and exocytotic activity that was associated with the presence of autophagic EVs markers (Atienzar-Aroca et al., 2016; Kannan et al., 2016). In this regard, it has been demonstrated that RPE cells release EVs under oxidative stress environment and this is accompanied with a high expression of VEGFR in their membrane and increased VEGFR mRNA cargo (Wang et al., 2009). Moreover, EVs can transport proteins related with essential signaling pathways such as mitogen-activated protein kinase (MAPK), nuclear factor kB (NFKB), and protein kinase B (AKT), as well as miRNAs (i.e., miR-294 or miR-302) into retinal microvascular endothelial cells (Tong et al., 2016). These miRNAs and proteins play important roles in processes associated with cell proliferation and, taking this into account, it has been suggested that RPE cells-derived EVs could contribute to the development of CVN (Tong et al., 2016). Beside the active role of RPE cells in exosomes secretion, the Hajrasouliha's study has revealed that exosomes from retinal astrocytes from non-pathologic mouse contain several antiangiogenic factors (PEDF and endostatin) that block the development of CNV in a laser-induced mouse model by targeting both macrophages and vascular endothelial cells (Hajrasouliha et al., 2013). Altogether, these results strongly suggest that, in the eye, exosomes derived from different ocular cells may play an important role for modulating the balance of anti- and pro-angiogenesis and the integrity of vision function.

EVs have also been involved in photoreceptor cells of the vertebrate retina which are continuously renewed by the addition of membranes at the base of the outer segments (OS) and removal of the older discs form the distal end (Besharse et al., 1977). Studies in retinas of other species such as *Xenopus* have shown that OS-bound proteins are continuously sorted and trafficked from the endoplasmic reticulum and trans-Golgi network as cargo in EVs from the inner segment toward the OS (Papermaster et al., 1985). The existence of interactions between EVs cargo proteins and proteins involved in the transport machinery is supported by several studies in animal models demonstrating that a defect in only one of these proteins can lead to a total loss of polarity in the protein trafficking resulting in photoreceptor death (Hagstrom et al., 1999; Deretic, 2006).

Actually, there are no studies in RP retinal degeneration disease linked to EVs secretion. Only a recent study which analyzes RP in combination with hearing loss describes a novel syndrome caused by biallelic mutations in the "exosome component 2" (*EXOSC2*) gene (Giunta et al., 2016). This study was performed in three patients from two German families without any relationship that were affected by a Mendelian disorder characterized by a progressive sensorineural hearing loss, childhood myopia, early onset RP, short stature, hypothyroidism, premature aging, recognizable facial gestalt, and mild intellectual disability. The exome sequencing identified homozygous or heterozygous missense variants in the *EXOSC2* gene in all three patients. *EXOSC2* encodes the "ribosomal RNA-processing protein 4" (RRP4), one of the core components of the RNA exosome. The phenotype associated to EXOSC2 was characterized by only a minimal overlap with previously reported diseases associated with mutations in the RNA exosome core component genes *EXOSC3* and *EXOSC8* (Giunta et al., 2016). The clinical consequences of altered RNA exosome function during RP is a novel condition which deserves further studies.

Although the role of EVs in the IGF-1 system in the retina is still unknown, it has been recently reported that mesenchymal stem cells-derived exosomes activate several signaling pathways which are involved in wound healing (AKT, MAPK, and STAT3) and are able to induce the expression of IGF-1 among other growth factors (Shabbir et al., 2015). In another study, exosomes derived from cardiomyocytes of a type 2 diabetic rat GK (GK-exosomes) inhibited mouse cardiac endothelial cells proliferation, migration and tube-like formation, whereas all these parameters were promoted by exosomes from non-diabetic rats (WT-exosomes) (Wang et al., 2014). Mechanistically, GK-exosomes encapsulated higher levels of miR-320 which functionally down-regulated target genes in mouse cardiac endothelial cells, one of which was IGF-1. Altogether these results conclude that in an experimental model of T2DM cardiomyocytes have an antiangiogenic function which is mediated by IGF-1 and involves the participation of exosomes in the transference of the miR-320 into endothelial cells. More research will be necessary to unravel cellular interactions via EVs within the retina and the processes modulated by IGF-1.

EMERGING INSIGHTS ON THE ROLE OF IGF-1 IN TARGETING INFLAMMATION IN RETINAL DISEASES

It has been reported that in the brain the antiinflammatory effects of some compounds rely in their capability to guide the polarization of microglia toward an antiinflammatory phenotype (Chio et al., 2015). In animal models of several diseases associated to inflammation, including neurodegenerative diseases, it has been described how microglia changes the polarization state under treatment with omega-3 polyunsaturated fatty acids by decreasing the production of neurotoxic and proinflammatory

molecules (Calviello et al., 2013; Serini and Calviello, 2016). Based on that, activation of microglial cells toward the antiinflammatory response and the subsequent reduction of proinflammatory cytokines are promising approaches for retinal neuroprotection in several models of retinal degeneration such as AMD, DR, and RP. In fact, several compounds from distinct origins elicit protective effects against inflammation, ischemia, light-, oxygen-, and age-associated pathologies of the neural retina in animal models (SanGiovanni and Chew, 2005; Tuo et al., 2009; Dornstauder et al., 2012) by their ability to resolve inflammation; all this effects being essential to avoid the progression of these retinal diseases. Not only the anti-inflammatory microglia is directly responsible of the protection against neuroinflammation since it has been described that microglia can elicit indirect effects. In this regard and, as stated above, we have demonstrated the requirement of the presence of microglia for the neuroprotective effect of IGF-1 in a mouse model of RP (Arroba et al., 2011).

AMD progression concurs with chronic and pathophysiological low-grade inflammation associated with a high cellular metabolism which contributes to generate ROS, oxidized lipoproteins, advanced glycation end-products, and apoptotic cells (Xu et al., 2009). Interestingly, in early AMD, microglia acts mainly with scavenger and antiinflammatory properties. However, in late AMD in diabetic rats, microglia promotes the angiogenic activity by increasing the expression of VEGF among different growth factors as well as ROS (Ma et al., 2007).

Recently, Cotter's laboratory and ours have reported that different kind of compounds (progesterone, sp^2-iminosugar dodecylsulfoxide or chemical inhibitors of protein tyrosine phosphatase 1B) are able to act on microglial cells to reduce the proinflammatory milieu by decreasing TNF-α, IL-1β, and iNOS levels and stimulate the antiinflammatory phenotypes by increasing CD206/MRC1 and arginase-1 in mouse models of models of RP (*rd10*) or DR (*db/db*), respectively (Arroba et al., 2016a; Roche et al., 2016). Regarding DR, the treatment of retinal explants from *db/db* mice with the sp^2-iminosugar derivative compound R-DS-ONJ ameliorated the reactive gliosis already detected in those retinas, as well as increased the antiinflammatory marker arginase-1, thereby reflecting a regression toward an early stage of DR (Arroba et al., 2016a). In this study, we also achieved the mechanism of action of the R-DS-ONJ compound by performing *in vitro* cell-based approaches aimed to mimic the proinflammatory environment associated to DR. By using Bv.2 mouse microglia cells stimulated with LPS, a proinflammatory stimulus that resembles the *in vivo* situation in *db/db* mice in the course of DR, we found

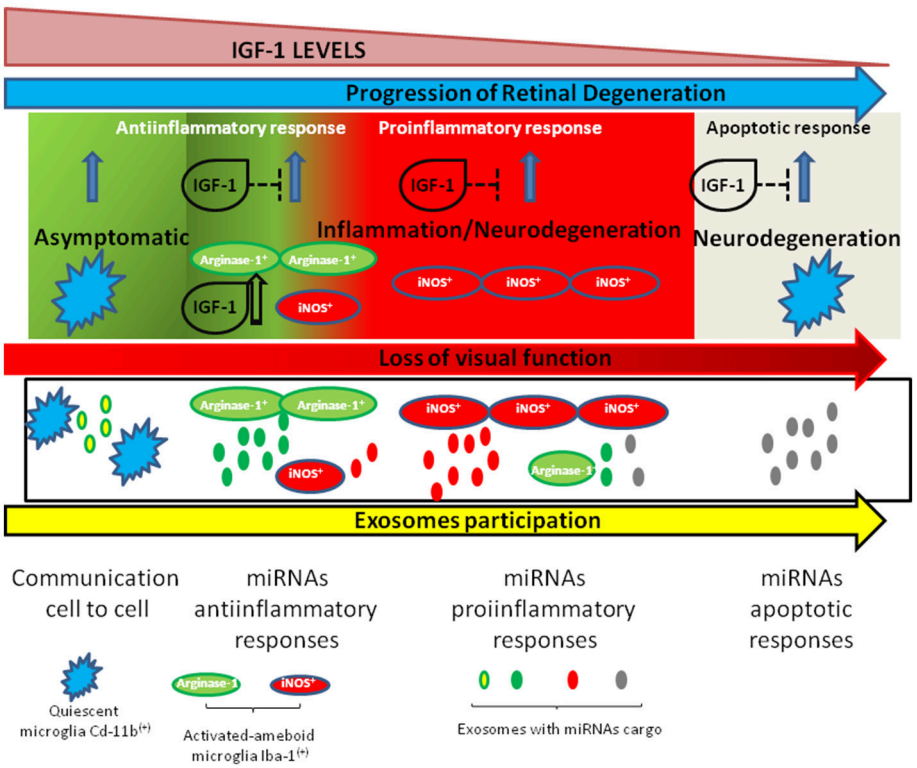


FIGURE 1 | Schematic representation of changes in microglia during retinal neurodegeneration and the role of IGF-1 in this process. IGF-1 levels decrease along aging and in some neurodegenerative diseases and this is associated to a switch in microglia polarization toward a proinflammatory status. In this context, exosomes participate into cell-to-cell communications as vehicles which transport anti- or proinflammatory miRNAS.

that the co-treatment of LPS with IL4/IL3 (M2 cytokines) or the sp^2-iminosugar dodecylsulfoxide *R*-DS-ONJ ameliorated the microglial proinflammatory phenotype induced by endotoxemia, as reflected by marked decreases in the levels of nitrites, iNOS mRNA and protein expression as well as by reductions in mRNAs encoding proinflammatory cytokines. At the molecular level, a cocktail of antiinflammatory cytokines or *R*-DS-ONJ reduced LPS-mediated activation of stress kinases (JNK and p38 MAPK) and prevented the degradation of IkBα and the nuclear translocation of the proinflammatory transcription factor NFκB. These two studies have provided new insights on targeting neuroinflammation in the retina by potentiating the polarization state of microglia that might be a promising therapeutic strategy to delay and/or prevent the deterioration of visual function in patients. This interesting issue deserves further research.

Although a direct role of IGF-1 as a potential therapy for targeting the polarization of microglia in the retina has not been reported, there are indirect evidences of its involvement in the dynamics of microglia polarization stages. For instance, mitochondrial toxins inhibited part of the IL-4-induced alternative activation in primary cultures of mouse microglia including the induction of arginase-1 and IGF-1 and, therefore, the counteraction of the LPS induced cytokine release was abolished (Ferger et al., 2010). Also, it is well known that IGF-1 actions are slowed during aging (Lee et al., 2013) and, as we have mentioned above, under this condition the deficiency of *Igf1* in mice reflects an unbalanced anti-inflammatory vs. proinflammatory response in the retina that parallels the retinal degeneration (Arroba et al., 2016a). In addition, new concepts on the modulation of microglial polarization by mitochondrial metabolism have emerged (Orihuela et al., 2016) and in this regard IGF-1-mediated metabolic actions may also play a key role. Future research in the field of neuroinflammation will provide new insights on this important issue.

CONCLUDING REMARKS

The progression of retinal degeneration is mediated by the dynamics of microglia polarization in the early steps of almost all retinal diseases. In this context, and as schematized in **Figure 1**, in the retina the immune responses must be tightly controlled since they are responsible for a better or worse prognostic of diseases such as DME, RP, or DR. Thus, targeting neuroinflammation through the IGF-1/IGF-1R system and/or additional pharmacological strategies might conduct the regression of retinal degeneration and represents a challenging therapeutic strategy.

AUTHOR CONTRIBUTIONS

AIA researched data and wrote and reviewed the manuscript. ÁMV provided funding, wrote and reviewed the manuscript. AC-C and MA-D reviewed the manuscript. ÁMV and AIA are responsible for the integrity of the work as a whole.

ACKNOWLEDGMENTS

We acknowledge I. Varela-Nieto, R. Simó, C. Hernández, E. Beltramo, A. Mazzeo, M. Porta, E. M. Sánchez-Fernández, C. Ortiz Mellet, J. M. García Fernández and L. Masgrau for their input and scientific collaboration. The work of AIA and ÁMV has been funded by grants from European Union (project H2020-MSCA-ITN TREATMENT Grant Agreement number: 721236 and project EUROCONDOR FP7 Grant Agreement number 278040), grant from the Spanish Ministry of Economy and Competitiveness: SAF2015-65267-R (MINECO/FEDER) and grants from the Spanish ISCIII (CIBERdem) and INFLAMES (ISCIII PIE14/00045, co-funded by ERDF, Investing in your future).

REFERENCES

Alarcon, C., Morales, A. V., Pimentel, B., Serna, J., and de Pablo, F. (1998). (Pro)insulin and insulin-like growth factor I complementary expression and roles in early development. *Compar. Biochem. Physiol. B Biochem. Mol. Biol.* 121, 13–17. doi: 10.1016/S0305-0491(98)10105-0

Antonucci, F., Turola, E., Riganti, L., Caleo, M., Gabrielli, M., Perrotta, C., et al. (2012). Microvesicles released from microglia stimulate synaptic activity via enhanced sphingolipid metabolism. *EMBO J.* 31, 1231–1240. doi: 10.1038/emboj.2011.489

Arroba, A. I., Alcalde-Estevez, E., Garcia-Ramirez, M., Cazzoni, D., de la Villa, P., Sanchez-Fernandez, E. M., et al. (2016a). Modulation of microglia polarization dynamics during diabetic retinopathy in db/db mice. *Biochim. Biophys. Acta* 1862, 1663–1674. doi: 10.1016/j.bbadis.2016.05.024

Arroba, A. I., Alvarez-Lindo, N., van Rooijen, N., and de la Rosa, E. J. (2011). Microglia-mediated IGF-I neuroprotection in the rd10 mouse model of retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 52, 9124–9130. doi: 10.1167/iovs.11-7736

Arroba, A. I., Alvarez-Lindo, N., van Rooijen, N., and de la Rosa, E. J. (2014). Microglia-Muller glia crosstalk in the rd10 mouse model of retinitis pigmentosa. *Adv. Exp. Med. Biol.* 801, 373–379. doi: 10.1007/978-1-4614-3209-8_47

Arroba, A. I., and Valverde, A. M. (2017). Modulation of microglia in the retina: new insights into diabetic retinopathy. *Acta Diabetol.* 54, 527–533. doi: 10.1007/s00592-017-0984-z

Arroba, A. I., Mazzeo, A., Cazzoni, D., Beltramo, E., Hernandez, C., Porta, M., et al. (2016b). Somatostatin protects photoreceptor cells against high glucose-induced apoptosis. *Mol. Vis.* 22, 1522–1531.

Arroba, A. I., Rodriguez-de la Rosa, L., Murillo-Cuesta, S., Vaquero-Villanueva, L., Hurle, J. M., Varela-Nieto, I., et al. (2016c). Autophagy resolves early retinal inflammation in Igf1-deficient mice. *Dis. Model. Mech.* 9, 965–974. doi: 10.1242/dmm.026344

Arroba, A. I., Wallace, D., Mackey, A., E. J., de la Rosa, and Cotter, T. G. (2009). IGF-I maintains calpastatin expression and attenuates apoptosis in several models of photoreceptor cell death. *Eur. J. Neurosci.* 30, 975–986. doi: 10.1111/j.1460-9568.2009.06902.x

Atienzar-Aroca, S., Flores-Bellver, M., Serrano-Heras, G., Martinez-Gil, N., Barcia, J. M., Aparicio, S., et al. (2016). Oxidative stress in retinal pigment epithelium cells increases exosome secretion and promotes angiogenesis in endothelial cells. *J. Cell. Mol. Med.* 20, 1457–1466. doi: 10.1111/jcmm.12834

Barilli, A., Rotoli, B. M., Visigalli, R., and and, V., Dall'Asta (2014). Gliadin activates arginase pathway in RAW264.7 cells and in human monocytes. *Biochim. Biophys. Acta* 1842, 1364–1371. doi: 10.1016/j.bbadis.2014.04.021

Bates, A. S., Evans, A. J., Jones, P., and Clayton, R. N. (1995). Assessment of GH status in adults with GH deficiency using serum growth hormone, serum

insulin-like growth factor-I and urinary growth hormone excretion. *Clin. Endocrinol.* 42, 425–430. doi: 10.1111/j.1365-2265.1995.tb02652.x

Besharse, J. C., Hollyfield, J. G., and Rayborn, M. E. (1977). Turnover of rod photoreceptor outer segments. II. Membrane addition and loss in relationship to light. *J. Cell Biol.* 75, 507–527.

Biasutto, L., Chiechi, A., Couch, R., Liotta, L. A., and Espina, V. (2013). Retinal pigment epithelium (RPE) exosomes contain signaling phosphoproteins affected by oxidative stress. *Exp. Cell Res.* 319, 2113–2123. doi: 10.1016/j.yexcr.2013.05.005

Bluthe, R. M., Kelley, K. W., and Dantzer, R. (2006). Effects of insulin-like growth factor-I on cytokine-induced sickness behavior in mice. *Brain Behav. Immun.* 20, 57–63. doi: 10.1016/j.bbi.2005.02.003

Bogdanov, P., Corraliza, L., Villena, J. A., Carvalho, A. R., Garcia-Arumi, J., Ramos, D., et al. (2014). The db/db mouse: a useful model for the study of diabetic retinal neurodegeneration. *PLoS ONE* 9:e97302. doi: 10.1371/journal.pone.0097302

Calviello, G., Su, H. M., Weylandt, K. H., Fasano, E., Serini, S., and Cittadini, A. (2013). Experimental evidence of omega-3 polyunsaturated fatty acid modulation of inflammatory cytokines and bioactive lipid mediators: their potential role in inflammatory, neurodegenerative, and neoplastic diseases. *Biomed Res. Int.* 2013:743171. doi: 10.1155/2013/743171

Campochiaro, P. A., and Mir, T. A. (2018). The mechanism of cone cell death in Retinitis Pigmentosa. *Prog. Retin. Eye Res.* 62, 24–37. doi: 10.1016/j.preteyeres.2017.08.004

Carro, E., Trejo, J. L., Nunez, A., and Torres-Aleman, I. (2003). Brain repair and neuroprotection by serum insulin-like growth factor I. *Mol. Neurobiol.* 27, 153–162. doi: 10.1385/MN:27:2:153

Cha, D. M., Woo, S. J., Kim, H. J., Lee, C., and Park, K. H. (2013). Comparative analysis of aqueous humor cytokine levels between patients with exudative age-related macular degeneration and normal controls. *Invest. Ophthalmol. Vis. Sci.* 54, 7038–7044. doi: 10.1167/iovs.13-12730

Chang, D. T., Colton, E., and Anderson, J. M. (2009). Paracrine and juxtacrine lymphocyte enhancement of adherent macrophage and foreign body giant cell activation. *J. Biomed. Mater. Res. A* 89, 490–498. doi: 10.1002/jbm.a.31981

Chang, G. Q., Hao, Y., and Wong, F. (1993). Apoptosis: final common pathway of photoreceptor death in rd, rds, and rhodopsin mutant mice. *Neuron* 11, 595–605. doi: 10.1016/0896-6273(93)90072-Y

Chen, Q., Qiu, F., Zhou, K., Matlock, H. G., Takahashi, Y. V., Rajala, R. V. S., et al. (2017). Pathogenic Role of microRNA-21 in diabetic retinopathy through downregulation of PPARalpha. *Diabetes* 66, 1671–1682. doi: 10.2337/db16-1246

Cheng, B., Liu, Y., Liu, X., Ge, J., Ling, Y., and Zheng, X. (2002). [Macular image changes of optical coherence tomography after phacoemulsification]. *[Zhonghua yan ke za zhi] Chin. J. Ophthalmol.* 38, 265–267.

Cherry, J. D., Olschowka, J. A., and O'Banion, M. K. (2014). Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. *J. Neuroinflamm.* 11:98. doi: 10.1186/1742-2094-11-98

Chio, C. C., Lin, M. T., and Chang, C. P. (2015). Microglial activation as a compelling target for treating acute traumatic brain injury. *Curr. Med. Chem.* 22, 759–770. doi: 10.2174/0929867321666141106124657

Chiu, C. J., Conley, Y. P., Gorin, M. B., Gensler, G., Lai, C. Q., Shang, F., et al. (2011). Associations between genetic polymorphisms of insulin-like growth factor axis genes and risk for age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* 52, 9099–9107. doi: 10.1167/iovs.11-7782

Colombo, M., Raposo, G., and Thery, C. (2014). Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell Dev. Biol.* 30, 255–289. doi: 10.1146/annurev-cellbio-101512-122326

Corrochano, S., Barhoum, R., Boya, P., Arroba, A. I., Rodriguez-Muela, N., Gomez-Vicente, V., et al. de la Rosa (2008). Attenuation of vision loss and delay in apoptosis of photoreceptors induced by proinsulin in a mouse model of retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 49, 4188–4194. doi: 10.1167/iovs.08-2182

Cuenca, N., Fernandez-Sanchez, L., Campello, L., Maneu, V., De la Villa, P., Lax, P., et al. (2014). Cellular responses following retinal injuries and therapeutic approaches for neurodegenerative diseases. *Prog. Retin. Eye Res.* 43, 17–75. doi: 10.1016/j.preteyeres.2014.07.001

Deretic, D. (2006). A role for rhodopsin in a signal transduction cascade that regulates membrane trafficking and photoreceptor polarity. *Vision Res.* 46, 4427–4433. doi: 10.1016/j.visres.2006.07.028

Dias, M. F., Joo, K., Kemp, J. A., Fialho, S. L., da Silva Cunha, A. Jr., Woo, S. J., et al. (2018). Molecular genetics and emerging therapies for retinitis pigmentosa: basic research and clinical perspectives. *Prog. Retin. Eye Res.* 63, 107–131. doi: 10.1016/j.preteyeres.2017.10.004

Dick, A. D. (2017). Doyne lecture 2016: intraocular health and the many faces of inflammation. *Eye* 31, 87–96. doi: 10.1038/eye.2016.177

Dong, N., Chang, L., Wang, B., and Chu, L. (2014). Retinal neuronal MCP-1 induced by AGEs stimulates TNF-alpha expression in rat microglia via p38, ERK, and NF-kappaB pathways. *Mol. Vis.* 20, 616–628.

Dornstauder, B., Suh, M., Kuny, S., Gaillard, F., Macdonald, I. M., Clandinin, M. T., et al. (2012). Dietary docosahexaenoic acid supplementation prevents age-related functional losses and A2E accumulation in the retina. *Invest. Ophthalmol. Vis. Sci.* 53, 2256–2265. doi: 10.1167/iovs.11-8569

Du, M., Phelps, E., Balangue, M. J., Dockins, A., Moiseyev, G., Shin, Y., et al. (2017). Transgenic Mice Over-Expressing RBP4 Have RBP4-Dependent and Light-Independent Retinal Degeneration. *Invest. Ophthalmol. Vis. Sci.* 58, 4375–4383. doi: 10.1167/iovs.17-22107

Eandi, C. M., Alovisi, C., De Sanctis, U., and Grignolo, F. M. (2016). Treatment for neovascular age related macular degeneration: the state of the art. *Eur. J. Pharmacol.* 787, 78–83. doi: 10.1016/j.ejphar.2016.03.002

Edwards, C. M., Mueller, G., Roelofs, A. J., Chantry, A., Perry, M., Russell, R. G., et al. (2007). Apomine, an inhibitor of HMG-CoA-reductase, promotes apoptosis of myeloma cells *in vitro* and is associated with a modulation of myeloma *in vivo*. *Int. J. Cancer* 120, 1657–1663. doi: 10.1002/ijc.22478

Eshaq, R. S., Aldalati, M., Z., Alexander, J. S., and Harris, N. R. (2017). Diabetic retinopathy: Breaking the barrier. *Pathophysiology* 24, 229–241. doi: 10.1016/j.pathophys.2017.07.001

Ferger, A. I., Campanelli, L., Reimer, V., Muth, K. N., Merdian, I., Ludolph, A. C., et al. (2010). Effects of mitochondrial dysfunction on the immunological properties of microglia. *J. Neuroinflam.* 7:45. doi: 10.1186/1742-2094-7-45

Feve, B., and Bastard, J. P. (2009). The role of interleukins in insulin resistance and type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* 5, 305–311. doi: 10.1038/nrendo.2009.62

Forrester, J. V., and Xu, H. (2012). Good news-bad news: the Yin and Yang of immune privilege in the eye. *Front. Immunol.* 3:338. doi: 10.3389/fimmu.2012.00338

Frade, J. M., Marti, E., Bovolenta, P., Rodriguez-Pena, M. A., Perez-Garcia, D., Rohrer, H., et al. (1996). Insulin-like growth factor-I stimulates neurogenesis in chick retina by regulating expression of the alpha 6 integrin subunit. *Development* 122, 2497–2506.

Funk, M., Karl, D., Georgopoulos, M., Benesch, T., Sacu, S., Polak, K., et al. (2009). Neovascular age-related macular degeneration: intraocular cytokines and growth factors and the influence of therapy with ranibizumab. *Ophthalmology* 116, 2393–2399. doi: 10.1016/j.ophtha.2009.05.039

Ginhoux, F., Lim, S., Hoeffel, G., Low, D., and Huber, T. (2013). Origin and differentiation of microglia. *Front. Cell. Neurosci.* 7:45. doi: 10.3389/fncel.2013.00045

Giunta, M., Edvardson, S., Xu, Y., Schuelke, M., Gomez-Duran, A., Boczonadi, V., et al. (2016). Altered RNA metabolism due to a homozygous RBM7 mutation in a patient with spinal motor neuropathy. *Hum. Mol. Genet.* 25, 2985–2996. doi: 10.1093/hmg/ddw149

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., and Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell* 140, 918–934. doi: 10.1016/j.cell.2010.02.016

Glybina, I. V., Kennedy, A., Ashton, P., Abrams, G. W., and Iezzi, R. (2010). Intravitreous delivery of the corticosteroid fluocinolone acetonide attenuates retinal degeneration in S334ter-4 rats. *Invest. Ophthalmol. Vis. Sci.* 51, 4243–4252. doi: 10.1167/iovs.09-4492

Gonzalez, H., Elgueta, D., Montoya, A., and Pacheco, R. (2014). Neuroimmune regulation of microglial activity involved in neuroinflammation and neurodegenerative diseases. *J. Neuroimmunol.* 274, 1–13. doi: 10.1016/j.jneuroim.2014.07.012

Gordon, N., Mullen, C. A., Tran, H., Worth, L., Gomez Almaguer, D., and Chan, K. W. (2003). Fludarabine and once-daily intravenous busulfan for allogeneic bone marrow transplantation for Chediak-Higashi syndrome. *J. Pediatr. Hematol. Oncol.* 25, 824–826. doi: 10.1097/00043426-200310000-00019

Gupta, N., Brown, K. E., and Milam, A. H. (2003). Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. *Exp. Eye Res.* 76, 463–471. doi: 10.1016/S0014-4835(02)00332-9

Hageman, G. S., Luthert, P. J., Victor Chong, N. H., Johnson, L. V., Anderson, D. H., and Mullins, R. F. (2001). An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog. Retin. Eye Res.* 20, 705–732. doi: 10.1016/S1350-9462(01)00010-6

Hagstrom, S. A., Duyao, M., North, M. A., and Li, T. (1999). Retinal degeneration in tulp1$^{-/-}$ mice: vesicular accumulation in the interphotoreceptor matrix. *Invest. Ophthalmol. Vis. Sci.* 40, 2795–2802.

Hajrasouliha, A. R., Jiang, G., Lu, Q., Lu, H., Kaplan, H. J., Zhang, H. G., et al. (2013). Exosomes from retinal astrocytes contain antiangiogenic components that inhibit laser-induced choroidal neovascularization. *J. Biol. Chem.* 288, 28058–28067. doi: 10.1074/jbc.M113.470765

Haurigot, V., Villacampa, P., Ribera, A., Llombart, C., Bosch, A., Nacher, V., et al. (2009). Increased intraocular insulin-like growth factor-I triggers blood-retinal barrier breakdown. *J. Biol. Chem.* 284, 22961–22969. doi: 10.1074/jbc.M109.014787

Heemskerk, V. H., Daemen, M. A., and Buurman, W. A. (1999). Insulin-like growth factor-1 (IGF-1) and growth hormone (GH) in immunity and inflammation. *Cytokine Growth Factor Rev.* 10, 5–14. doi: 10.1016/S1359-6101(98)00022-7

Hellstrom, A., Carlsson, B., Niklasson, A., Segnestam, K., Boguszewski, M., de Lacerda, L., et al. (2002). IGF-I is critical for normal vascularization of the human retina. *J. Clin. Endocrinol. Metab.* 87, 3413–3416. doi: 10.1210/jcem.87.7.8629

Hernandez, C., Bogdanov, P., Corraliza, L., Garcia-Ramirez, M., Sola-Adell, C., Arranz, J. A., et al. (2016). Topical administration of GLP-1 receptor agonists prevents retinal neurodegeneration in experimental diabetes. *Diabetes* 65, 172–187. doi: 10.2337/db15-0443.

Hernandez-Sanchez, C., Lopez-Carranza, A., Alarcon, C., de La Rosa, E. J., and de Pablo, F. (1995). Autocrine/paracrine role of insulin-related growth factors in neurogenesis: local expression and effects on cell proliferation and differentiation in retina. *Proc. Natl. Acad. Sci. U.S.A.* 92, 9834–9838. doi: 10.1073/pnas.92.21.9834

Hernandez-Zimbron, L. F., Zamora-Alvarado, R., Ochoa-De la Paz, L., Velez-Montoya, R., Zenteno, E., Gulias-Canizo, R., et al. (2018). Age-related macular degeneration: new paradigms for treatment and management of AMD. *Oxid. Med. Cell. Longev.* 2018:8374647. doi: 10.1155/2018/8374647

Herrera, A. J., Espinosa-Oliva, A. M., Carrillo-Jimenez, A., Oliva-Martin, M. J., Garcia-Revilla, J., Garcia-Quintanilla, A., et al. (2015). Relevance of chronic stress and the two faces of microglia in Parkinson's disease. *Front. Cell. Neurosci.* 9:312. doi: 10.3389/fncel.2015.00312

Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259, 87–91. doi: 10.1126/science.7678183

Jung, H. J., and Suh, Y. (2014). Regulation of IGF−1 signaling by microRNAs. *Front. Genet.* 5:472. doi: 10.3389/fgene.2014.00472

Kalani, A., Tyagi, A., and Tyagi, N. (2014). Exosomes: mediators of neurodegeneration, neuroprotection and therapeutics. *Mol. Neurobiol.* 49, 590–600. doi: 10.1007/s12035-013-8544-1

Kang, G. Y., Bang, J. Y., Choi, A. J., Yoon, J., Lee, W. C., Choi, S., et al. (2014). Exosomal proteins in the aqueous humor as novel biomarkers in patients with neovascular age-related macular degeneration. *J. Proteome Res.* 13, 581–595. doi: 10.1021/pr400751k

Kannan, R., Sreekumar, P. G., and Hinton, D. R. (2016). Alpha crystallins in the retinal pigment epithelium and implications for the pathogenesis and treatment of age-related macular degeneration. *Biochim. Biophys. Acta* 1860, 258–268. doi: 10.1016/j.bbagen.2015.05.016

Karlstetter, M., Scholz, R., Rutar, M., Wong, W. T., Provis, J. M., and Langmann, T. (2015). Retinal microglia: just bystander or target for therapy? *Prog. Retin. Eye Res.* 45, 30–57. doi: 10.1016/j.preteyeres.2014.11.004

Kauppinen, A., Paterno, J. J., Blasiak, J., Salminen, A., and Kaarniranta, K. (2016). Inflammation and its role in age-related macular degeneration. *Cell. Mol. Life Sci.* 73, 1765–1786. doi: 10.1007/s00018-016-2147-8

Kermer, P., Klocker, N., Labes, M., and Bahr, M. (2000). Insulin-like growth factor-I protects axotomized rat retinal ganglion cells from secondary death via PI3-K-dependent Akt phosphorylation and inhibition of caspase-3 *in vivo*. *J. Neurosci.* 20, 2–8. doi: 10.1523/JNEUROSCI.20-02-00722.2000

Labandeira-Garcia, J. L., Costa-Besada, M. A., Labandeira, C. M., Villar-Cheda, B., and Rodriguez-Perez, A. I. (2017). Insulin-like growth factor-1 and neuroinflammation. *Front. Aging Neurosci.* 9:365. doi: 10.3389/fnagi.2017.00365

Lambooij, A. C., van Wely, K. H., Lindenbergh-Kortleve, D. J., Kuijpers, R. W., Kliffen, M., and Mooy, C. M. (2003). Insulin-like growth factor-I and its receptor in neovascular age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* 44, 2192–2198. doi: 10.1167/iovs.02-0410

Lavalette, S., Raoul, W., Houssier, M., Camelo, S., Levy, O., Calippe, B., et al. (2011). Interleukin-1beta inhibition prevents choroidal neovascularization and does not exacerbate photoreceptor degeneration. *Am. J. Pathol.* 178, 2416–2423. doi: 10.1016/j.ajpath.2011.01.013

Lee, J. E., Liang, K. J., Fariss, R. N., and Wong, W. T. (2008). *Ex vivo* dynamic imaging of retinal microglia using time-lapse confocal microscopy. *Invest. Ophthalmol. Vis. Sci.* 49, 4169–4176. doi: 10.1167/iovs.08-2076

Lee, M. J., Shin, D. H., Ko, K. I., Koo, H. M., Kim, C. H., Doh, F. M., et al. (2013). Association between the ratio of insulin-like growth factor-I to insulin-like growth factor binding protein-3 and inflammation in incident automated peritoneal dialysis patients. *Growth Horm. IGF Res.* 23, 170–174. doi: 10.1016/j.ghir.2013.06.004

Legacy, J., Hanea, S., Theoret, J., and Smith, P. D. (2013). Granulocyte macrophage colony-stimulating factor promotes regeneration of retinal ganglion cells *in vitro* through a mammalian target of rapamycin-dependent mechanism. *J. Neurosci. Res.* 91, 771–779. doi: 10.1002/jnr.23205

Li, L., Eter, N., and Heiduschka, P. (2015). The microglia in healthy and diseased retina. *Exp. Eye Res.* 136, 116–130. doi: 10.1016/j.exer.2015.04.020

Li, Q., and Puro, D. G. (2002). Diabetes-induced dysfunction of the glutamate transporter in retinal Muller cells. *Invest. Ophthalmol. Vis. Sci.* 43, 3109–3116.

Ma, H. J., Luo, Y., Wang, M., Liang, X. L., Huang, C. X., Li, T., et al. (2007). [Changes of tight junction protein and GFAP in the retina of experimental diabetic rats and their relationship with blood aqueous barrier]. *[Zhonghua yan ke za zhi] Chin. J. Ophthalmol.* 43, 397–401.

MacCormick, I. J., Czanner, G., and Faragher, B. (2015). Developing retinal biomarkers of neurological disease: an analytical perspective. *Biomark. Med.* 9, 691–701. doi: 10.2217/bmm.15.17

Machalinska, A., Safranow, K., Dziedziejko, V., Mozolewska-Piotrowska, K., Paczkowska, E., Klos, P., et al. (2011). Different populations of circulating endothelial cells in patients with age-related macular degeneration: a novel insight into pathogenesis. *Invest. Ophthalmol. Vis. Sci.* 52, 93–100. doi: 10.1167/iovs.10-5756

McKeever, P. M., Schneider, R., Taghdiri, F., Weichert, A., Multani, N., Brown, R. A., et al. (2018). MicroRNA expression levels are altered in the cerebrospinal fluid of patients with young-onset Alzheimer's disease. *Mol. Neurobiol.* doi: 10.1007/s12035-018-1032-x. [Epub ahead of print].

Mori, Y., Murakami, T., Suzuma, K., Ishihara, K., Yoshitake, S., Fujimoto, M., et al. (2017). Relation between macular morphology and treatment frequency during twelve months with ranibizumab for diabetic macular edema. *PLoS ONE* 12:e0175809. doi: 10.1371/journal.pone.0175809

Mulcahy, L. A., Pink, R. C., and Carter, D. R. (2014). Routes and mechanisms of extracellular vesicle uptake. *J. Extracel. Vesic.* 3:24641. doi: 10.3402/jev.v3.24641

Nakano, I., Garnier, D., Minata, M., and Rak, J. (2015). Extracellular vesicles in the biology of brain tumour stem cells–Implications for inter-cellular communication, therapy and biomarker development. *Semin. Cell Dev. Biol.* 40, 17–26. doi: 10.1016/j.semcdb.2015.02.011

Narayan, D. S., Wood, J. P., Chidlow, G., and Casson, R. J. (2016). A review of the mechanisms of cone degeneration in retinitis pigmentosa. *Acta ophthalmologica* 94, 748–754. doi: 10.1111/aos.13141

Nayak, D., Roth, T. L., and McGavern, D. B. (2014). Microglia development and function. *Annu. Rev. Immunol.* 32, 367–402. doi: 10.1146/annurev-immunol-032713-120240

Nazari, H., Zhang, L., Zhu, D., Chader, G. J., Falabella, P., Stefanini, F., et al. (2015). Stem cell based therapies for age-related macular degeneration: the promises and the challenges. *Prog. Retin. Eye Res.* 48, 1–39. doi: 10.1016/j.preteyeres.2015.06.004

Nentwich, M. M., and Ulbig, M. W. (2015). Diabetic retinopathy - ocular complications of diabetes mellitus. *World J. Diabetes* 6, 489–499. doi: 10.4239/wjd.v6.i3.489

Netchine, I., Azzi, S., Houang, M., Seurin, D., Perin, L., Ricort, J. M., et al. (2009). Partial primary deficiency of insulin-like growth factor (IGF)-I activity associated with IGF1 mutation demonstrates its critical role in growth and brain development. *J. Clin. Endocrinol. Metab.* 94, 3913–3921. doi: 10.1210/jc.2009-0452

Nigro, A., Colombo, F., Casella, G., Finardi, A., Verderio, C., and Furlan, R. (2016). Myeloid extracellular vesicles: messengers from the demented brain. *Front. Immunol.* 7:17. doi: 10.3389/fimmu.2016.00017

Nishikawa, S., Kunikata, H., Aizawa, N., and Nakazawa, T. (2017). Bullous exudative retinal detachment after retinal pattern scan laser photocoagulation in diabetic retinopathy. *Case Rep. Ophthalmol.* 8, 475–481. doi: 10.1159/000480723

Noailles, A., Fernandez-Sanchez, L., Lax, P., and Cuenca, N. (2014). Microglia activation in a model of retinal degeneration and TUDCA neuroprotective effects. *J. Neuroinflammation* 11:186. doi: 10.1186/s12974-014-0186-3

Noailles, A., Maneu, V., Campello, L., Gomez-Vicente, V., Lax, P., and Cuenca, N. (2016). Persistent inflammatory state after photoreceptor loss in an animal model of retinal degeneration. *Sci. Rep.* 6:33356. doi: 10.1038/srep33356

Oellers, P., and Mahmoud, T. H. (2016). Surgery for proliferative diabetic retinopathy: new tips and tricks. *J. Ophthalmic Vis. Res.* 11, 93–99. doi: 10.4103/2008-322X.180697

Orihuela, R., McPherson, C. A., and Harry, G. J. (2016). Microglial M1/M2 polarization and metabolic states. *Br. J. Pharmacol.* 173, 649–665. doi: 10.1111/bph.13139

Papermaster, D. S., Schneider, B. G., and Besharse, J. C. (1985). Vesicular transport of newly synthesized opsin from the Golgi apparatus toward the rod outer segment. Ultrastructural immunocytochemical and autoradiographic evidence in *Xenopus retinas*. *Invest. Ophthalmol. Vis. Sci.* 26, 1386–1404.

Penfold, P. L., Madigan, M. C., Gillies, M. C., and Provis, J. M. (2001). Immunological and aetiological aspects of macular degeneration. *Prog. Retin. Eye Res.* 20, 385–414. doi: 10.1016/S1350-9462(00)00025-2

Penfold, P. L., Provis, J. M., and Billson, F. A. (1987). Age-related macular degeneration: ultrastructural studies of the relationship of leucocytes to angiogenesis. *Graefes Arch. Clin. Exp. Ophthalmol.* 225, 70–76. doi: 10.1007/BF02155808

Peng, B., Xiao, J., Wang, K., So, K. F., Tipoe, G. L., and Lin, B. (2014). Suppression of microglial activation is neuroprotective in a mouse model of human retinitis pigmentosa. *J. Neurosci.* 34, 8139–8150. doi: 10.1523/JNEUROSCI.5200-13.2014

Prada, C. E., Jousma, E., Rizvi, T. A., Wu, J., Dunn, R. S., Mayes, D. A., et al. (2013). Neurofibroma-associated macrophages play roles in tumor growth and response to pharmacological inhibition. *Acta Neuropathol.* 125, 159–168. doi: 10.1007/s00401-012-1056-7

Prada, I., Furlan, R., Matteoli, M., and Verderio, C. (2013). Classical and unconventional pathways of vesicular release in microglia. *Glia* 61, 1003–1017. doi: 10.1002/glia.22497

Ramirez, A. I., de Hoz, R., Salobrar-Garcia, E., Salazar, J. J., Rojas, B., Ajoy, D., et al. (2017). The Role of Microglia in Retinal Neurodegeneration: Alzheimer's Disease, Parkinson, and Glaucoma. *Front. Aging Neurosci.* 9:214. doi: 10.3389/fnagi.2017.00214

Roche, S. L., Wyse-Jackson, A. C., Gomez-Vicente, V., Lax, P., Ruiz-Lopez, A. M., Byrne, A. M., et al. (2016). Progesterone attenuates microglial-driven retinal degeneration and stimulates protective fractalkine-CX3CR1 signaling. *PLoS ONE* 11:e016 5197. doi: 10.1371/journal.pone.0165197

Rodriguez-de la Rosa, L., Fernandez-Sanchez, L., Germain, F., Murillo-Cuesta, S., Varela-Nieto, I., de la Villa, P., et al. (2012). Age-related functional and structural retinal modifications in the Igf1$^{-/-}$ null mouse. *Neurobiol. Dis.* 46, 476–485. doi: 10.1016/j.nbd.2012.02.013

Sancho-Pelluz, J., Arango-Gonzalez, B., Kustermann, S., Romero, F. J., van Veen, T., Zrenner, E., et al. (2008). Photoreceptor cell death mechanisms in inherited retinal degeneration. *Mol. Neurobiol.* 38, 253–269. doi: 10.1007/s12035-008-8045-9

SanGiovanni, J. P., and Chew, E. Y. (2005). The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog. Retin. Eye Res.* 24, 87–138. doi: 10.1016/j.preteyeres.2004.06.002

Sappington, R. M., Chan, M., and Calkins, D. J. (2006). Interleukin-6 protects retinal ganglion cells from pressure-induced death. *Invest. Ophthalmol. Vis. Sci.* 47, 2932–2942. doi: 10.1167/iovs.05-1407

Schneider, A., and Simons, M. (2013). Exosomes: vesicular carriers for intercellular communication in neurodegenerative disorders. *Cell Tissue Res.* 352, 33–47. doi: 10.1007/s00441-012-1428-2

Semeraro, F., Russo, A., Delcassi, L., Romano, M. R., Rinaldi, M., Chiosi, F., et al. (2015). Treatment of exudative age-related macular degeneration with ranibizumab combined with ketorolac eyedrops or photodynamic therapy. *Retina* 35, 1547–1554. doi: 10.1097/IAE.0000000000000525

Serini, S., and Calviello, G. (2016). Reduction of Oxidative/nitrosative stress in brain and its involvement in the neuroprotective effect of n-3 PUFA in Alzheimer's disease. *Curr. Alzheimer Res.* 13, 123–134. doi: 10.2174/1567205012666150921101147

Shabbir, A., Cox, A., Rodriguez-Menocal, L., Salgado, M., and Van Badiavas, E. (2015). Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis *in vitro*. *Stem Cells Dev.* 24, 1635–1647. doi: 10.1089/scd.2014.0316

Sharma, P., Ludwig, S., Muller, L., Hong, C. S., Kirkwood, J. M., Ferrone, S., et al. (2018). Immunoaffinity-based isolation of melanoma cell-derived exosomes from plasma of patients with melanoma. *J. Extracell. Vesic.* 7:1435138. doi: 10.1080/20013078.2018.1435138

Shaw, P. X., Sang, A., Wang, Y., Ho, D., Douglas, C., Dia, L., et al. (2017). Topical administration of a Rock/Net inhibitor promotes retinal ganglion cell survival and axon regeneration after optic nerve injury. *Exp. Eye Res.* 158, 33–42. doi: 10.1016/j.exer.2016.07.006

Shen, Y., Liu, K., and Xu, X. (2016). Correlation between visual function and photoreceptor integrity in diabetic macular edema: spectral-domain optical coherence tomography. *Curr. Eye Res.* 41, 391–399. doi: 10.3109/02713683.2015.1019003

Sica, A., and Mantovani, A. (2012). Macrophage plasticity and polarization: *in vivo* veritas. *J. Clin. Invest.* 122, 787–795. doi: 10.1172/JCI59643

Sohn, J. H., Kaplan, H. J., Suk, H. J., Bora, P. S., and Bora, N. S. (2000). Chronic low level complement activation within the eye is controlled by intraocular complement regulatory proteins. *Invest. Ophthalmol. Vis. Sci.* 41, 3492–3502.

Sokolovic, A., Rodriguez-Ortigosa, C. M., Bloemendaal, L. T., Oude Elferink, R. P., Prieto, J., and Bosma, P. J. (2013). Insulin-like growth factor 1 enhances bile-duct proliferation and fibrosis in Abcb4(-/-) mice. *Biochim. Biophys. Acta* 1832, 697–704. doi: 10.1016/j.bbadis.2013.02.005

Spadoni, I., Fornasa, G., and Rescigno, M. (2017). Organ-specific protection mediated by cooperation between vascular and epithelial barriers. *Nat. Rev. Immunol.* 17, 761–773. doi: 10.1038/nri.2017.100

Tong, Y., Zhou, Y. L., Wang, Y. X., Zhao, P. Q., and Wang, Z. Y. (2016). Retinal pigment epithelium cell-derived exosomes: possible relevance to CNV in wet-age related macular degeneration. *Med. Hypotheses* 97, 98–101. doi: 10.1016/j.mehy.2016.10.027

Tuo, J., Ross, R. J., Herzlich, A. A., Shen, D., Ding, X., Zhou, M., et al. (2009). A high omega-3 fatty acid diet reduces retinal lesions in a murine model of macular degeneration. *Am. J. Pathol.* 175, 799–807. doi: 10.2353/ajpath.2009.090089

van Norren, D., and Vos, J. J. (2016). Light damage to the retina: an historical approach. *Eye* 30, 169–172. doi: 10.1038/eye.2015.218

Varela-Nieto, I., Murillo-Cuesta, S., L., Rodriguez-de la Rosa, Lassatetta, L., and Contreras, J. (2013). IGF-I deficiency and hearing loss: molecular clues and clinical implications. *Pediatr. Endocrinol. Rev.* 10, 460–472.

Varnum, M. M., and Ikezu, T. (2012). The classification of microglial activation phenotypes on neurodegeneration and regeneration in Alzheimer's disease brain. *Arch. Immunol. Ther. Exp. (Warsz).* 60, 251–266. doi: 10.1007/s00005-012-0181-2

Verderio, C., Muzio, L., Turola, E., Bergami, A., Novellino, L., Ruffini, F., et al. (2012). Myeloid microvesicles are a marker and therapeutic target for neuroinflammation. *Ann. Neurol.* 72, 610–624. doi: 10.1002/ana.23627

Vestergaard, P. F., Hansen, M., Frystyk, J., Espelund, U., Christiansen, J. S., Jorgensen, J. O., et al. (2014). Serum levels of bioactive IGF1 and physiological

markers of ageing in healthy adults. *Eur. J. Endocrinol.* 170, 229–236. doi: 10.1530/EJE-13-0661

Villacampa, P., Ribera, A., Motas, S., Ramirez, L., Garcia, M., de la Villa, P., et al. (2013). Insulin-like growth factor I (IGF-I)-induced chronic gliosis and retinal stress lead to neurodegeneration in a mouse model of retinopathy. *J. Biol. Chem.* 288, 17631–17642. doi: 10.1074/jbc.M113.468819

Walenkamp, M. J., Karperien, M., Pereira, A. M., Hilhorst-Hofstee, Y., van Doorn, J., Chen, J. W., et al. (2005). Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. *J. Clin. Endocrinol. Metab.* 90, 2855–2864. doi: 10.1210/jc.2004-1254

Wan, J., Zhao, X. F., Vojtek, A., and Goldman, D. (2014). Retinal injury, growth factors, and cytokines converge on beta-catenin and pStat3 signaling to stimulate retina regeneration. *Cell Rep.* 9, 285–297. doi: 10.1016/j.celrep.2014.08.048

Wang, A. L., Lukas, T. J., Yuan, M., Du, N., Tso, M. O., and Neufeld, A. H. (2009). Autophagy, exosomes and drusen formation in age-related macular degeneration. *Autophagy* 5, 563–564. doi: 10.4161/auto.5.4.8163

Wang, X., Huang, W., Liu, G., Cai, W., Millard, R. W., Wang, Y., et al. (2014). Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. *J. Mol. Cell. Cardiol.* 74, 139–150. doi: 10.1016/j.yjmcc.2014.05.001

Whitcup, S. M., Nussenblatt, R. B., Lightman, S. L., and Hollander, D. A. (2013). Inflammation in retinal disease. *Int. J. Inflam.* 2013:724648. doi: 10.1155/2013/724648

Woods, K. A., Camacho-Hubner, C., Savage, M. O., and Clark, A. J. (1996). Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N. Engl. J. Med.* 335, 1363–1367. doi: 10.1056/NEJM199610313351805

Xu, H., Chen, M., and Forrester, J. V. (2009). Para-inflammation in the aging retina. *Prog. Retin. Eye Res.* 28, 348–368. doi: 10.1016/j.preteyeres.2009.06.001

Yamamoto, N., Nakagawa, T., and Ito, J. (2014). Application of insulin-like growth factor-1 in the treatment of inner ear disorders. *Front. Pharmacol.* 5:208. doi: 10.3389/fphar.2014.00208

Yoshida, N., Ikeda, Y., Notomi, S., Ishikawa, K., Murakami, Y., Hisatomi, T., et al. (2013). Laboratory evidence of sustained chronic inflammatory reaction in retinitis pigmentosa. *Ophthalmology* 120, e5–e12. doi: 10.1016/j.ophtha.2012.07.006

Zeng, H. Y., Zhu, X. A., Zhang, C., Yang, L. P., Wu, L. M., and Tso, M. O. (2005). Identification of sequential events and factors associated with microglial activation, migration, and cytotoxicity in retinal degeneration in rd mice. *Invest. Ophthalmol. Vis. Sci.* 46, 2992–2999. doi: 10.1167/iovs.05-0118

Zhang, G., Yu, L., Chen, Z. Y., Zhu, J. S., Hua, R., Qin, X., et al. (2016). Activation of corticotropin-releasing factor neurons and microglia in paraventricular nucleus precipitates visceral hypersensitivity induced by colorectal distension in rats. *Brain Behav. Immun.* 55, 93–104. doi: 10.1016/j.bbi.2015.12.022

Zhang, S., Wang, J., Song, C., Zhu, L., and Yu, Y. (2017). Lower prevalence of proliferative diabetic retinopathy in elderly onset patients with diabetes. *Diabetes Res. Clin. Pract.* 125, 47–52. doi: 10.1016/j.diabres.2016.09.009

Zhao, L., Zabel, M. K., Wang, X., Ma, W., Shah, P., Fariss, R. N., et al. (2015). Microglial phagocytosis of living photoreceptors contributes to inherited retinal degeneration. *EMBO Mol. Med.* 7, 1179–1197. doi: 10.15252/emmm.201505298

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 15 February 2018
doi: 10.3389/fnagi.2018.00015

The Complement System Is Critical in Maintaining Retinal Integrity during Aging

Ryo Mukai[1,2], *Yoko Okunuki*[1], *Deeba Husain*[1], *Clifford B. Kim*[1], *John D. Lambris*[3] and *Kip M. Connor*[1]*

[1] Angiogenesis Laboratory, Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Harvard University, Boston, MA, United States, [2] Department Ophthalmology, Graduate School of Medicine, Gunma University, Maebashi, Japan, [3] Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, United States

The complement system is a key component of innate immunity comprised of soluble components that form a proteolytic cascade leading to the generation of effector molecules involved in cellular clearance. This system is highly activated not only under general inflammatory conditions such as infections, collagen diseases, nephritis, and liver diseases, but also in focal ocular diseases. However, little is known about the role of the complement system in retinal homeostasis during aging. Using young (6-week-old) and adult (6-month-old) mice in wild type (C57BL/6) and complement knockout strains ($C1q^{-/-}$, *Mbl a/c*$^{-/-}$, $Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$), we compared amplitudes of electroretinograms (ERG) and thicknesses of retinal layers in spectral domain optical coherence tomography between young and adult mice. The ERG amplitudes in adult mice were significantly decreased ($p < 0.001$, $p < 0.0001$) compared to that of young mice in all complement knockout strains, and there were significant decreases in the inner nuclear layer (INL) thickness in adult mice compared to young mice in all complement knockout strains ($p < 0.0001$). There were no significant differences in ERG amplitude or thickness of the INL between young and adult control mice. These data suggest that the complement system plays an important role in maintaining normal retinal integrity over time.

Keywords: aging, complement system, retina, ERG, OCT, EM

OPEN ACCESS

Edited by:
Isabel Varela-Nieto, Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
Antje Grosche, Ludwig-Maximilians-Universität München, Germany
Enrica Strettoi, Istituto di Neuroscienze (CNR), Italy

***Correspondence:**
Kip M. Connor
kip_connor@meei.harvard.edu

Received: *12 October 2017*
Accepted: *12 January 2018*
Published: *15 February 2018*

Citation:
Mukai R, Okunuki Y, Husain D, Kim CB, Lambris JD and Connor KM (2018) The Complement System Is Critical in Maintaining Retinal Integrity during Aging. Front. Aging Neurosci. 10:15. doi: 10.3389/fnagi.2018.00015

INTRODUCTION

Retinal degeneration is a common form of neurodegenerative disease and a leading cause of vision loss worldwide. In the United States alone, about 3.4 million people are estimated to have vision loss due to retinal degeneration, and its prevalence is predicted to increase in industrialized countries with aging populations (Wert et al., 2014). Retinal degeneration contributes to vision loss in many conditions, including age-related macular degeneration, retinal detachment, and retinitis pigmentosa. There is considerable genotypic and phenotypic variability

Abbreviations: ERG, Electroretinogram; EM, electron microscopy; INL, inner nuclear layer; RD, retinal degeneration; SD-OCT, spectral domain optical coherence tomography; IPL/GC, inner plexiform layer/ganglion cell layer; ONL, outer nuclear layer; ONH, optic nerve head.

with retinal degeneration which complicates the identification of mechanisms of degeneration and development of potential therapeutic approaches for this debilitating condition (Wert et al., 2014). The complement system has recently become a topic of intense investigation, and is both a key mediator of developmental neuronal function and homeostasis, and contributes to a number of retinal disease pathologies (Sweigard et al., 2014, 2015; Kim et al., 2016). Cumulatively, these seminal studies suggest that the complement system is vital to normal retinal physiology.

The complement system is an essential component of the innate immune system and plays an important role in immune function and disease pathogenesis. The complement system is a hub-like surveillance network of the innate immune system that plays a vital role in the modulation of immune and inflammatory responses (Walport, 2001a,b; Harboe and Mollnes, 2008; Ricklin et al., 2010; Yanai et al., 2012). Activation of the complement system involves the sequential cleavage of proteins that resembles the coagulation cascade (Barnum, 2017). There are a number of proteins that comprise the complement system's three main upstream activation pathways—classical (complement components C1–C4), alternative (Factors B and D), and lectin (MASP-1 to 3 and MBL)—as well as the terminal activation pathway (complement components C5–C9). Additionally, the system is regulated by soluble (e.g., Factor H, I) and membrane-bound (e.g., CD55, CD59) complement regulatory proteins (Barnum, 2017). While liver hepatocytes are the primary source of complement synthesis, several other tissues and cell types, ranging from macrophages to endothelial cells to neurons, are capable of complement synthesis when induced, and may synthesize some components constitutively under certain circumstances (Barnum, 2017). Based on both synthesis location and function, it is evident that the complement system can affect all parts of the body, so it is an important consideration in various focal and systemic pathologies.

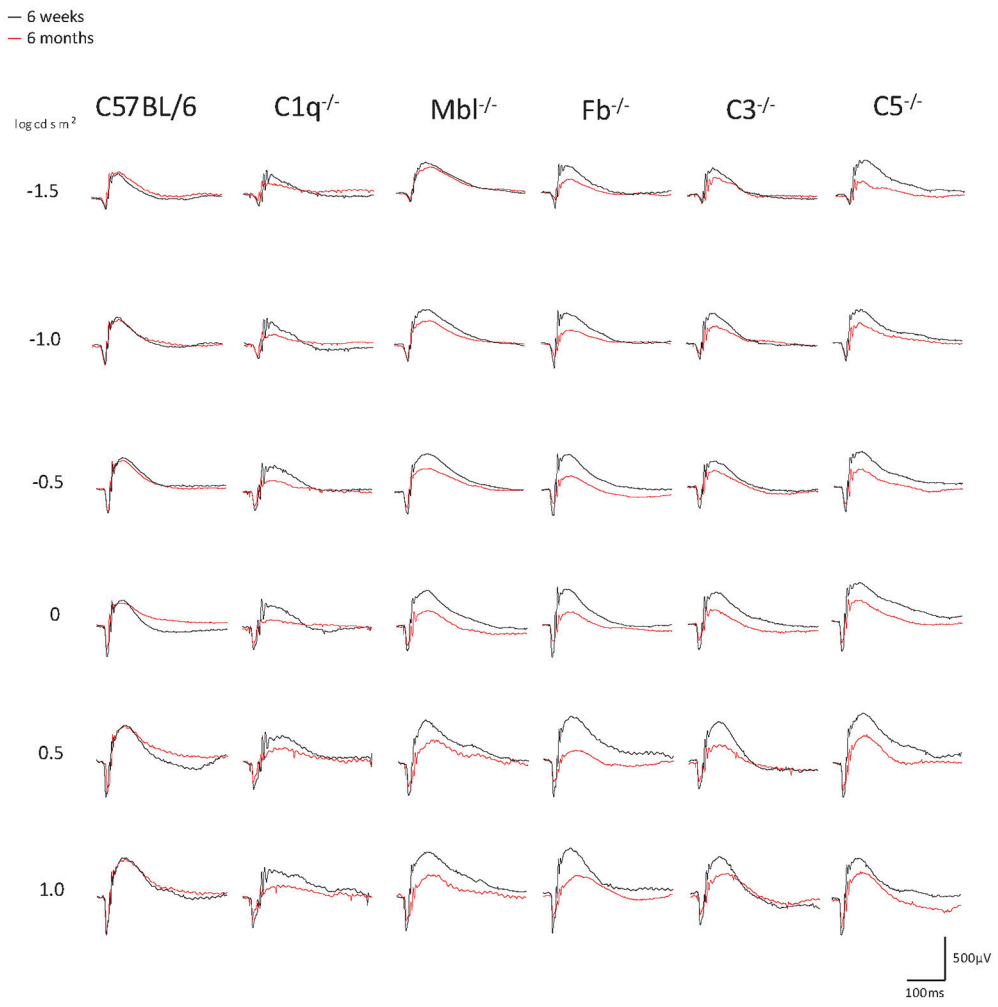


FIGURE 1 | Representative examples of ERG a- and b-wave responses in young (6 weeks) and adult (6 months) mice in each experimental group. In C57BL/6 mice, the amplitudes of a- and b-waves between young and adult mice were almost identical. On the other hand, both amplitudes were impaired in aged *C1q*$^{-/-}$, *Mbl*$^{-/-}$, *Fb*$^{-/-}$, *C3*$^{-/-}$, and *C5*$^{-/-}$ mice. A scale was shown at the bottom right.

The complement system is complex, with numerous complement effectors and a variety of receptors with different effects (McGeer et al., 2017). Thus, any dysfunction or defect in this complex network will likely be of consequence, as evidenced by the identification of complement involvement in numerous systemic (McGeer et al., 2017), inflammatory (Zhuang and Lyga, 2014), and retinal (Zhuang and Lyga, 2014; McHarg et al., 2015; Xu and Chen, 2016) pathologies. Indeed, the complement system has recently been implicated in a variety of pathophysiological processes, including photoreceptor degeneration, vascular regression, ischemia/reperfusion injury, sepsis, stroke, autoimmunity, and inflammatory disorders (Guo et al., 2005; Walsh et al., 2005; Sjoberg et al., 2009; Langer et al., 2010; Ricklin et al., 2010; Sweigard et al., 2014, 2015). Unsurprisingly, there is considerable interest in the development of complement-based therapeutics, with numerous clinical trials conducted and underway (Melis et al., 2015; Ricklin and Lambris, 2016).

The complement system is thought to contribute to general homeostasis by eliminating immune complexes and apoptotic cells, as well as mediating cross-talk with immune cells for adaptive immune functions (Ricklin et al., 2010). During synapse development and plasticity, C1q, the initiating protein of the classical complement pathway, is believed to play an important role in synapse elimination (Stevens et al., 2007). Moreover, C1q protein levels dramatically increase during normal aging in mice and the human brain (Stephan et al., 2013). In the study of sleep and circadian rhythms, the plasma level of complement components C3, C4, C3a, and C5a appear to fluctuate with the sleep-awake cycle, thereby suggesting that circadian rhythms can affect the immune regulatory properties of the complement system (Reis et al., 2011). In retinal detachment models in mice, deficiencies in key complement components (i.e., Factor b, or C3) resulted in a decreased number of apoptotic cells compared to wild type C57BL/6J mice with retinal detachment (Sweigard et al., 2015). A similar result was observed in endothelial cell death in a model of ocular ischemia (Sweigard et al., 2014). Recent studies of mice deficient in key complement receptors ($C3aR^{-/-}$ and $C5aR^{-/-}$) have found that a- and b-wave signals from electroretinograms (ERGs) of these strains were impaired in mice that were 14 weeks, 6 months, and 12 months old compared to ERG readings from strain-matched mice that were 6 weeks old (Yu et al., 2012). Additionally, light microscopy in $C3aR^{-/-}$ and $C5aR^{-/-}$ mice revealed that inner nuclear layer (INL) and outer nuclear layer (ONL) thickness in 14-month-old mice were diminished compared to that of 3-month-old

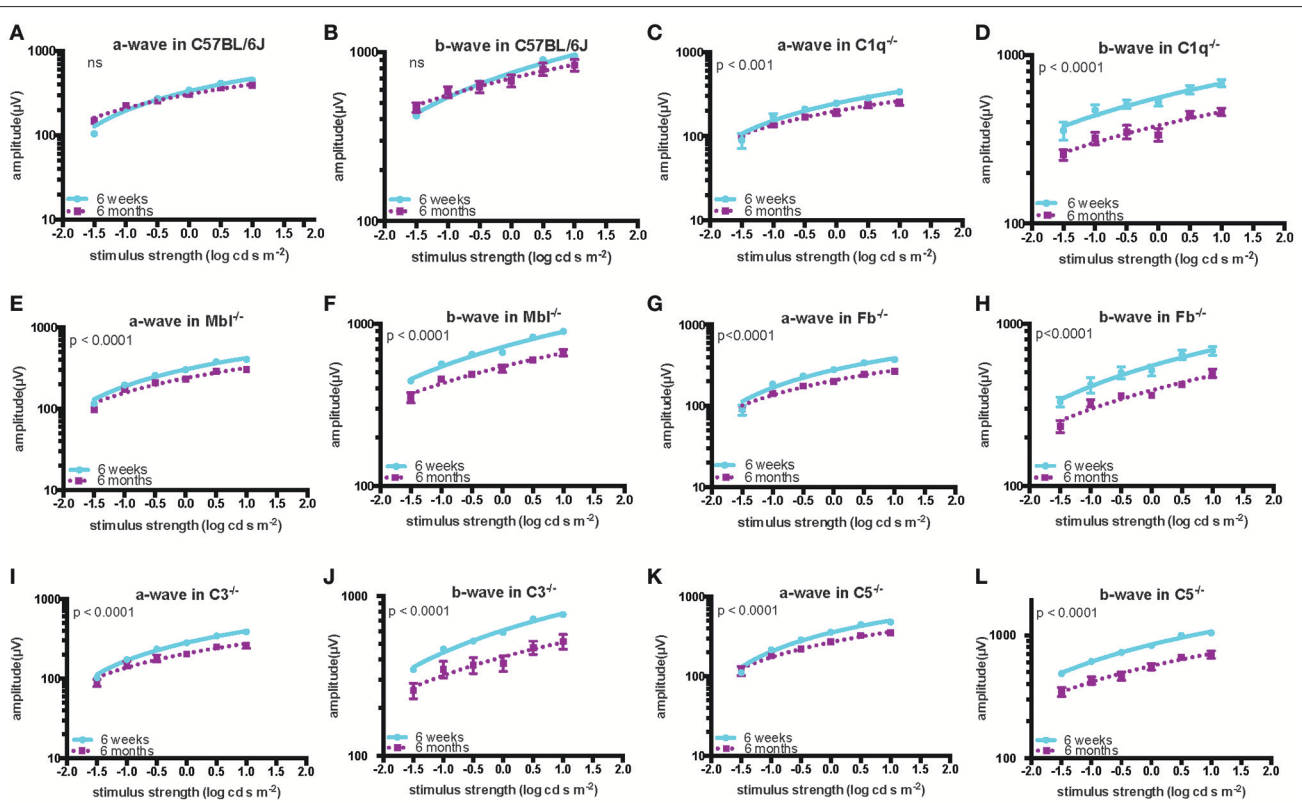


FIGURE 2 | Quantification of ERG amplitudes in a- and b-waves of young (6 weeks) and adult (6 months) mice in each experimental group. In C57BL/6 mice **(A,B)**, there was no significant difference in the amplitude of a- and b-waves between the young ($n = 5$) and old ($n = 5$) mice. By contrast, both amplitudes were significantly decreased in old compared to in young $C1q^{-/-}$ mice (a-wave: $p < 0.001$, b-wave: $p < 0.0001$; young $n = 3$, adult $n = 3$), $Mbl^{-/-}$ mice (a-wave: $p < 0.0001$, b-wave: $p < 0.0001$; young $n = 6$, adult $n = 5$), $Fb^{-/-}$ mice (a-wave: $p < 0.001$, b-wave: $p < 0.0001$; young $n = 4$, adult $n = 8$), $C3^{-/-}$ mice (a-wave: $p < 0.001$, b-wave: $p < 0.0001$; young $n = 6$, adult $n = 4$), and $C5^{-/-}$ mice (a-wave: $p < 0.001$, b-wave: $p < 0.0001$; young $n = 3$, adult $n = 3$) **(C–L)**.

mice. These results suggest that C3aR and C5aR are necessary in maintaining normal retinal structure and function (Yu et al., 2012).

The complement system is active in the retina, RPE, and choroid under endogenous conditions. Immunohistochemistry has revealed that C1q is expressed in the retinal ganglion cell (RGC) layer even during the developmental stage (Stevens et al., 2007). Furthermore, Factor b (Collier et al., 2011), C3 (Anderson et al., 2010; Collier et al., 2011; Luo et al., 2011), and C5 (Copland et al., 2010) components have been detected in normal retinas. In mRNA analysis, a key component of the mannose-binding-lectin (Mbl) pathway was also identified in normal retinas (Luo et al., 2011). However, the role of these complement factors play under normal conditions is unclear. To fill this knowledge gap, this study focused on the effects of the three main complement pathways—classical, lectin, and alternative pathways—on retinal function and morphology during the normal aging process using complement-pathway deficient mice.

MATERIALS AND METHODS

Animals

The Massachusetts Eye and Ear Animal Care Committees approved all animal procedures and experiments prior to beginning, and all animals were treated in accordance with the guidelines set forth by the Association of Research for Vision and Ophthalmology.

In this study, C57BL/6 (stock no. 000664) mice were purchased from Jackson Laboratories (Bar Harbor ME, USA). *C3*$^{-/-}$, *C5*$^{-/-}$, *Fb*$^{-/-}$, and *C1q*$^{-/-}$ mice were a gift from J.D.L. at the University of Pennsylvania. *Mbl A/C*$^{-/-}$ mice were a gift from G. Stahl at Brigham and Women's Hospital. All complement-deficient strains were in a C57BL/6 background. Each strain was maintained as a breeding colony in the Massachusetts Eye and Ear animal facility. Mice of all complement knockout stains utilized were screened for: RD1, RD2, RD3, RD6, RD7, RD8, and RD 10 mutations (TransnetYX®), and all strains were negative for these retinal degenerative genes.

Electroretinography (ERG) Recording

Full-field ERGs were recorded simultaneously from both eyes. Animals were dark-adapted overnight (>12 h). Mice were weighed and anesthetized with intraperitoneal injections of a mixture of ketamine (60 mg/kg) and xylazine (9 mg/kg). Mydriasis was achieved with one drop of 0.5% tropicamide with 5% phenylephrine. Corneal anesthesia was performed with a single drop of 0.5% proparacaine hydrochloride ophthalmic solution (Akorn Inc. Illinois). A warm heating pad was used to maintain body temperature (37°C).

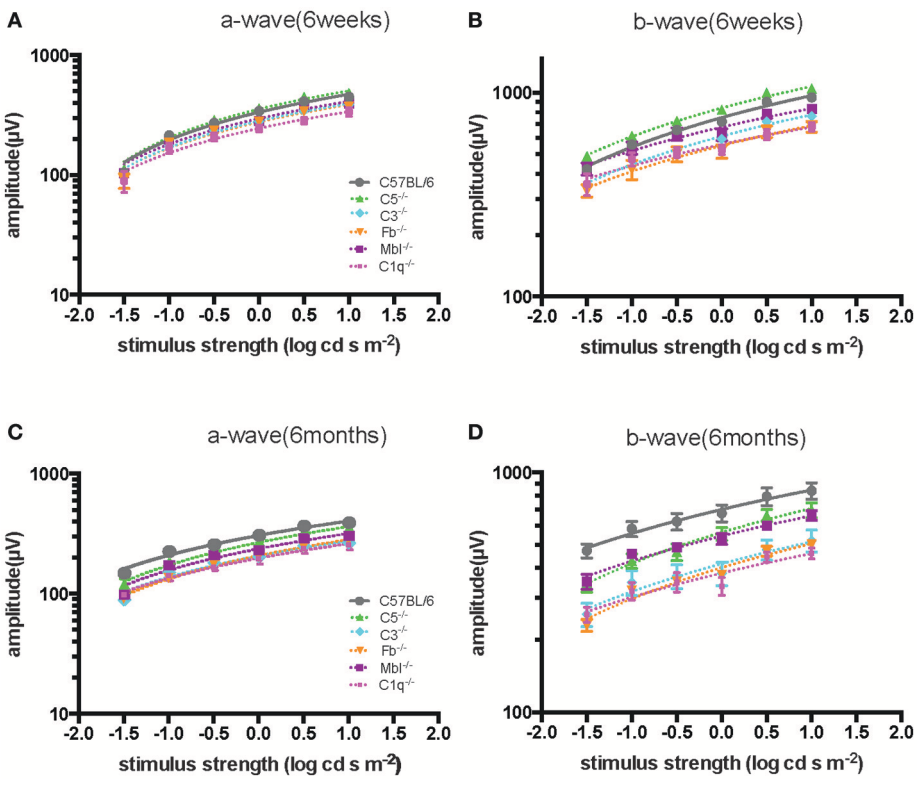


FIGURE 3 | Comparison of ERG a- and b-wave amplitudes among experimental mouse strains at 6 weeks **(A,B)** and 6 months **(C,D)** of age. The amplitude of a- and b-waves in complement knockout strains in *C1q*$^{-/-}$, *Mbl*$^{-/-}$, *Fb*$^{-/-}$, and *C3*$^{-/-}$ mice were lower than those of C57BL/6 mice at each age, except for *C5*$^{-/-}$ mice at 6 weeks of age.

ERGs were recorded using HMS's ERG LAB system (OcuScience, Nevada). Stimulus flashes were presented in a Ganzfeld bowl. Stimulus intensities ranging from −1.5 to 1.0 log cd s/m^2 in 0.5-log units steps were used under dark-adapted conditions. Light stimuli were presented with a 1-min interval between successive stimuli.

SD-OCT Imaging

Before optical coherence tomography (OCT) imaging was performed, each animal was anesthetized by intraperitoneal Avertin injection (125 mg/kg), and the pupils were dilated with a drop of 0.5% tropicamide with 5% phenylephrine. Two repeated volumetric images, centered on the optic nerve head, were acquired in both eyes using spectral-domain OCT (SD-OCT; Bioptigen, Inc., Davis, NC). All SD-OCT images consisted of 1,000 A-scans and 100-averaged B scans (each B-scan was the average of three B-scans). These parameters correspond to the area of approximately 1.4 × 1.4 mm.

SD-OCT Image Analysis

The RGC layer, inner plexiform layer and ganglion cell complex (IPL/GC), INL, and ONL were measured using SD-OCT B-scan cross-sectional images (Supplementary Figure 1A). To evaluate the cross-sectional thickness (in microns, μm) of each layer, manual calipers included in the Bioptigen software program were used. Retinal en-face scans were divided into inferior, nasal, temporal, and superior quadrants surrounding the central retinal subfield containing the optic nerve heads. One B-scan obtained approximately 400 μm from the optic nerve head (ONH) superiorly or inferiorly was selected for measurement of each retinal layer (Supplementary Figure 1B superior and inferior). Three B-scans that included the ONH or approximately 140 μm from the ONH superiorly and inferiorly were selected for measurement of each retinal layer in the nasal and temporal regions of the fundus. In the superior and inferior B-scan images, three measurement points were selected as follows: the central measurement point was determined as just superior or inferior of the ONH, and the other two points were defined at 300 μm nasally or temporally from this central measurement point. To measure the retinal thickness in the nasal or temporal area, three points were selected at 300 μm nasally or temporally from the ONH in the three B-scan images. Therefore, a total of 12 points were used to average the thickness of each retinal layer (Supplementary Figure 1B).

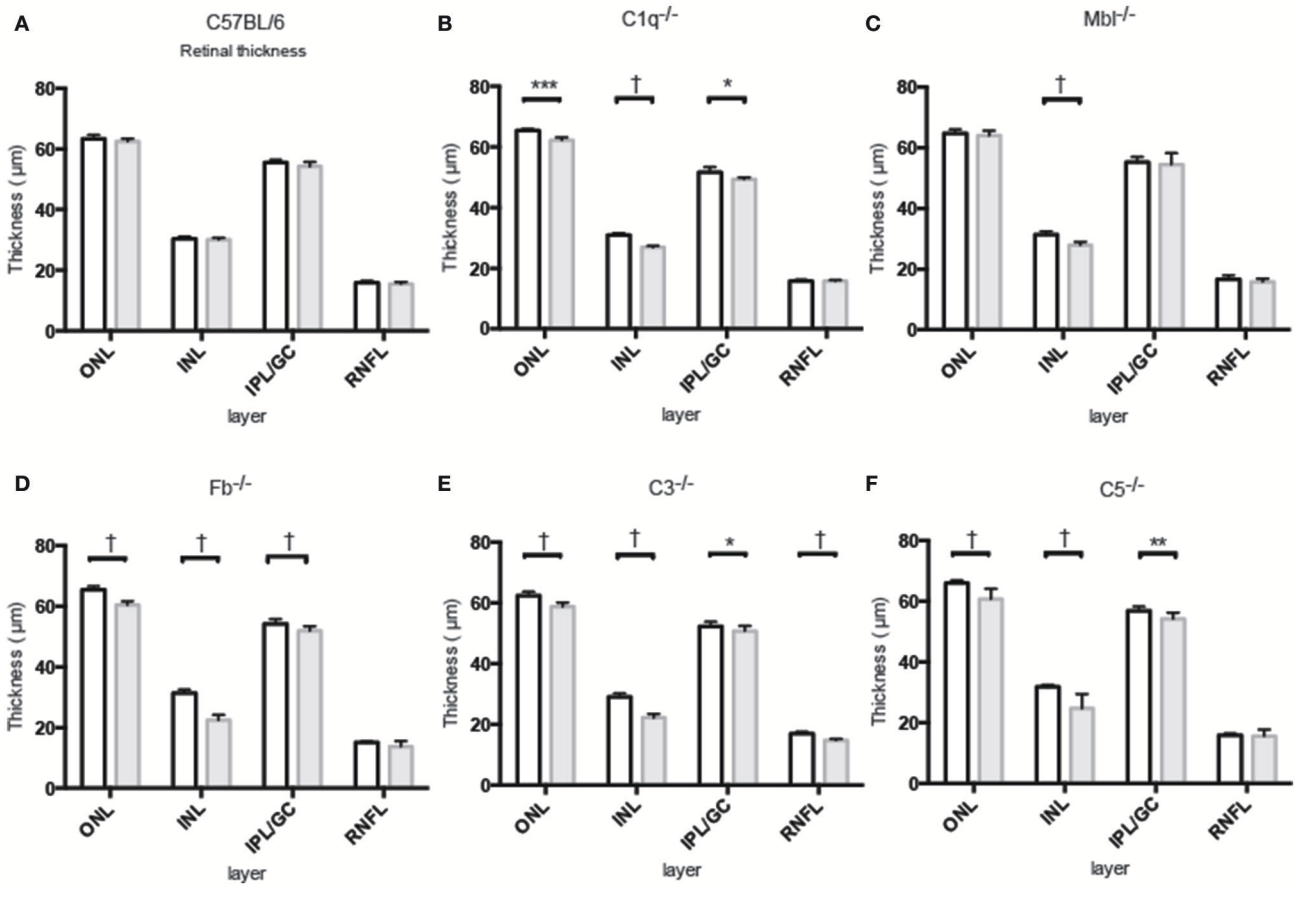


FIGURE 4 | Mean thickness of retinal sub-layers measured by Spectral Domain Optical Coherence Tomography in young (6 weeks) and adult (6 months) mice in each experimental group. **(A)** There was no significant thinning in any retinal layer between 6-week-old and 6-month-old C57BL/6 mice. **(B–F)** Contrastingly, INL thinning was identified in all complement knockout strains at the age of 6 months. Values and statistical analysis are summarized in **Table 1**. RNFL, retinal nerve fiber layer; IPL/GC, inner plexiform layer/ganglion cell; INL, inner nuclear layer; ONL, outer nuclear layer. Bar = 100 μm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, †$p < 0.0001$.

Light Microscopy and Electron Microscopy

Mouse eyes were enucleated and immersed in half strength Karnovsky's fixative (2% formaldehyde + 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4; Electron Microscopy Sciences, Hatfield, Pennsylvania) at room temperature. An eyecup was created with each eye by dissecting away the anterior portion from the posterior portion. Eyecup samples were then placed back into half-strength Karnovsky's fixative for a minimum of 24 h under refrigeration. After fixation, samples were rinsed with 0.1 M sodium cacodylate buffer, post-fixed with 2% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1.5 h, en bloc stained with 2% aqueous uranyl acetate for 30 min, then dehydrated with graded ethyl alcohol solutions, transitioned with propylene oxide, and resin infiltrated in tEPON-812 epoxy resin (Tousimis, Rockville, Maryland) utilizing an automated EMS Lynx 2 EM tissue processor (Electron Microscopy Sciences, Hatfield, Pennsylvania). The whole posterior eye cup was processed and embedded as a single block in transparent epoxy resin to prevent outer segment detachment during subsequent processing and sectioning procedures. The mid optic nerve head plane within the embedded eyecup was targeted for sectioning through the use of a stereoscope observing all directions of the transparent embedded block, then scored for sectioning using the optic nerve as an exterior reference. An initial precision saw cut was made into the embedded block away from the mid-level plane then ground down using a frosted slide. The edges of the eyecup tissue were trimmed using razor blades for ultramicrotomy. Sections were subsequently generated at 1 micrometer thickness and stained with toluidine blue stain. The orientation of photoreceptor outer segments was assessed in addition to the level through optic nerve head. The angle of cut and orientation in the both the x and y directions where made to generate a mid-level plane with regions of retinal photoreceptor outer segments oriented longitudinally prior to thin sectioning at 80 nanometers thickness for electron microscopy. Ultrathin sections (70–90 nm) were cut from the epoxy block using a Leica EM UC7 ultramicrotome (Leica Microsystems, Buffalo Grove, IL) and a diamond knife, collected onto 2 × 1 mm single slot formvar/carbon coated grids, and were stained with aqueous 25% Uranyl Acetate Replacement stain (Electron Microscopy Sciences, Hatfield, Pennsylvania) and Sato's lead citrate using a modified Hiraoka grid staining system. Grids were imaged using a FEI Tecnai G2 Spirit transmission electron microscope (TEM) (FEI, Hillsboro, Oregon) at 80 kV interfaced with an AMT XR41 digital CCD camera (Advanced Microscopy Techniques, Woburn, Massachusetts) for digital TIFF file image acquisition. TEM imaging of all layers of the retina was used to capture representative regions. When analyzing the number of dense inclusions in the outer plexiform layer (OPL), 10 images taken at x11,000 magnification were selected every 50 μm from the optic nerve head in each mouse strain.

Statistical Analysis

All data are expressed as means ± SE. ERG comparisons in a-wave or b-wave signals were made between 6-week-old and 6-month-old mice using two-way analysis of variance (ANOVA), and comparisons of retinal thickness were analyzed using a two-tailed student's *t*-test. The Pearson *r*-test was used to estimate the correlation between retinal thickness and ERG amplitude. $P < 0.05$ were considered statistically significant.

RESULTS

Reduced Retinal Function with Age in Complement Knockout Mice

Significant decreases in the ERG amplitude of both a-wave and b-waves were detected in all strains of complement knockout mice that were 6 months old, compared to that of complement knockout mice (strain-matched mice) at 6 weeks of age; however, no such age-related differences were noted in C57BL/6 mice (**Figures 1**, **2**). The extent of signal loss was more severe in the classical pathway knockout ($C1q^{-/-}$) and alternative pathway knockout ($Fb^{-/-}$) strains (**Figure 3**).

Thinning of Retinal Layers with Age in Complement Knockout Mice

Inner nuclear layer (INL) thickness was significantly decreased in $C1q^{-/-}$, $Mbl^{-/-}$, $Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$ mice at 6 months

TABLE 1 | Changes in thickness of different retinal layers in 6-week-old or 6-month-old mice from each experimental strain.

Layer	Strain	Thickness (SE) μm				*P*-values
		6 weeks	*n*	6 months	*n*	
ONL						
	C57BL/6	63.35(0.38)	10	62.53(0.38)	6	0.18
	$C1q^{-/-}$	65.46(0.22)	4	62.25(0.36)	6	**<0001**
	$Mbl^{-/-}$	64.73(0.51)	7	64.03(0.51)	10	0.34
	$Fb^{-/-}$	65.50(0.48)	5	61.03(0.32)	14	**<0.0001**
	$C3^{-/-}$	62.45(0.34)	14	58.83(0.46)	8	**<0.0001**
	$C5^{-/-}$	65.94(0.40)	6	60.76(0.55)	7	**<0.0001**
INL						
	C57BL/6	30.38(0.24)	10	30.07(0.27)	6	0.42
	$C1q^{-/-}$	30.90(0.34)	4	26.88(0.22)	6	**<0.0001**
	$Mbl^{-/-}$	31.34(0.42)	7	27.88(0.34)	10	**<0.0001**
	$Fb^{-/-}$	31.43(0.48)	5	23.32(0.46)	14	**<0.0001**
	$C3^{-/-}$	29.03(0.31)	14	22.14(0.46)	8	**<0.0001**
	$C5^{-/-}$	31.78(0.30)	6	24.68(0.80)	7	**<0.0001**
IPL/GC						
	C57BL/6	55.47(0.31)	10	54.22(0.61)	6	0.06
	$C1q^{-/-}$	51.67(0.84)	4	49.39(0.24)	6	**<0.05**
	$Mbl^{-/-}$	55.22(0.64)	7	54.53(1.17)	10	0.64
	$Fb^{-/-}$	54.25(0.68)	5	52.32(0.41)	14	**<0.01**
	$C3^{-/-}$	52.32(0.40)	14	50.79(0.57)	8	**<0.05**
	$C5^{-/-}$	56.93(0.56)	6	54.24(0.34)	7	**<0.01**
RNFL						
	C57BL/6	15.93(0.20)	10	15.51(0.26)	6	0.22
	$C1q^{-/-}$	15.75(0.23)	4	15.76(0.15)	6	0.98
	$Mbl^{-/-}$	16.72(0.44)	7	15.83(0.31)	10	0.11
	$Fb^{-/-}$	15.08(0.14)	5	15.15(0.48)	14	0.86
	$C3^{-/-}$	16.91(0.17)	14	14.77(0.16)	8	**<0.0001**
	$C5^{-/-}$	15.90(0.25)	6	15.54(0.36)	7	0.70

RNFL, Retinal nerve fiber layer; IPL/GC, inner plexiform layer/ganglion cell; INL, inner nuclear layer; ONL, outer nuclear layer. Significant differences in each layer between young and old animals for each KO strain were detected and noted in bold.

of age compared to that in strain-matched mice at 6 weeks of age; however, no such age-related differences in INL thickness were found between 6-week-old and 6-month-old C57BL/6 mice (**Figure 4**). Specifically, we observed the following reductions in INL thickness in 6-month-old mice compared to 6-week-old mice: 23% reduction in $C1q^{-/-}$, 21% in $Mbl^{-/-}$, 36% in $Fb^{-/-}$, 34% in $C3^{-/-}$, and 23% in $C5^{-/-}$ mice. There were significant decreases in the thickness of the IPL/GC in C57BL/6, $C1q^{-/-}$, $Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$ mice; however, only a 1.3–4.7% reduction was identified (**Table 1**).

Positive Correlation between the Amplitude of B-Waves and the Thickness of the INL and IPL/GC

According to retinal single cell recordings, it is thought that the ERG is a mass retinal response in which the a-wave is generated by photoreceptors and the b-wave primarily reflects activation of bipolar cells. Thus, we analyzed the correlation between a-wave amplitude and ONL thickness, the correlation between b-wave amplitude and INL thickness, and the correlation between b-wave amplitude and IPL/GC thickness. A positive correlation between the b-wave amplitude and INL thickness ($r^2 = 0.5263$, $p < 0.01$), as well as a correlation between b-wave amplitude and IPL/GC thickness ($r^2 = 0.7608$, $p < 0.001$), were noted. However, no significant correlation was identified between the a-wave amplitude and ONL thickness (Supplementary Figures 2, 3).

Histologic Analysis

Light microscopic and electron microscopic images were obtained in each mouse strain in order to define the morphological changes induced in the retina when there was a deficiency in complement system activity. INL thinning was observed via electron and light microscopy in $C1q^{-/-}$ and $C3^{-/-}$ mice that were 6 months of age relative to mice at 6 weeks of age (**Figures 5**, **6**). In $Fb^{-/-}$, $C5^{-/-}$, and $Mbl^{-/-}$ mice, there was no difference between 6-week-old and 6-month-old mice (**Figure 7**). In particular, phagocytized cells were identified in the INL in the $C1q^{-/-}$ strain (**Figure 8**). Dense inclusions in the OPL have previously been identified as the degeneration of the junction between photoreceptors and bipolar cells (Wang et al., 2016). Our retinal samples also contained dense inclusions in the OPL in $Mbl^{-/-}$, $Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$ mice that were 6 months old. Furthermore, similar dense inclusions were identified in strain-matched mice that were 6 weeks old (**Figure 9**, Supplementary Figure 4, **Table 2**).

DISCUSSION

Retinal functional abnormalities were detected in $C1q^{-/-}$, $Mbl^{-/-}$, $Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$ mice at 6 months of age compared to in mice at 6 weeks of age under normal, disease-free conditions. This functional deterioration seemed to correlate with thinning of the inner retina in these strains.

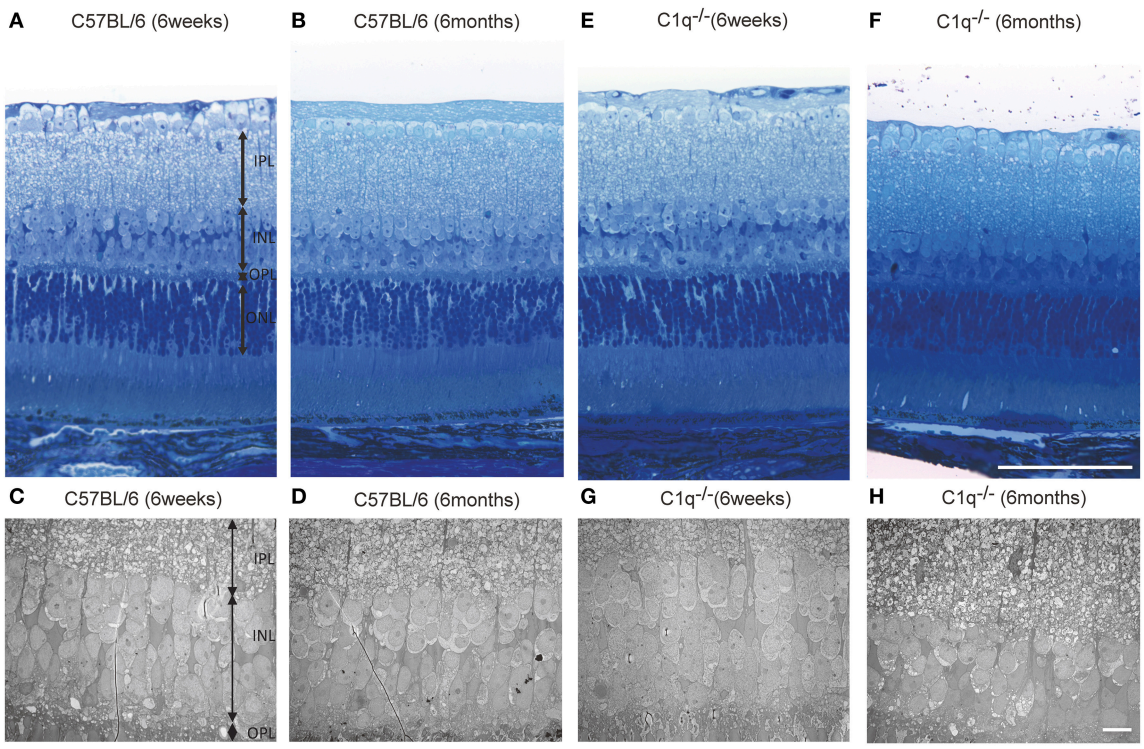


FIGURE 5 | Representative light microscopic and electron microscopic images comparing the retinal layer thickness of C57BL/6 **(A–D)** and $C1q^{-/-}$ **(E–H)** mice at 6 weeks **(A,C,E,G)** and 6 months **(B,D,F,H)** of age. **(A–D)** No thinning in any retinal layer were observed between 6-week-old and 6-month-old C57BL/6 mice. **(E–H)** In $C1q^{-/-}$ mice at 6 months of age, the thickness of inner nuclear layer seemed to be thinner than that in $C1q^{-/-}$ mice at 6 weeks of age. Bar = 100 μm, **(F)** Bar = 10 μm **(H)**.

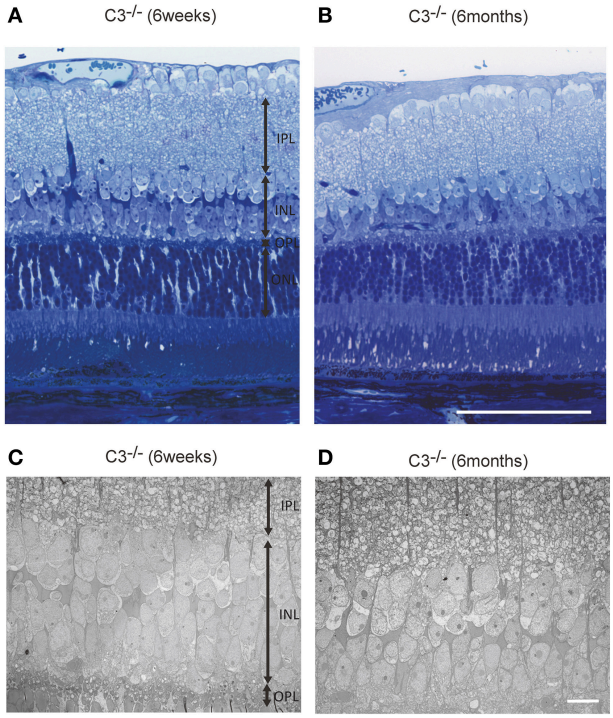


FIGURE 6 | Representative light microscopic **(A,B)** and electron microscopic images **(C,D)** comparing the retinal layer thickness of $C3^{-/-}$ mice at 6 weeks **(A,C)** and 6 months of age **(B,D)**. **(A–D)** The thickness of the inner nuclear layer in $C3^{-/-}$ mice at 6 months of age **(B,D)** appears thinner than that in $C3^{-/-}$ mice at 6 weeks of age **(A,C)**. **(A,B)** Bar = 100 μm. **(C,D)** Bar = 10 μm.

Additionally, dense inclusions in the OPL were observed via electron microscopy in $Mbl^{-/-}$, $Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$ mice at 6 months of age. These structural changes in the OPL may potentially contribute to retinal functional abnormalities, at least in some complement knockout mice.

In C57BL/6 mice, a mild decrease in a-wave and b-wave amplitudes was observed in 6-month-old mice compared to in 6-week-old mice; however, the changes were not significant in this study, indicating that retinal function is preserved from 6 weeks to 6 months in C57BL/6 mice. A previous study detected significant reductions in a-wave and b-wave amplitudes in 12-month-old mice, but such reductions were limited in 6-month-old mice, as compared to 2-month-old mice in the C57BL/6 strain (Li et al., 2001). In another study, authors compared ERGs from 1-, 4-, and 17-month-old mice, showing significantly decreased ERG amplitudes in 17-month-old mice compared to in 1-month-old mice; however, no major differences in ERG amplitude were detected at least in b-waves between 1-month-old and 4-month-old mice in same strain (Gresh et al., 2003). These trends are quite similar to those found in our study.

Decreased amplitudes of both a- and b-waves at 6 months of age were identified not only in alternative pathway knockout mice ($Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$), but also in classical ($C1q^{-/-}$) as well as mannose-binding-lectin pathway ($Mbl^{-/-}$) knockout mice. A previous study in 2012 explored how deficiencies in alternative pathway components such as C3, C3aR, and C5aR impacted ERG amplitude in mice (Yu et al., 2012). The authors identified decreased b-wave amplitudes in $C3^{-/-}$ mice at 12 months of age, as well as decreased a- and b-wave amplitudes in $C3aR^{-/-}$ and $C3aR^{-/-}C5aR^{-/-}$ mice at 14 weeks, 6 months, and 12 months of age. In addition, thinning of the INL and ONL was observed by 14 months of age in C3aR-deficient mouse strains, as analyzed by retinal histological sections (Yu et al., 2012). These results seem to support our ERG and OCT data in alternative pathway-deficient mice ($Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$).

In this study, we discovered that INL thickness in SD-OCT was significantly decreased in $C1q^{-/-}$, $Mbl^{-/-}$, $Fb^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$ mice at 6 months of age, compared to strain-matched mice, at 6 weeks of age and that this significantly correlated with b-wave amplitude. In addition, light microscopy revealed thinning of the INL in $C3^{-/-}$ and $C1q^{-/-}$ mice in our study. Specifically, we found that thinning in the INL inner region was more severe than that of the INL outer region. We therefore postulate that thinning of inner retinal layer at 6 months of age may be a key contributor to the deteriorating retinal function observed in these strains. However, there were some discrepancies in the results of INL thickness between SD-OCT and light microscopic finding among KO lines (**Figures 4–7**, **Table 1**). Thinning of the INL observed by SD-OCT in $C3^{-/-}$ and $C1q^{-/-}$ corresponded to thinning observed by light microscopic analysis, although there were some discrepancies in results of INL thickness between SD-OCT and light microscopic findings in other strains. We regarded thickness in OCT (**Figure 4**) as more accurate than thickness in HE or EM (**Figures 5–7**), due to the possibility that fixation can modify the thickness of tissues.

The mechanism for neuronal loss in the INL and ONL in complement knockout strains, remains unclear. Yu et al. demonstrated that decreased PKCα staining in the INL correlated with a reduction in the number of Calbundin D28 positive cells, and found swelling of the outer segment in aged $C3aR^{-/-}$ and $C3^{-/-}$ mice in comparison to wild type mice (Yu et al., 2012). Hoh and colleagues revealed that $C3^{-/-}$ mice had significant photoreceptor loss and thickening of the Bruch's membrane as well as reduced amplitudes in photopic and scotopic responses in 12-month-old mice, as compared to C57BL/6 mice of the same age (Hoh Kam et al., 2013). These results suggested that deficiency of complement components might cause impairment of signaling function in the inner and outer retinal layers.

Hoh therefore concluded that inhibition of C3 for treatment of age-related macular degeneration (AMD) patients might be deleterious (Hoh Kam et al., 2013). Our ERG findings in $C3^{-/-}$ mice are in accordance with this study, and strengthen the possibility that lack of C3 in the retina might contribute to reduced retinal function over time.

Interestingly, we detected dense inclusions in the OPL via electron microscopy, which may correspond to the degeneration of synapses between photoreceptors and bipolar cells (Somogyi et al., 1977; Linder et al., 1987; Wang et al., 2016), in

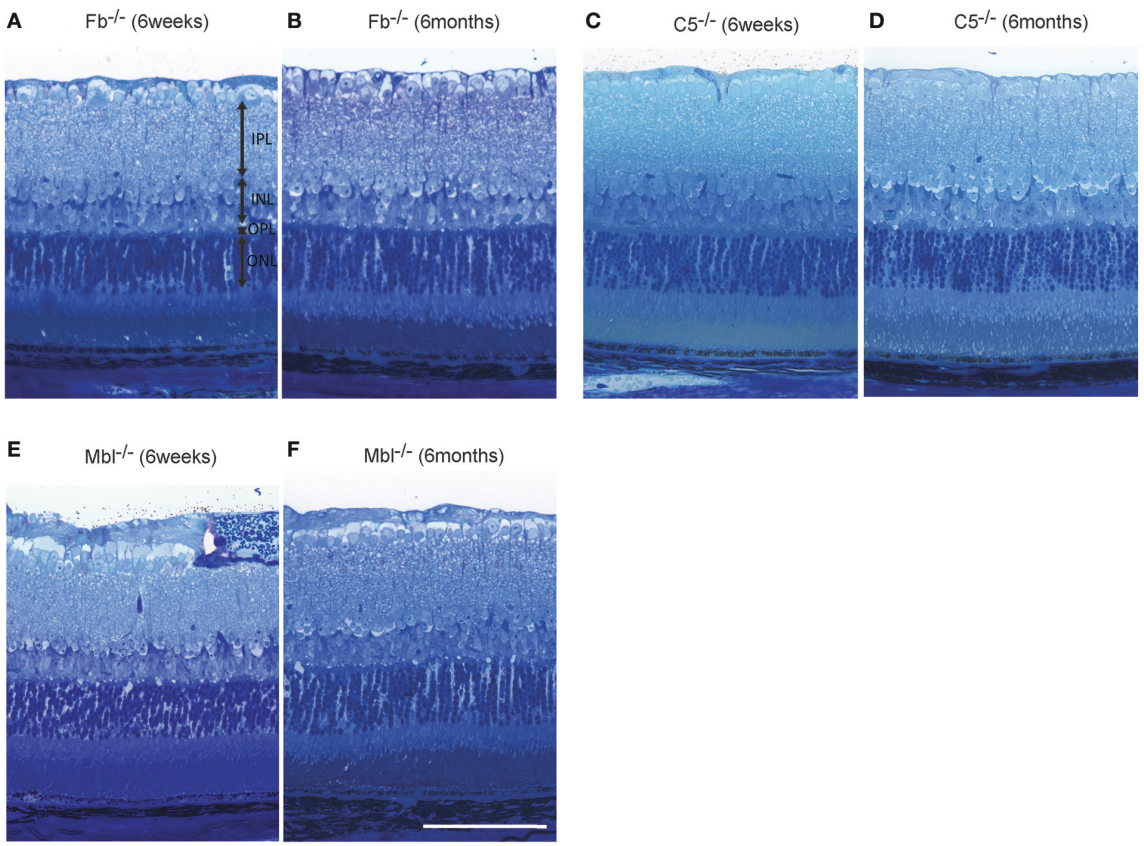


FIGURE 7 | Representative comparison of retinal layer thickness between 6-week-old and 6-month-old $Fb^{-/-}$ **(A,B)**, $C5^{-/-}$ **(C,D),** and $Mbl^{-/-}$ **(E,F)** mice. **(A–F)** There was no apparent difference in retinal thickness of each layer between mice at 6 weeks of age and those at 6 months of age. **(A–F)** Bar = 100 μm.

complement pathway-deficient ($Fb^{-/-}$, $Mbl^{-/-}$, $C3^{-/-}$, and $C5^{-/-}$) mice at 6 months of age. Dense inclusions in the OPL have been reported previously in microglia-depleted retinas, with diminished scotopic and photopic responses due to the destruction of pre-synaptic termini in the OPL (Wang et al., 2016). These structural changes in the OPL and electrophysiological changes in b-wave amplitude in microglia-depleted retinas were similar to our results in normal aging eyes (Wang et al., 2016). Based on our findings, we hypothesize that the complement system may maintain retinal integrity under normal homeostatic conditions. Nevertheless, it is still unclear why complement depletion may lead to degeneration in the retina. Dense inclusions were observed in OPL, but their number was limited (**Figure 9**, **Table 2**, Supplementary Figure 4). We evaluated the number of dense inclusions around the disc area (and not centrally) as we sought to evaluate the number of dense inclusions at the same position in all of our complement-deficient mice. Thus, this could be why the number of dense inclusions was limited.

When analyzing morphological changes in INL and IPL by electron microscopy, we focused on morphological changes in amacrine cells, bipolar cells and horizontal cells. We recognized the star-like shape of nuclei in amacrine cells as an index. The Kolmer's organelle is highly important in order to identify horizontal cells (Hogan et al., 1971; Richard et al., 2001). However, there were no obvious changes in these cells in both young and old complement-deficient strains. Next, we focused on synaptic ribbons between photoreceptors and bipolar cells, and compared the number of synaptic ribbons between young and old mice in complement-deficient strains. Still, there were no changes in the number of synapse ribbons in all complement-deficient strains (Supplementary Figure 5). In addition, although we tried to identify bipolar cells synapses by focusing on the numerous number of synaptic vesicles in axons of bipolar cells, it was difficult to identify and compare them among complement-deficient strains in a quantitative analysis. Moreover, we did not observe any distinct morphological changes in Müller cells in any of our complement-deficient strains. In C1q strains, we identified microglial cells at the inner surface of INL, which appeared to include phagocytosed cells. From the aspect of the location of these phagocytosing cells, it is possible that microglial cells had engulfed bipolar or amacrine cells in at least the C1q strain (**Figure 8**).

Of note, our study has shown the functional (**Figure 2**) and morphological changes (**Figure 7D**, Supplementary Figures 6, 7) in $C5^{-/-}$ mice at 6 months of age. In addition, there appeared

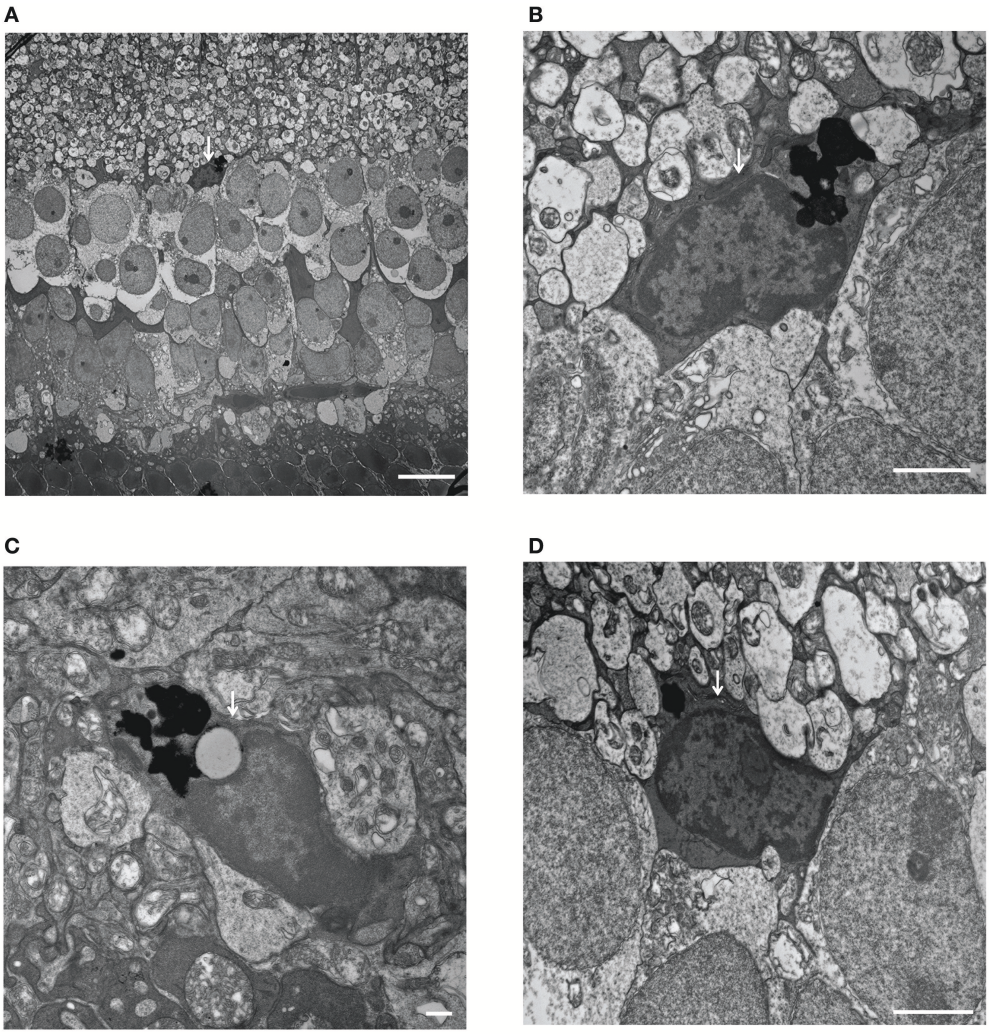


FIGURE 8 | Phagocytized microglial cells in the inner nuclear layer of $C1q^{-/-}$ mice at 6 months of age. (**A–D**, white arrows) **(A)** A microglial cell phagocytizing dead cells, with dense particles observed in the microglial cell body at the border between the inner nuclear layer and inner plexiform layer (magnification: x1,400). **(B)** High magnification image of **(A)** (magnification: x9,300). **(C,D)** Microglial cells containing dense particles were detected in other parts of the inner nuclear layer. (**C**: x13,000, **D**: x9,300). **(A)** Bar = 10 μm, **(B)** Bar = 2 μm, **(C)** Bar = 500 nm, **(D)** Bar = 2 μm.

to be swelling and folding of the outer segment in $C5^{-/-}$ mice at 6 months of age (**Figure 7D**, Supplementary Figures 6, 7). By contrast, previous studies did not show significant reductions in ERG amplitude and retinal thickness in $C5aR^{-/-}$ mice with increasing age (Yu et al., 2012). We suspect this may be due to C5 signaling being transmitted through two receptors (Ward, 2009, 2010; Bosmann et al., 2013; Li et al., 2013), such as C5aR1 and C5L2, which is suggestive that severe dysfunction only in the absence of both receptors.

Recently, evidence has been accumulating regarding the association between genetic alterations in complement factor H (CFH) with the development of age-related macular degeneration (AMD) in clinical studies. CFH has also been a topic of intense investigation in basic research (Anderson et al., 2010; Kondo et al., 2011). Photoreceptor loss and decreased amplitude in ERG scotopic responses were observed in 12-month-old CFH knockout mice ($Cfh^{-/-}$) (Ding et al., 2015). Additionally, the study noted an apparent decrease in INL thickness. Outer retinal degeneration resembling retinal changes seen in AMD was observed in 10-month-old mice lacking CD46, which is thought to regulate the aggregation of C3b and C4 (Lyzogubov et al., 2016). Basal laminar deposits were observed in $Fb^{-/-}$ mice at the age of 6 months in our study (Supplementary Figure 8). These results suggest that a lack of alternative pathway components or an imbalanced complement system could result in retinal degeneration in younger animals, even under normal conditions. Although a deficiency in CFH or CD46 could potentially enhance alternative pathway activity and cause retinal degeneration observed with AMD, it is also possible that the lack of a fully functional complement system could equally contribute to alteration of normal retinal homeostasis in the context of aging.

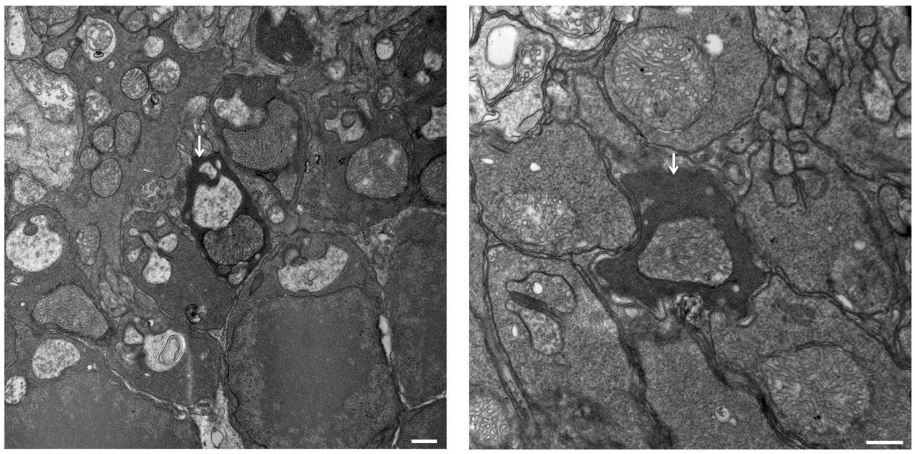


FIGURE 9 | Assessment of dense inclusions in the outer plexiform layer (OPL) (**A,B**, white arrows). Dense inclusions in the OPL have been reported previously in microglia-depleted retinas, and were due the destruction of pre-synaptic termini in the OPL (Wang et al., 2016). The presence of dense inclusions was identified in alternative complement pathway deficient mice and corresponded to the border between the tip of photoreceptors and neighboring bipolar cells. **(A)** $Fb^{-/-}$ mice at 6 weeks of age. Bar = 500 nm. **(B)** $Fb^{-/-}$ mice at 6 months of age. Bar = 500 nm.

TABLE 2 | The number of dense inclusions in each mouse strain at 6-weeks or 6-months of age.

Strains	Age	
	Young (6 weeks)	Old (6 months)
C57BL/6J	0	0
$Fb^{-/-}$	3	5
$C3^{-/-}$	16	9
$C5^{-/-}$	5	11
$C1q^{-/-}$	0	0
$Mbl^{-/-}$	2	3

To the best of our knowledge, this is the first study to suggest that, in addition to C3, C3aR, and C5aR(Yu et al., 2012), Factor b, C5, C1q, and Mbl A/C of the complement system are involved in the maintenance of retinal integrity under normal aging conditions. Further studies are needed to detail the association between the complement system and other immune components endogenous conditions, which would enhance our understanding of the mechanisms of age-related retinal diseases.

AUTHOR CONTRIBUTIONS

Drafting the article and conception or design of the work: RM and KC. Critical revision of the article, data analysis and interpretation: RM, YO, CK, DH, JL, and KC.

ACKNOWLEDGMENTS

The authors are grateful to G. Stahl at Brigham and Women's Hospital, who gave us *Mbl A/C*$^{-/-}$ mice. This study was supported by NIH grants R01EY022084–01/S1 (KC); Special thanks to the Harvard Department of Ophthalmology and Massachusetts Eye and Ear Infirmary for supporting this research (KC); Research to Prevent Blindness Medical Student Fellowship Grant (to CK); as well as to the Massachusetts Lions Eye Research Fund (KC).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2018.00015/full#supplementary-material

REFERENCES

Anderson, D. H., Radeke, M. J., Gallo, N. B., Chapin, E. A., Johnson, P. T., Curletti, C. R., et al. (2010). The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited. *Prog. Retin. Eye Res.* 29, 95–112. doi: 10.1016/j.preteyeres.2009.11.003

Barnum, S. R. (2017). Complement: a primer for the coming therapeutic revolution. *Pharmacol. Ther.* 172, 63–72. doi: 10.1016/j.pharmthera.2016.11.014

Bosmann, M., Haggadone, M. D., Zetoune, F. S., Sarma, J. V., and Ward, P. A. (2013). The interaction between C5a and both C5aR and C5L2 receptors is required for production of G-CSF during acute inflammation. *Eur. J. Immunol.* 43, 1907–1913. doi: 10.1002/eji.201243075

Collier, R. J., Wang, Y., Smith, S. S., Martin, E., Ornberg, R., Rhoades, K., et al. (2011). Complement deposition and microglial activation in the outer retina in light-induced retinopathy: inhibition by a 5-HT1A agonist. *Invest. Ophthalmol. Vis. Sci.* 52, 8108–8116. doi: 10.1167/iovs.10-6418

Copland, D. A., Hussain, K., Baalasubramanian, S., Hughes, T. R., Morgan, B. P., Xu, H., et al. (2010). Systemic and local anti-C5 therapy reduces the disease

severity in experimental autoimmune uveoretinitis. *Clin. Exp. Immunol.* 159, 303–314. doi: 10.1111/j.1365-2249.2009.04070.x

Ding, J. D., Kelly, U., Landowski, M., Toomey, C. B., Groelle, M., Miller, C., et al. (2015). Expression of human complement factor H prevents age-related macular degeneration-like retina damage and kidney abnormalities in aged Cfh knockout mice. *Am. J. Pathol.* 185, 29–42. doi: 10.1016/j.ajpath.2014.08.026

Gresh, J., Goletz, P. W., Crouch, R. K., and Rohrer, B. (2003). Structure-function analysis of rods and cones in juvenile, adult, and aged C57bl/6 and Balb/c mice. *Vis. Neurosci.* 20, 211–220. doi: 10.1017/S0952523803202108

Guo, M., Wu, M. H., Granger, H. J., and Yuan, S. Y. (2005). Focal adhesion kinase in neutrophil-induced microvascular hyperpermeability. *Microcirculation* 12, 223–232. doi: 10.1080/10739680590905251

Harboe, M., and Mollnes, T. E. (2008). The alternative complement pathway revisited. *J. Cell. Mol. Med.* 12, 1074–1084. doi: 10.1111/j.1582-4934.2008.00350.x

Hogan, M. J., Alvarado, J. A., and Weddell J. E. (1971). *Histology of the Human Eye*. Philadelphia, PA: Saunders.

Hoh Kam, J., Lenassi, E., Malik, T. H., Pickering, M. C., and Jeffery, G. (2013). Complement component C3 plays a critical role in protecting the aging retina in a murine model of age-related macular degeneration. *Am. J. Pathol.* 183, 480–492. doi: 10.1016/j.ajpath.2013.04.008

Kim, C., Smith, K. E., Castillejos, A., Diaz-Aguilar, D., Saint-Geniez, M., and Connor, K. M. (2016). The alternative complement pathway aids in vascular regression during the early stages of a murine model of proliferative retinopathy. *FASEB J.* 30, 1300–1305. doi: 10.1096/fj.15-280834

Kondo, N., Bessho, H., Honda, S., and Negi, A. (2011). Complement factor H Y402H variant and risk of age-related macular degeneration in Asians: a systematic review and meta-analysis. *Ophthalmology* 118, 339–344. doi: 10.1016/j.ophtha.2010.06.040

Langer, H. F., Chung, K. J., Orlova, V. V., Choi, E. Y., Kaul, S., Kruhlak, M. J., et al. (2010). Complement-mediated inhibition of neovascularization reveals a point of convergence between innate immunity and angiogenesis. *Blood* 116, 4395–4403. doi: 10.1182/blood-2010-01-261503

Li, C., Cheng, M., Yang, H., Peachey, N. S., and Naash, M. I. (2001). Age-related changes in the mouse outer retina. *Optom. Vis. Sci.* 78, 425–430. doi: 10.1097/00006324-200106000-00015

Li, R., Coulthard, L. G., Wu, M. C., Taylor, S. M., and Woodruff, T. M. (2013). C5L2: a controversial receptor of complement anaphylatoxin, C5a. *FASEB J.* 27, 855–864. doi: 10.1096/fj.12-220509

Linder, J. C., Klemfuss, H., and Groves, P. M. (1987). Acute ultrastructural and behavioral effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice. *Neurosci. Lett.* 82, 221–226. doi: 10.1016/0304-3940(87)90134-0

Luo, C., Chen, M., and Xu, H. (2011). Complement gene expression and regulation in mouse retina and retinal pigment epithelium/choroid. *Mol. Vis.* 17, 1588–1597.

Lyzogubov, V. V., Bora, P. S., Wu, X., Horn, L. E., De Roque, R., Rudolf, X. V., et al. (2016). The complement regulatory protein CD46 deficient mouse spontaneously develops dry-type age-related macular degeneration-like phenotype. *Am. J. Pathol.* 186, 2088–2104. doi: 10.1016/j.ajpath.2016.03.021

McGeer, P. L., Lee, M., and McGeer, E. G. (2017). A review of human diseases caused or exacerbated by aberrant complement activation. *Neurobiol. Aging* 52, 12–22. doi: 10.1016/j.neurobiolaging.2016.12.017

McHarg, S., Clark, S. J., Day, A. J., and Bishop, P. N. (2015). Age-related macular degeneration and the role of the complement system. *Mol. Immunol.* 67, 43–50. doi: 10.1016/j.molimm.2015.02.032

Melis, J. P., Strumane, K., Ruuls, S. R., Beurskens, F. J., Schuurman, J., and Parren, P. W. (2015). Complement in therapy and disease: regulating the complement system with antibody-based therapeutics. *Mol. Immunol.* 67, 117–130. doi: 10.1016/j.molimm.2015.01.028

Reis, E. S., Lange, T., Kohl, G., Herrmann, A., Tschulakow, A. V., Naujoks, J., et al. (2011). Sleep and circadian rhythm regulate circulating complement factors and immunoregulatory properties of C5a. *Brain Behav. Immun.* 25, 1416–1426. doi: 10.1016/j.bbi.2011.04.011

Richard, S. S., Simon, W. M. J., Patsy, M. N., and John, P. S. (2001). *Systemic Evaluation of the Mouse Eye*. Boca Raton, FL: CRC Press.

Ricklin, D., Hajishengallis, G., Yang, K., and Lambris, J. D. (2010). Complement: a key system for immune surveillance and homeostasis. *Nat. Immunol.* 11, 785–797. doi: 10.1038/ni.1923

Ricklin, D., and Lambris, J. D. (2016). New milestones ahead in complement-targeted therapy. *Semin. Immunol.* 28, 208–222. doi: 10.1016/j.smim.2016.06.001

Sjoberg, A. P., Trouw, L. A., and Blom, A. M. (2009). Complement activation and inhibition: a delicate balance. *Trends Immunol.* 30, 83–90. doi: 10.1016/j.it.2008.11.003

Somogyi, G., Hajdu, F., and Hassler, R. (1977). Electron microscopic study of terminal degeneration in the anterodorsal thalamic nucleus of the cat. *Cell Tissue Res.* 182, 455–467. doi: 10.1007/BF00219829

Stephan, A. H., Madison, D. V., Mateos, J. M., Fraser, D. A., Lovelett, E. A., Coutellier, L., et al. (2013). A dramatic increase of C1q protein in the CNS during normal aging. *J. Neurosci.* 33, 13460–13474. doi: 10.1523/JNEUROSCI.1333-13.2013

Stevens, B., Allen, N. J., Vazquez, L. E., Howell, G. R., Christopherson, K. S., Nouri, N., et al. (2007). The classical complement cascade mediates CNS synapse elimination. *Cell* 131, 1164–1178. doi: 10.1016/j.cell.2007.10.036

Sweigard, J. H., Matsumoto, H., Smith, K. E., Kim, L. A., Paschalis, E. I., Okonuki, Y., et al. (2015). Inhibition of the alternative complement pathway preserves photoreceptors after retinal injury. *Sci. Transl. Med.* 7:297ra116. doi: 10.1126/scitranslmed.aab1482

Sweigard, J. H., Yanai, R., Gaissert, P., Saint-Geniez, M., Kataoka, K., Thanos, A., et al. (2014). The alternative complement pathway regulates pathological angiogenesis in the retina. *FASEB J.* 28, 3171–3182. doi: 10.1096/fj.14-251041

Walport, M. J. (2001a). Complement. First of two parts. *N. Engl. J. Med.* 344, 1058–1066. doi: 10.1056/NEJM200104053441406

Walport, M. J. (2001b). Complement. Second of two parts. *N. Engl. J. Med.* 344, 1140–1144. doi: 10.1056/NEJM200104123441506

Walsh, M. C., Bourcier, T., Takahashi, K., Shi, L., Busche, M. N., Rother, R. P., et al. (2005). Mannose-binding lectin is a regulator of inflammation that accompanies myocardial ischemia and reperfusion injury. *J. Immunol.* 175, 541–546. doi: 10.4049/jimmunol.175.1.541

Wang, X., Zhao, L., Zhang, J., Fariss, R. N., Ma, W., Kretschmer, F., et al. (2016). Requirement for microglia for the maintenance of synaptic function and integrity in the mature retina. *J. Neurosci.* 36, 2827–2842. doi: 10.1523/JNEUROSCI.3575-15.2016

Ward, P. A. (2009). Functions of C5a receptors. *J. Mol. Med.* 87, 375–378. doi: 10.1007/s00109-009-0442-7

Ward, P. A. (2010). The harmful role of c5a on innate immunity in sepsis. *J. Innate Immun.* 2, 439–445. doi: 10.1159/000317194

Wert, K. J., Lin, J. H., and Tsang, S. H. (2014). General pathophysiology in retinal degeneration. *Dev. Ophthalmol.* 53, 33–43. doi: 10.1159/000357294

Xu, H., and Chen, M. (2016). Targeting the complement system for the management of retinal inflammatory and degenerative diseases. *Eur. J. Pharmacol.* 787, 94–104. doi: 10.1016/j.ejphar.2016.03.001

Yanai, R., Thanos, A., and Connor, K. M. (2012). Complement involvement in neovascular ocular diseases. *Adv. Exp. Med. Biol.* 946, 161–183. doi: 10.1007/978-1-4614-0106-3_10

Yu, M., Zou, W., Peachey, N. S., McIntyre, T. M., and Liu, J. (2012). A novel role of complement in retinal degeneration. *Invest. Ophthalmol. Vis. Sci.* 53, 7684–7692. doi: 10.1167/iovs.12-10069

Zhuang, Y., and Lyga, J. (2014). Inflammaging in skin and other tissues - the roles of complement system and macrophage. *Inflamm. Allergy Drug Targets* 13, 153–161. doi: 10.2174/18715281136661405 22112003

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 04 January 2018
doi: 10.3389/fnagi.2017.00428

Age-Related Differences in Hearing Function and Cochlear Morphology between Male and Female Fischer 344 Rats

Zuzana Balogová[1], Jiří Popelář[1], Francesca Chiumenti[2], Tetyana Chumak[1], Jana Svobodová Burianová[1], Natalia Rybalko[1] and Josef Syka[1]*

[1]*Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czechia,* [2]*Faculty of Medicine, University of Padova, Padova, Italy*

Fischer 344 (F344) rats represent a strain that is frequently used as a model for fast aging. In this study, we systematically compare the hearing function during aging in male and female F344 rats, by recording auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). In addition to this, the functional parameters are correlated with the cochlear histology. The parameters of the hearing function were not different in the young (3-month-old) male and female F344 rats; the gender differences occurred only in adult and aged animals. In 8–24-month-old males, the ABR thresholds were higher and the ABR amplitudes were smaller than those measured in females of the same age. There were no gender differences in the neural adaptation tested by recording ABRs, elicited by a series of clicks with varying inter-click interval (ICI). Amplitudes of DPOAEs in both the males and females decreased with age, but in the males, the decrease of DPOAE amplitudes was faster. In males older than 20 months, the DPOAEs were practically absent, whereas in 20–24-month-old females, the DPOAEs were still measurable. There were no gender differences in the number of surviving outer hair cells (OHC) and the number of inner hair cell ribbon synapses in aged animals. The main difference was found in the stria vascularis (SV). Whereas the SV was well preserved in females up to the age of 24 months, in most of the age-matched males the SV was evidently deteriorated. The results demonstrate more pronounced age-related changes in the cochlear morphology, hearing thresholds, ABR amplitudes and DPOAE amplitudes in F344 males compared with females.

Keywords: Fischer 344 rats, aging, gender differences, hearing function, cochlear morphology, stria vascularis

OPEN ACCESS

Edited by:
Isabel Varela-Nieto, Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
George M. Strain, Louisiana State University, United States
Juan Carlos Alvarado, Universidad de Castilla-La Mancha, Spain
Anna R. Fetoni, Università Cattolica del Sacro Cuore, Italy

****Correspondence:***
Jiří Popelář
jpopelar@biomed.cas.cz

Received: *17 October 2017*
Accepted: *13 December 2017*
Published: *04 January 2018*

Citation:
Balogová Z, Popelář J, Chiumenti F, Chumak T, Burianová JS, Rybalko N and Syka J (2018) Age-Related Differences in Hearing Function and Cochlear Morphology between Male and Female Fischer 344 Rats. Front. Aging Neurosci. 9:428. doi: 10.3389/fnagi.2017.00428

INTRODUCTION

Age-related hearing loss (presbycusis) is the most prevalent sensory disorder in the elderly population (Gates and Mills, 2005; Fetoni et al., 2011). Presbycusis is a complex phenomenon characterized by a combination of the deteriorated function in the periphery, as well as in the central auditory system. In the development of presbycusis mutation of the mitochondrial DNA, other genetic disorders and systemic diseases (hypertension, diabetes, metabolic diseases) may contribute (Gates and Mills, 2005; Huang and Tang, 2010; Fetoni et al., 2011). The state of presbycusis is also influenced by the effects of noise, consumption of ototoxic drugs

and diet during the life-time. Pathological processes leading to the occurrence of presbycusis, may be present in different parts of the auditory system. In the inner ear losses are often present: of inner and outer hair cells (OHC), of ribbon synapses between inner hair cells (IHC) and auditory nerve fibers of spiral ganglion neurons- and atrophy of the stria vascularis (SV), resulting in a decline of the endolymphatic potential. The central component of presbycusis, i.e., the pathological processes occuring in neurons of the central auditory system, affect the temporal processing of acoustical stimuli and cause a cognitive decline. This results in deteriorated speech-understanding and particularly in speech-perception difficulties in a noisy environment. Presbycusis is the factor that markedly affects patients' quality of life and represents a serious social and economic problem (World Health Organization, 2017).

The Fisher 344 (F344) strain is an inbred strain frequently used in the studies of cancer and toxicity. F344 rats develop a fast progressing hearing loss, which is demonstrated by a hearing threshold increase starting at the age of 12 months (Popelar et al., 2006). However, only 10%–20% of the IHC or OHC are missing in aged F344 rats with a severe hearing loss (Popelar et al., 2006; Bielefeld et al., 2008). Similarly, a 29% spiral ganglion cell loss was observed in elderly F344 rats (Buckiova et al., 2006). A serious age-related degeneration of the SV was reported in aging animals with a damaged layer of marginal cells, loss of pigmented cells and reduced vascularity (Gratton and Schulte, 1995; Buckiova et al., 2006; Ohlemiller et al., 2006). These changes in the peripheral hearing organ are accompanied by pathologies in the central auditory system, represented mainly by a significant decline in the glutamic acid decarboxylase (GAD) level and changes in the expression of parvalbumin-immunoreactive neurons in the central auditory system (Burianová et al., 2009; Ouda et al., 2012, 2015). For a review of age-related changes in the auditory system of F344 rats, see Syka (2010).

Most of our previous results were obtained in F344 males. Preliminary experiments performed on F344 females, indicated that females possess better parameters of hearing function than age-matched males. The aim of this study was to characterize the structural and functional differences in the auditory system of male and female F344 rats of different ages, by comparing their auditory brainstem responses (ABRs), distortion products of otoacoustic emissions (DPOAEs), and the changes in cochlear morphology.

MATERIALS AND METHODS

Animals

Hearing function was investigated in male and female F344 rats in five age groups: 3-month-, 8-month-, 12-month-, 22–24-month- and 27–30-month-old animals. The animals in all of the age groups were in good health. HEINE mini 2000 otoscope was used to exclude any eardrum pathology. Fischer 344 rats were obtained from Charles River Deutschland (Sulzfeld, Germany) and subsequently maintained in a local facility through inhouse breeding. All of the animals were housed in age-matched groups of two or three per cage, under standard laboratory conditions, in a constant environment and a 12/12 h normal light/dark cycle; food and water were available *ad libitum*. All experimental procedures were approved by the Ethical Committee of the Institute of Experimental Medicine of the Czech Academy of Sciences, and followed the guidelines of the EU Directive 2010/63/EU for animal experiments.

Auditory Brainstem Responses (ABRs) Recording

ABRs were recorded in animals placed in a sound proof and anechoic room using acoustical free-field stimulation. The walls and ceiling inside the room were covered by cones from phono-absorbent material. Acoustic measurements demonstrated that the attenuation inside the room against the noise level outside the room, was 55 dB at 250 Hz and 60–70 dB for frequencies above 500 Hz. The acoustic system inside the room was calibrated with a Bruel&Kjaer® 4939 microphone, a ZC0020 preamplifier and a B&K 2231 sound level meter (BRÜEL & KJÆR SOUND & VIBRATION MEASUREMENT A/S, Nærum, Denmark). The frequency–response curve of this system was relatively flat and varied by less than ±9 dB between 0.15 kHz and 40 kHz.

The hearing thresholds in rats were assessed by recording the ABRs in the animals under light anesthesia (ketamine 35 mg/kg, xylazine 6 mg/kg), using subcutaneous needle electrodes placed on the vertex (an active electrode) and in the neck muscles (ground and reference electrodes). The signal from the electrode was amplified 10,000-times using RA16PA RA4LI preamplifier (Tucker Davis Technologies, SystemIII, Alachua, FL, USA), band-pass filtered over the range of frequencies from 300 Hz to 3 kHz, processed with a TDT RX5-2 Pentusa Base Station (Tucker Davis Technologies, SystemIII, Alachua, FL, USA) and analyzed with BioSig software.

ABRs were evoked by tonal stimuli (3-ms duration, 1 ms rise/fall times) in the frequency range from 2 kHz to 40 kHz, or by clicks generated with a RP2.1 enhanced real-time processor (Tucker Davis Technologies, SystemIII, Alachua, FL, USA). To test the neural adaptability, a series of four clicks with variable inter-click interval (ICI) ranging from 1 ms to 50 ms were used. Acoustic stimuli were delivered in free-field conditions via a two-way loudspeaker system [Jamo® 22496 woofer (Jamo Denmark ApS, Niva, Denmark) and SEAS Excel® T25CF 002 tweeter (SEAS FABRIKKER AS, Moss, Norway)], placed 70 cm in front of the animal's head. The ABR threshold to each frequency was determined as the minimal tone intensity that still evoked a noticeable potential peak in the expected time window of the recorded signal.

Distortion Product Otoacoustic Emissions (DPOAEs) Recording

To test the functional status of the OHC, the DPOAEs were recorded with a low-noise microphone system (Etymotic probe ER-10B+; Etymotic Research, Elk Grove Village, IL, USA). DP-grams (the function of the DPOAE level on increasing stimulus frequency) were recorded with a resolution of four points per octave over the frequency range from 1 kHz to

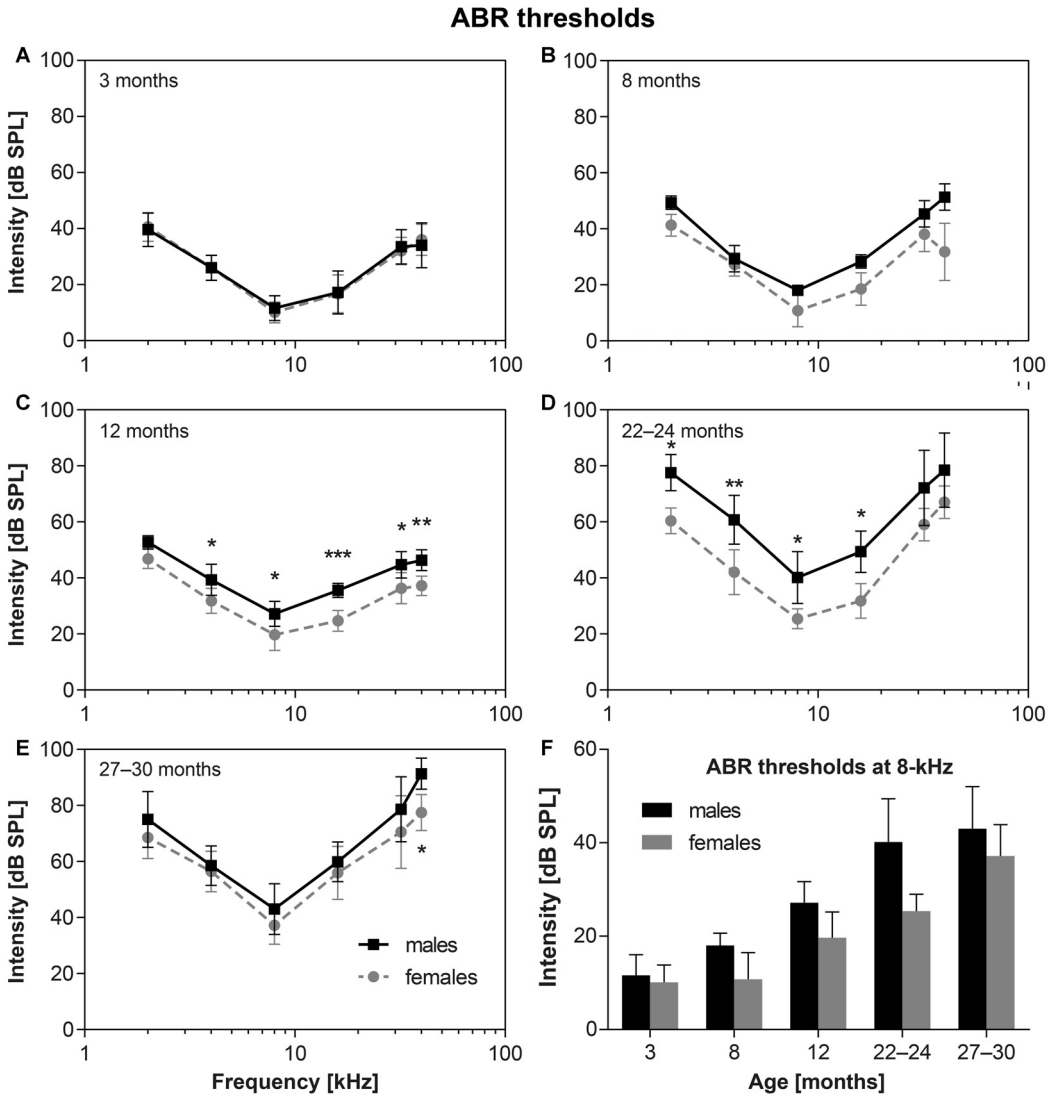


FIGURE 1 | (A–E) The average auditory brainstem response (ABR) thresholds in Fischer 344 (F344) males and females of different ages. $^{*}p < 0, 05$; $^{**}p < 0, 01$; $^{***}p < 0.001$; two-way ANOVA, Bonferroni *post hoc* tests. Mean ± SEM. **(F)** Comparison of ABR thresholds at 8 kHz for F344 males and females of individual age category. Mean ± SEM.

38 kHz. During DPOAE recording, in addition to DPOAE amplitude, also the F1 and F2 intensities in the outer ear canal were monitored to confirm the correct placement of the recording probe. Acoustic stimuli (two primary tones with frequency ratio F_2/F_1 = 1.21 and level $L_1 = L_2$ = 65 dB) were generated by a TDT System III (RP2 processor; sampling rate 100 kHz) and presented to the ear canal with two custom-made piezoelectric stimulators connected to the probe with 10-cm-long silastic tubes. The signal from the microphone was analyzed by a TDT System III (RP2 processor; sampling rate 100 kHz). DPOAEs were measured in both ears of the animals. The recording of DPOAEs were performed in an anechoic and sound proof room.

During ABR and DPOAE recordings, the rats were placed on a heating pad that automatically maintained body temperature at 38°C.

Cochlear Histology

Deeply anesthetized animals (ketamine 55 mg/kg and xylazine 8 mg/kg i.m.) were sacrificed and cochleas were quickly removed from the skull. Holes were made in the apical turn, round and oval windows, and cochleas were immersed in 4% paraformaldehyde solution for 3 h (whole mount preparation, $n = 4$ per group) or overnight (paraffin sectioning, $n = 8$ in young groups, $n = 20$ in old groups). One ear from each animal was used for cochlear cross-section preparation; the opposite ear was used for cochlear whole mounts in some animals.

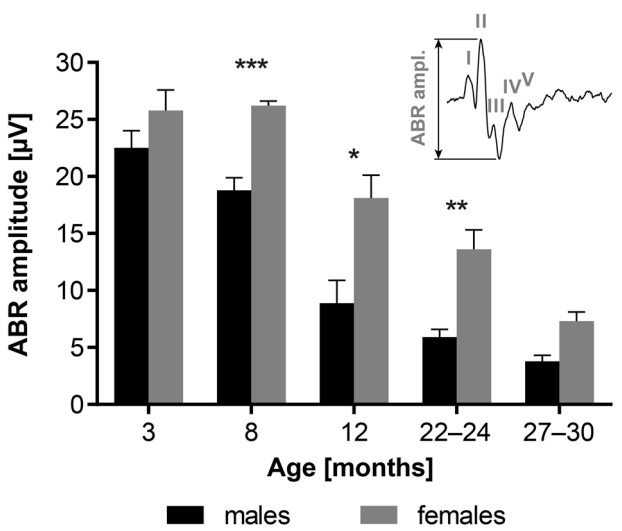


FIGURE 2 | Age-related changes in the average ABR amplitudes in F344 rats, evoked by clicks at 100 dB SPL. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; unpaired *t*-test. Mean ± SEM.

For whole mount preparation the bone of the cochlear wall and tectorial membrane were removed gradually from the cochlear apex to the base and basilar membrain together with organ of Corti was microdissected into four pieces per each cochlea. Cochlear whole mounts then were stained with anti-C-terminal binding protein 2 antibodies (anti-CtBP2, BDTransduction Laboratories, San Jose, CA, USA) for presynaptic hair cell ribbons analysis, phalloidin (# 59033, Dyomics) for hair cell visualization, and DAPI to counterstain cell nuclei. Cochlear preparations were visualized using confocal microscope Zeiss LSM 510 Duo. For each analyzed cochlea, low magnification images of all of the whole mount parts were acquired and localization of certain frequency was determined, based on a percentage distance from base using ImageJ Plugin Measure_Line[1].

The numbers of OHCs and IHC ribbons were counted using ImageJ software (Schneider et al., 2012). Part of the basilar membrane, hosting 60 OHCs (20 in each raw) in the proximity of certain frequency was analyzed. Present and missing cells were counted and the percentage of cells present was calculated. The mean value for each frequency from four cochleas per group was plotted in the resulting graph. IHC ribbon synapses were counted in image stacks. Five adjacent IHC in the proximity of the particular frequency were chosen and the synaptic puncta relating to them were counted, using Cell counter plugin of ImageJ software (Schneider et al., 2012). The number of synaptic puncta per one IHC was calculated for each frequency in each cochlea.

[1]http://www.masseyeandear.org/research/otolaryngology/investigators/laboratories/eaton-peabody-laboratories/epl-histology-resources/imagej-plugin-for-cochlear-frequency-mapping-in-whole-mounts

To analyze the SV, cochlear cross-sections were used. The fixed tissues were decalcified in ethylendiaminetetraacetic acid (0.5 M, pH 8.0) for 2 weeks, dehydrated through a graded alcohol series, cleared in toluene, and embedded in parafin. The paraffin blocks were cut into 5-μm horizontal serial sections. The slices were deparaffinized and hydrated. Tissue sections were treated with Bouin's solution to enhance the final coloration and stained with Weigert's iron hematoxylin and Masson's trichrome staining kit (HT15-1KT, Sigma-Aldrich) visualizing collagen. The changes of stria vascularis morphology were analyzed using light microscope Leica DMRXA.

Statistical Analysis

The differences between ABR hearing thresholds, DPOAE amplitudes, number of OHCs and IHC ribbon synapses in F344 males and females were tested using two-way ANOVA and Bonferroni *post hoc* test. The differences between ABR amplitudes and ABR thresholds at 8 kHz tones were tested using an unpaired *t*-test.

RESULTS

ABR Hearing Thresholds

The average ABR hearing thresholds (ABR audiograms) for F344 male and female rats of different ages are shown in **Figure 1**. The ABR thresholds in young male and female F344 rats (3-months-old) were found to be almost identical (**Figure 1A**, n = 10m + 10f). In the higher age categories the ABR thresholds increased, but the hearing alterations started later in the F344 females and progressed more slowly than in the F344 males. In contrast, the ABR thresholds in females aged 8 months (**Figure 1B**, n = 4m + 4f) were the same as in 3-month-old females, while the ABR thresholds in 8-month-old males increased in comparison with 3-month-old males, and they were significantly higher at 32–40 kHz in comparison with 8-month-old females ($p < 0.05$, Bonferroni *post hoc* test). In 12-month-old males (**Figure 1C**, n = 6m + 6f) the ABR thresholds were significantly higher, in comparison with age-matched females at frequencies 4–32 kHz ($p < 0.05$ and $p < 0.001$, Bonferroni *post hoc* test). In 22–24-month-old males (**Figure 1D**, n = 8m + 6f), the ABR thresholds were significantly higher at frequencies 2–16 kHz in comparison with females ($p < 0.05$ and $p < 0.001$, Bonferroni *post hoc* test). In 27–30-month-old males (**Figure 1E**, n = 10m + 8f), the hearing loss increased more rapidly in females than in males which resulted in a decrease of differences between hearing thresholds in these oldest females and males. Even though the ABR thresholds in F344 males and females were significantly different ($p < 0.0001$, two-way ANOVA), the differences in individual frequencies was significant only at frequency 40 kHz ($p < 0.05$, Bonferroni *post hoc* test).

Figure 1F demonstrates the comparison of the time pattern of ABR threshold changes at 8 kHz for males and females. In males the ABR threshold at 8 kHz increased continuously, and almost linearly, from the age of 3 months to the age of 27–30 months. In females this threshold increase started

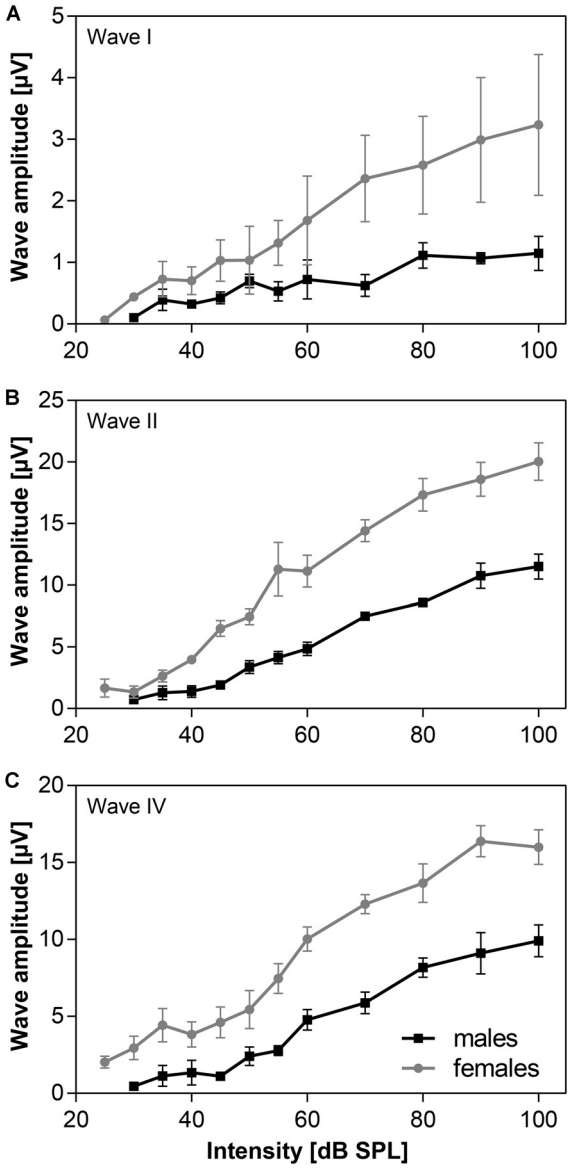


FIGURE 3 | The averaged click-evoked ABR amplitude-intensity functions, n = 5m + 5f. **(A)** ABR wave I, **(B)** ABR wave II, **(C)** ABR wave IV. Bars represent mean ± SEM.

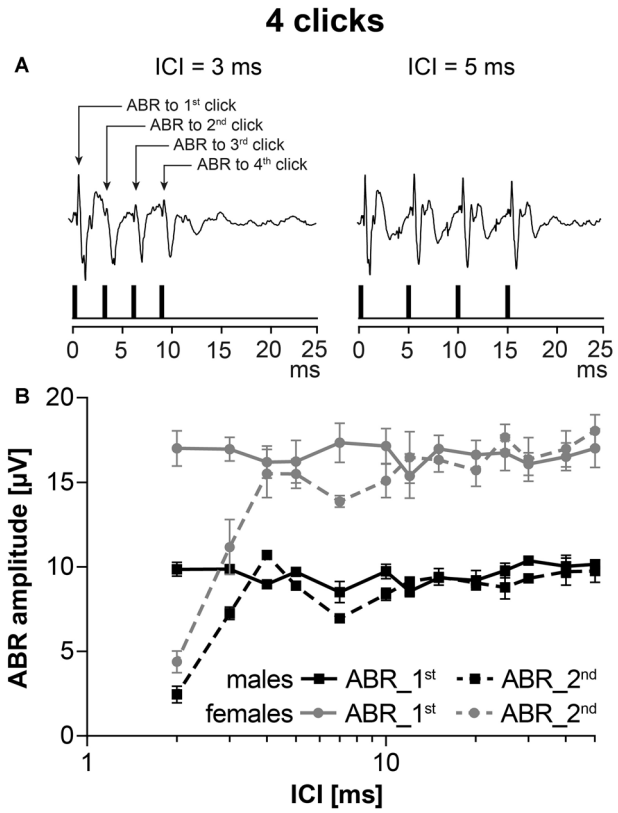


FIGURE 4 | ABRs evoked by stimulation with the series of clicks, intensity 70 dB SPL, with variable interclick interval (ICI). **(A)** Two examples of ABRs to click stimulation with ICI = 3 ms and ICI = 5 ms. **(B)** The averaged ABR amplitude-ICI functions to 1st click stimulation and to 2nd click stimulation in 24-month-old F344 males and females. Bars represent mean ± SEM.

later (in females older than 8 months) and continued to the age category 22–24 months. However, the ABR threshold at 8 kHz in the F344 females aged 22–24 months started to increase with a faster rate than in males and at the age of 27–30 months, ABR thresholds in females almost reached the thresholds in the age-matched F344 males. Whereas ABR thresholds at 8 kHz in 22–24 months old males and females were significantly different ($p < 0.0023$, unpaired t-test), ABR thresholds at 8 kHz in 27–30 month old males and females were not significantly different ($p < 0.114$, unpaired t-test; **Figure 1F**).

ABR Amplitudes

Figure 2 shows age-related changes in the ABR amplitudes, evoked by clicks at 100 dB SPL, and measured as the difference between the value of the positive peak II and the negative through between the peaks II and IV (see inserted schema). The results indicate that ABR amplitudes are significantly smaller in F344 males aged 8–24 months than in age-matched females ($p < 0.05$ to $p < 0.001$; unpaired t-test).

Amplitudes of individual ABR waves, evoked by click stimulation of variable intensity, were measured in five males and five females of 24-month old F344 rats. The averaged amplitude-intensity functions for the ABR wave I, II and IV are demonstrated in **Figure 3**. Maximal wave I amplitude (**Figure 3A**) in females was three times the maximal wave I amplitude in males. The difference in maximal wave amplitudes is less expressed in later ABR waves. The maximal wave II amplitude (**Figure 3B**) in females is twice that of males and the maximal wave IV amplitude (**Figure 3C**) is only 1.6 times that in males. These results indicate that the difference between ABR wave I amplitude, originating at the periphery, in males and females, is larger than the difference between ABR wave IV amplitude, originating in higher auditory structures.

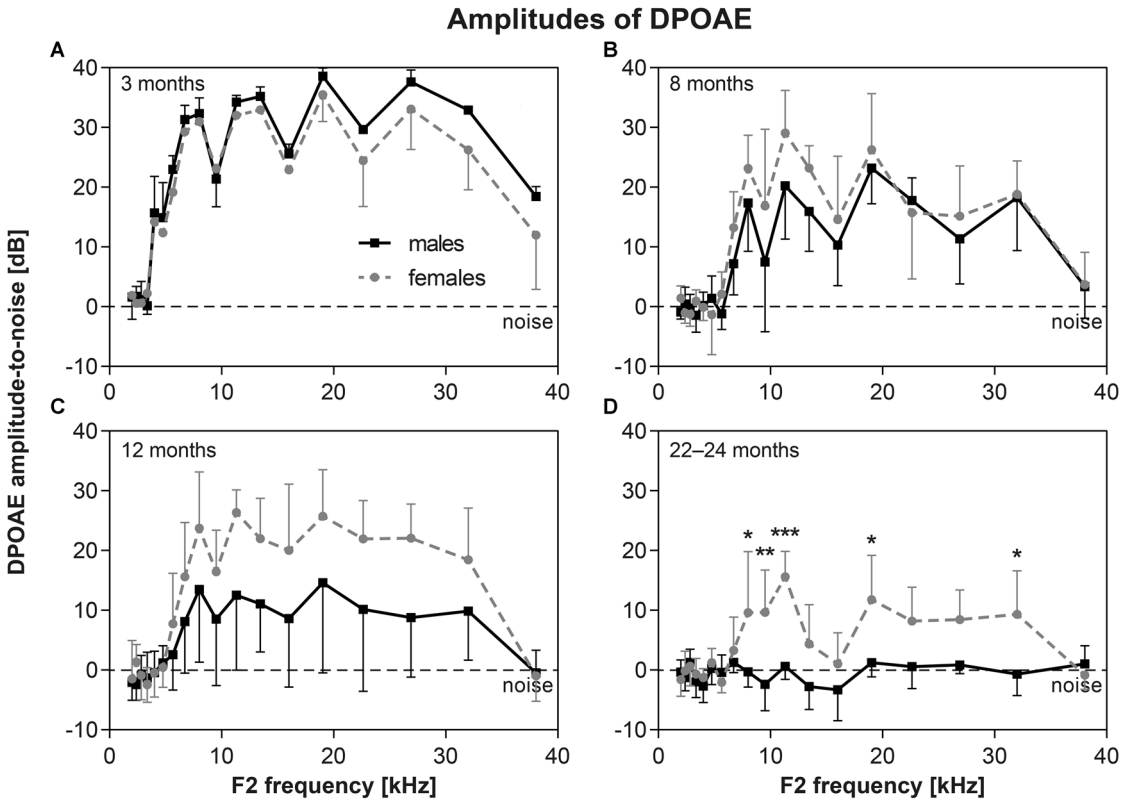


FIGURE 5 | (A–D) DP-grams in F344 rats (distortion products of otoacoustic emissions (DPOAE) amplitudes above background noise level) measured in males (n = 6–8) and females (n = 6–8) of different age categories. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; two-way ANOVA, Bonferroni *post hoc* tests. Mean ± SEM.

Neural Adaptation

To test the neural adaptation in five males and five females of 24-month old F344 rats, stimulation with a series of four clicks, intensity 70 dB SPL, with variable interclick interval (ICI) was used. **Figure 4A** demonstrates the schema of an experiment. When the ICI was short (ICI = 3 ms), ABR amplitudes to 2nd, 3rd and 4th clicks were smaller than ABR amplitude to 1st click stimulation. When the ICI was longer (ICI = 5 ms), ABR amplitudes to 2nd, 3rd and 4th click were the same as ABR amplitudes to 1st click stimulation. The function of ABR amplitudes on ICI intervals in aged males and females is demonstrated in **Figure 4B**. As the ABR amplitudes to the 2nd, 3rd and 4th click stimulation within a particular click series were similar, **Figure 4B** compares the ABR amplitudes to the 1st and 2nd click stimulation in the F344 males and females. Regardless of the smaller click-evoked ABR amplitude obtained in males, the function of the ABR amplitude on ICI was similar in both sexes, i.e., the amplitude of ABR evoked by the second click, reached the ABR amplitude of the first click at ICI of 4 ms.

DPOAEs

The functional status of cochlear OHC were checked by the recording of DPOAEs. The average DP grams (i.e., the function of DPOAE amplitudes above background noise level on F2 stimulus frequency) in six to eight F344 males and females of different age category are present in **Figure 5**. The DPOAE amplitudes of both males and females is maximal in young, 3-month-old animals (**Figure 5A**). At a higher age the DPOAE amplitudes of both males and females decreased. In animals aged 12 months (**Figure 5C**), the DPOAE amplitudes in F344 males were significantly smaller than those in F344 females ($p < 0.0001$, two-way ANOVA), but Bonferroni *post hoc* test did not show any significant difference at individual F2 frequencies. In males older than 20 months, the DPOAEs were practically absent, whereas in some of the 22–24-month-old females, the DPOAEs were still measurable (**Figure 5D**). In the age category 27–30 months no DPOAE were detectable in either male or female F344 rats (not presented).

Cochlear Morphology

Cochlear whole mount preparations were used to calculate the number of OHCs and the number of ribbon synapses docking vesicles with neurotransmitter glutamate in the IHCs in 27-month-old F344 males (n = 4) and females (n = 4). In **Figure 6**, examples of cochlear whole mounts from aged rats, showing IHC ribbon synapses immunostained with CtBP2 protein (green), in males (**Figure 6A**) and females (**Figure 6B**), and IHCs and OHCs stained with phalloidin (red) in males

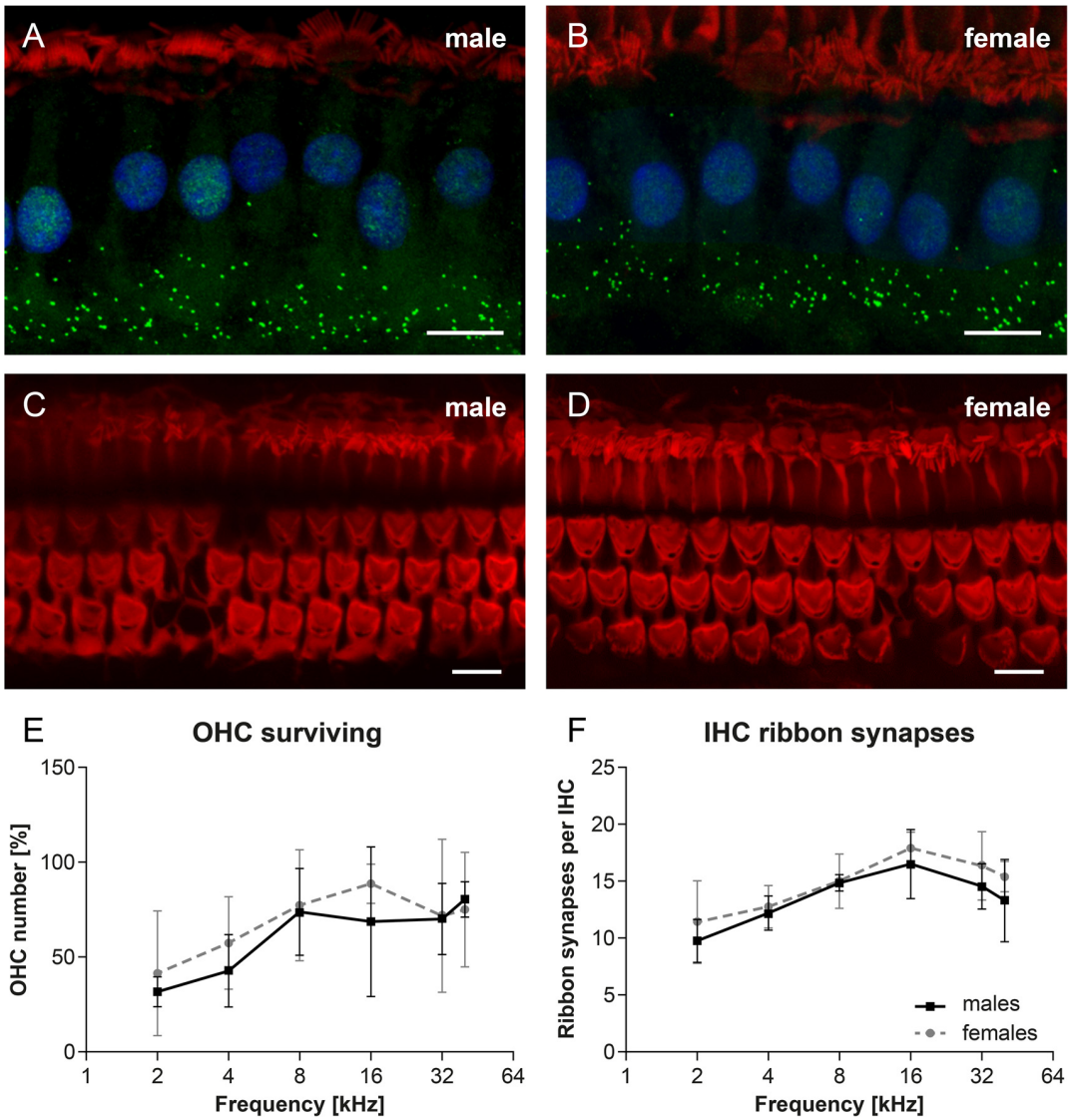


FIGURE 6 | Examples of cochlear whole mounts from 27-month-old rats, showing inner hair cell (IHC) ribbon synapses immunostained with CtBP2 protein (green), in F344 males **(A)** and females **(B)**, and IHCs and OHCs stained with phalloidin (red) in F344 males **(C)** and females **(D)**. Cell nuclei are counterstained with DAPI (blue). **(E)** The average number of surviving OHCs in individual tonotopical regions of the organ of Corti in F344 males and females. **(F)** The average number of IHC ribbon synapses per one IHC localized in individual tonotopical regions of the organ of Corti in F344 males and females. Bars represent mean ± SD. Scale bar = 10 μm.

(**Figure 6C**) and females (**Figure 6D**), are presented. The average number of surviving OHCs in individual tonotopical regions of the organ of Corti are presented in **Figure 6E**. The pronounced OHC loss was observed mainly at the apical, low-frequency end of the cochlea (50%–60%), whereas only a few OHCs were missing at the basal, high-frequency part of the cochlea (less than 10%). However, the difference between both curves is not statistically significant (p = 0.364; two-way ANOVA). The quantitative analysis of the number of IHC ribbon synapses per one IHC (**Figure 6F**) demonstrated that the average number of ribbon synapses per IHC is almost identical in both F344 males and females (p = 0.071; two-way ANOVA).

Stria Vascularis

The main structural difference between males and females has been found in the morphology of the marginal layer of the SV. Single-cell marginal layer of the SV (marked by an arrow in **Figure 7A**) consists of marginal cells located on the surface of the SV. The examples of histological section of SV in young (4 month old) and aged (27-month-old) rats are presented in **Figure 7**. Most of SV samples were taken from the middle cochlear turn. SV in young animals (**Figures 7A,B**, n = 4m + 4f) is well preserved, the layer of the marginal cells is covered by a thin layer of collagen fibers. SV in aged female rats, presented in **Figures 7C,E**, is also well preserved with a continuous layer of marginal cells. In contrast to the old F344 females, the SV in old

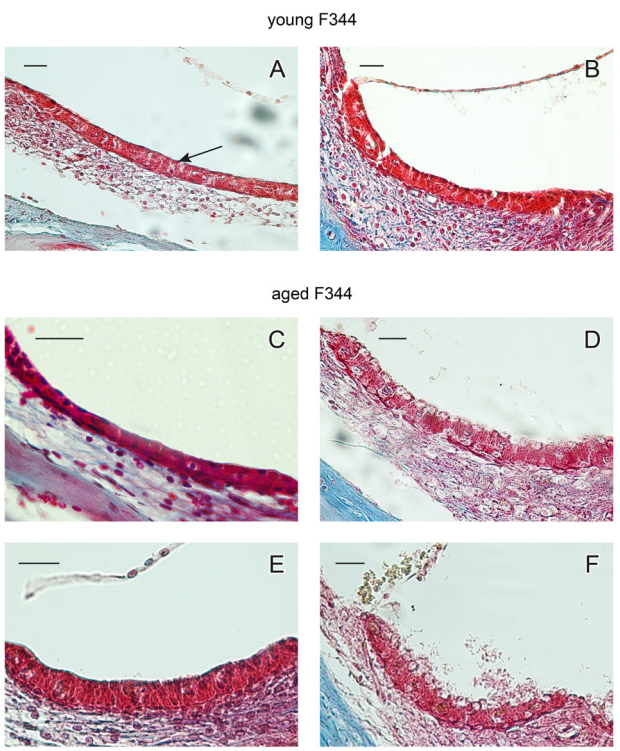


FIGURE 7 | Examples of cross-sections of the stria vascularis (SV) in young **(A,B)** and 27-month-old F344 females **(C,E)** and males **(D,F)**. The arrow in **(A)** marks the marginal layer of the SV. Scale bar = 25 μm.

males (**Figures 7D,F**) shows many degenerative changes, mainly in marginal cells that are destroyed and vacuolized.

Degenerative changes in the marginal layer of the SV in aged males were usually present in the whole extent of the cochlea. However, in two males (out of 10), the SV was damaged in only several parts of the cochlear coils, whereas in other parts of the cochlea, the SV degenerative changes were less evident. In the other hand, in three aged F344 females (out of 10) the signs of degenerative changes were present in isolated parts of the cochlea. Complete degenerative SV changes were observed in 80% of aged males and partial degeneration was found in 20% of aged males. The marginal layer of the SV was fully preserved in 70% of aged females, whereas in 30% of aged females the signs of degenerative changes in isolated parts of the cochlea were observed.

DISCUSSION

The results of this study demonstrate the different pattern of the gradual deterioration of the hearing function during aging in F344 males and females. The hearing alterations started later in F344 females (after 8th month of age) than in F344 males (after 3rd month of age) which resulted in the ABR threshold differences at most of frequencies between F344 males and females at 12 months and 22–24 months age categories. However, in age category 27–30 months the ABR threshold in the females increased with a faster rate than in males and almost reached the ABR thresholds in the age-matched males.

Similar gender differences were observed in DPOAEs. The decrease of DPOAE amplitudes during aging was faster in males than in females. In males older than 20 months, the DPOAEs were practically absent, whereas in some of the 22–24-month-old females, the DPOAEs were still measurable. In the oldest group of animals (27–30 months old), no DPOAEs were measurable in any males or females.

DPOAEs reflect *in vivo* OHC motility. However, morphological analysis of the inner ear structures demonstrated that the majority of OHCs were present and morphologically intact in 24-month-old F344 females and males, although dysfunctional. Under normal conditions the proper function of the cochlear hair cells is satisfied by the SV, the epithelial structure on the lateral wall of the cochlear duct. SV produces endolymph for the scala media, it is involved in the potassium transport to the endolymphatic space and generates positive endocochlear potential (EP; +80 to 100 mV). In transgenic mice with serious morphological defects of stria such as homozygous transgenic mice with microphthalmia-associated transcription factor gene mutation (Mitf), expressed by missing intermediate cells in the SV, the EP and ABRs were markedly reduced or almost absent (Liu et al., 2016). As previous results demonstrated normal EP in F344 rats (Bielefeld et al., 2008), we do not expect serious morphological alterations of intermediate cells in the SV. Instead, we observed pathological changes of marginal cells, that can be caused by a marked decrease of collagen fibers in the SV and spiral ligament, as previously demonstrated in F344 rats by Buckiova et al. (2006). However, the reason for evident degenerative changes in the SV in aged F344 males, in comparison with age-matched females, are not known. Chen et al. (2009) demonstrated in F344 rats the disruption of OHC motor protein prestin that can be responsible for the suppression of DPOAE amplitudes and reduced cochlear sensitivity. However, these authors did not mention the gender of the investigated F344 rats. It is probable that in our aged F344 males, the pathological state of SV marginal cells evokes functional changes resulting in a dysfunction of the auditory sensory epithelium.

Our results have demonstrated that the maximal ABR wave I amplitude, originating from the activity of auditory nerve fibers (**Figure 3A**) in females, was much larger than the maximal wave I amplitude in males. However, the difference in the maximal amplitudes of later ABR waves (**Figures 3B,C**), originating in higher auditory structures, was much smaller in males than in females.

Neural adaptation in both sexes, tested by ABR evoked by a series of clicks, was found to be comparable in both sexes. These results confirmed our hypothesis that the major reason for deterioration of the hearing function is not localized in the central part of the auditory system, but in the peripheral part.

It is known that the function of the SV is similar to some extent to the function of the tubular system in the kidney.

For example, diuretics like ethacrynic acid or furosemide, which are known to suppress the function of the Henle loop in the kidney, also suppress the function of the SV (Sellick and Johnstone, 1974; Melichar and Syka, 1978). In many strains of rats, including Fischer 344 rats, renal disease occurs earlier in life and to a more severe extent in males than in females (Abrass et al., 1995; Baylis and Corman, 1998). Two recent studies (Kwekel et al., 2013, 2015) showed sex differences in kidney gene expression in Fischer 344 rats during aging.

Sexual dimorphism was described in Fischer 344 rats in several other functions. In males, in contrast to females, a drastically accelerated rate of peripheral retinal degeneration was observed (DiLoreto et al., 1994). The males were found more likely to lose spatial reference memory with aging than females (Bizon et al., 2009).

The reasons for the earlier occurrence of the above presented pathologies in F344 male rats, in comparison with females, are not known. An important role in the sex difference is played by the estrogen receptors alpha (ERα) and beta (ERβ). Both of these subtypes of estrogen receptors have been identified in the cochlea at locations where hearing impulses are transmitted (inner and OHC, spiral ganglion cells) and in areas responsible for inner ear homeostasis (SV and spiral ligament) and there are indications that they have neuroprotective effects (Stenberg et al., 2001; Meltser et al., 2008). On the other hand, hyperandrogenism in patients with polycystic ovary syndrome was demonstrated to produce high-frequency hearing loss (Oghan and Coksuer, 2012). The gender differences in the hearing function of F344 rats may be due to combination of the damaging effect of testosterone, as well as the protective effects of estrogen and progesterone, which continue to be secreted after the cessation of the estrous cycle in aging females (Markham and Juraska, 2002). In our experiments, the prolonged secretion of sex hormones in females may be the reason for the later start of the hearing threshold increase in females, in comparison with males.

Prolactin (PRL) was recently found in the cochlea in the aged female mice (Marano et al., 2012). Histological sectioning of the cochlea from the aged female mice, followed by immunostaining with antibodies specific to PRL, revealed that spiral ganglion cells and marginal cells of the SV are the sole sites of PRL expression (Marano et al., 2013).

It has previously been shown that prolactin treatment in guinea pigs results in hyperprolactinemia, producing bone dysmorphology of the otic capsule and hearing loss (Horner et al., 2007; Seriwatanachai et al., 2008). Hyperprolactinemia, leading to hearing loss, has been also detected in menopausal women taking hormone replacement therapy containing prolactin (Metka et al., 1994).

This data provided an indication that PRL expression in aged female mice can be potentially linked to abnormal bone metabolism or degeneration of the cochlear structures leading to an age-related hearing loss. In the present study, the hearing threshold in very old F344 females (aged 27–30 months) demonstrates an accelerated increase in comparison with younger females, that can be produced by increased PRL expression in the cochlea in these very old females. Such an accelerated hearing loss was not observed in age-matched males where no PRL expresion was previously observed.

In conclusion, our results have demonstrated the different pattern of the gradual deterioration of the hearing function during aging in F344 males and females. In F344 females the hearing alterations started later in comparison with males which resulted in the ABR threshold and ABR amplitude differences at most of frequencies between F344 males and females at 12 months and 22–24 months of age. In the group of the oldest animals, the differences between the F344 males and females were reduced. The most probable and most evident differences were observed in the morphological deterioration of the SV. Whereas the SV was well preserved in most 24-month-old females, in most aged-matched males the marginal layer of the SV was degenerated and distorted. Another reason for age-related gender differences in the hearing function in F344 rats, could be the different time pattern of the secretion of sex hormones in males and females or the expression of prolactin in very old females. This study presents evidence that the gender of F344 rats should be taken into account when reporting the results of an experimental study.

AUTHOR CONTRIBUTIONS

JS and JP designed the study, interpreted the data and wrote the manuscript. ZB, FC, JSB and NR performed recording of the ABR and DPOAE. TC and JSB performed cochlear histology. JP, ZB and JSB performed data processing and statistical analysis.

ACKNOWLEDGMENTS

This study was supported by Czech Science Foundation grant P30/12/G069, by European Union Seventh Framework Programme grant FP7-PEOPLE-2013-IAPP-TARGEAR and by the by European Union, the Operational Programme Research, Development and Education in the framework of the project "Centre of Reconstructive Neuroscience", registration number CZ.02.1.01/0.0./0.0/15_003/0000419.

REFERENCES

Abrass, C. K., Adcox, M. J., and Raugi, G. J. (1995). Aging-associated changes in renal extracellular matrix. *Am. J. Pathol.* 146, 742–752.

Baylis, C., and Corman, B. (1998). The aging kidney: insights from experimental studies. *J. Am. Soc. Nephrol.* 9, 699–709.

Bielefeld, E. C., Coling, D., Chen, G. D., Li, M., Tanaka, C., Hu, B. H., et al. (2008). Age-related hearing loss in the Fischer 344/NHsd rat substrain. *Hear. Res.* 241, 26–33. doi: 10.1016/j.heares.2008.04.006

Bizon, J. L., LaSarge, C. L., Montgomery, K. S., McDermott, A. N., Setlow, B., and Griffith, W. H. (2009). Spatial reference and working memory across the lifespan of male Fischer 344 rats.

Neurobiol. Aging 30, 646–655. doi: 10.1016/j.neurobiolaging.2007.08.004

Buckiova, D., Popelar, L., and Syka, J. (2006). Collagen changes in the cochlea of aged Fischer 344 rats. *Exp. Gerontol.* 41, 296–302. doi: 10.1016/j.exger.2005.11.010

Burianová, J., Ouda, L., Profant, O., and Syka, J. (2009). Age-related changes in GAD levels in the central auditory system of the rat. *Exp. Gerontol.* 44, 161–169. doi: 10.1016/j.exger.2008.09.012

Chen, G. D., Li, M., Tanaka, C., Bielefeld, E. C., Hu, B. H., Kermany, M. H., et al. (2009). Aging outer hair cells (OHCs) in the Fischer 344 rat cochlea: function and morphology. *Hear. Res.* 248, 39–47. doi: 10.1016/j.heares.2008.11.010

DiLoreto, D. Jr., Cox, C., Grover, D. A., Lazar, E., del Cerro, C., and del Cerro, M. (1994). The influences of age, retinal topography, and gender on retinal degeneration in the Fischer 344 rat. *Brain Res.* 647, 181–191. doi: 10.1016/0006-8993(94)91316-1

Fetoni, A. R., Picciotti, P. M., Paludetti, G., and Troiani, D. (2011). Pathogenesis of presbycusis in animal models: a review. *Exp. Gerontol.* 46, 413–425. doi: 10.1016/j.exger.2010.12.003

Gates, G. A., and Mills, J. H. (2005). Presbycusis. *Lancet* 366, 1111–1120. doi: 10.1016/S0140-6736(05)67423-5

Gratton, M. A., and Schulte, B. A. (1995). Alterations in microvasculature are associated with atrophy of the stria vascularis in quiet-aged gerbils. *Hear. Res.* 82, 44–52. doi: 10.1016/0378-5955(94)00161-i

Horner, K. C., Cazals, Y., Guieu, R., Lenoir, M., and Sauze, N. (2007). Experimental estrogen-induced hyperprolactinemia results in bone-related hearing loss in the guinea pig. *Am. J. Physiol. Endocrinol. Metab.* 293, E1224–E1232. doi: 10.1152/ajpendo.00279.2007

Huang, Q., and Tang, J. (2010). Age-related hearing loss or presbycusis. *Eur. Arch. Otorhinolaryngol.* 267, 1179–1191. doi: 10.1007/s00405-010-1270-7

Kwekel, J. C., Desai, V. G., Moland, C. L., Vijay, V., and Fuscoe, J. C. (2013). Life cycle analysis of kidney gene expression in male F344 rats. *PLoS One* 8:e75305. doi: 10.1371/journal.pone.0075305

Kwekel, J. C., Vijay, V., Desai, V. G., Moland, C. L., and Fuscoe, J. C. (2015). Age and sex differences in kidney microRNA expression during the life span of F344 rats. *Biol. Sex Differ.* 6:1. doi: 10.1186/s13293-014-0019-1

Liu, H., Li, Y., Chen, L., Zhang, Q., Pan, N., Nichols, D. H., et al. (2016). Organ of corti and stria vascularis: is there an interdependence for survival? *PLoS One* 11:e0168953. doi: 10.1371/journal.pone.0168953

Marano, R. J., Tickner, J., and Redmond, S. L. (2012). Age related changes in gene expression within the cochlea of C57BL/6J mice. *Aging Clin. Exp. Res.* 24, 603–611. doi: 10.3275/8590

Marano, R. J., Tickner, J., and Redmond, S. L. (2013). Prolactin expression in the cochlea of aged BALB/c mice is gender biased and correlates to loss of bone mineral density and hearing loss. *PLoS One* 8:e63952. doi: 10.1371/journal.pone.0063952

Markham, J. A., and Juraska, J. M. (2002). Aging and sex influence the anatomy of the rat anterior cingulate cortex. *Neurobiol. Aging* 23, 579–588. doi: 10.1016/s0197-4580(02)00004-0

Melichar, I., and Syka, J. (1978). The effects of ethacrynic acid upon the potassium concentration in guinea pig cochlear fluids. *Hear. Res.* 1, 35–41. doi: 10.1016/0378-5955(78)90007-2

Meltser, I., Tahera, Y., Simpson, E., Hultcrantz, M., Charitidi, K., Gustafsson, J. A., et al. (2008). Estrogen receptor β protects against acoustic trauma in mice. *J. Clin. Invest.* 118, 1563–1570. doi: 10.1172/JCI32796

Metka, M., Holzer, G., Raimann, H., Heytmanek, G., Hartmann, B., and Kurz, C. (1994). The role of prolactin in the menopause. *Maturitas* 20, 151–154. doi: 10.1016/0378-5122(94)90011-6

Oghan, F., and Coksuer, H. (2012). Does hyperandrogenism have an effect on hearing loss in patients with polycystic ovary syndrome? *Auris Nasus Larynx* 39, 365–368. doi: 10.1016/j.anl.2011.06.006

Ohlemiller, K. K., Lett, J. M., and Gagnon, P. M. (2006). Cellular correlates of age-related endocochlear potential reduction in a mouse model. *Hear. Res.* 220, 10–26. doi: 10.1016/j.heares.2006.06.012

Ouda, L., Burianova, J., and Syka, J. (2012). Age-related changes in calbindin and calretinin immunoreactivity in the central auditory system of the rat. *Exp. Gerontol.* 47, 497–506. doi: 10.1016/j.exger.2012.04.003

Ouda, L., Profant, O., and Syka, J. (2015). Age-related changes in the central auditory system. *Cell Tissue Res.* 361, 337–358. doi: 10.1007/s00441-014-2107-2

Popelar, J., Groh, D., Pelánová, J., Canlon, B., and Syka, J. (2006). Age-related changes in cochlear and brainstem auditory functions in Fischer 344 rats. *Neurobiol. Aging* 27, 490–500. doi: 10.1016/j.neurobiolaging.2005.03.001

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. doi: 10.1038/nmeth.2089

Sellick, P. M., and Johnstone, B. M. (1974). Differential effects of ouabain and ethacrynic acid on the labyrinthine potentials. *Pflugers Arch.* 352, 339–350. doi: 10.1007/bf00585686

Seriwatanachai, D., Thongchote, K., Charoenphandhu, N., Pandaranandaka, J., Tudpor, K., Teerapornpuntakit, J., et al. (2008). Prolactin directly enhances bone turnover by raising osteoblast-expressed receptor activator of nuclear factor κB ligand/osteoprotegerin ratio. *Bone* 42, 535–546. doi: 10.1016/j.bone.2007.11.008

Stenberg, A., Wang, H., Fish, J. III., Schrott-Fischer, A., Sahlin, L., and Hultcrantz, M. (2001). Estrogen receptors in the normal adult and developing human inner ear and in Turner syndrome. *Hear. Res.* 157, 87–92. doi: 10.1016/s0378-5955(01)00280-5

Syka, J. (2010). The Fischer 344 rat as a model of presbycusis. *Hear. Res.* 264, 70–78. doi: 10.1016/j.heares.2009.11.003

World Health Organization. (2017). Deafness and hearing loss. Available online at: http://www.who.int/mediacentre/factsheets/fs300/en/

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

MINI REVIEW
published: 12 December 2017
doi: 10.3389/fnagi.2017.00411

The Role of Insulin-Like Growth Factor 1 in the Progression of Age-Related Hearing Loss

Lourdes Rodríguez-de la Rosa[1,2,3], *Luis Lassaletta*[1,2,3,4], *Miryam Calvino*[3,4], *Silvia Murillo-Cuesta*[1,2,3*†] *and Isabel Varela-Nieto*[1,2,3†]

[1] *"Alberto Sols" Biomedical Research Institute CSIC-UAM, Madrid, Spain,* [2] *Centro de Investigación Biomédica en Red de Enfermedades Raras, Instituto de Salud Carlos III, Madrid, Spain,* [3] *Hospital La Paz Institute for Health Research (IdiPAZ), Madrid, Spain,* [4] *Otorhinolaryngology Department, Hospital La Paz, Madrid, Spain*

Aging is associated with impairment of sensorial functions and with the onset of neurodegenerative diseases. As *pari passu* circulating insulin-like growth factor 1 (IGF-1) bioavailability progressively decreases, we see a direct correlation with sensory impairment and cognitive performance in older humans. Age-related sensory loss is typically caused by the irreversible death of highly differentiated neurons and sensory receptor cells. Among sensory deficits, age-related hearing loss (ARHL), also named presbycusis, affects one third of the population over 65 years of age and is a major factor in the progression of cognitive problems in the elderly. The genetic and molecular bases of ARHL are largely unknown and only a few genes related to susceptibility to oxidative stress, excitotoxicity, and cell death have been identified. IGF-1 is known to be a neuroprotective agent that maintains cellular metabolism, activates growth, proliferation and differentiation, and limits cell death. Inborn IGF-1 deficiency leads to profound sensorineural hearing loss both in humans and mice. IGF-1 haploinsufficiency has also been shown to correlate with ARHL. There is not much information available on the effect of IGF-1 deficiency on other human sensory systems, but experimental models show a long-term impact on the retina. A secondary action of IGF-1 is the control of oxidative stress and inflammation, thus helping to resolve damage situations, acute or made chronic by aging. Here we will review the primary actions of IGF-1 in the auditory system and the underlying molecular mechanisms.

Keywords: ARHL, GH, IGF system, presbycusis, rare diseases

OPEN ACCESS

Edited by:
Filippo Tempia,
Università degli Studi di Torino, Italy

Reviewed by:
Fabio Mammano,
Università degli Studi di Padova, Italy
Jacques Epelbaum,
Institut National de la Santé et de la Recherche Médicale, France

***Correspondence:**
Silvia Murillo-Cuesta
smurillo@iib.uam.es

† These authors have contributed equally to this work and are both senior authors.

Received: *13 October 2017*
Accepted: *27 November 2017*
Published: *12 December 2017*

Citation:
Rodríguez-de la Rosa L, Lassaletta L, Calvino M, Murillo-Cuesta S and Varela-Nieto I (2017) The Role of Insulin-Like Growth Factor 1 in the Progression of Age-Related Hearing Loss. Front. Aging Neurosci. 9:411. doi: 10.3389/fnagi.2017.00411

IGF SYSTEM, UPSTREAM REGULATION, AND DOWNSTREAM IGF-1 SIGNALING

The mammalian IGF system is comprised of insulin-like growth factors (IGF), receptors and binding proteins (IGFBP). IGFs and insulin are small polypeptides produced as pre-pro-peptides that can bind the insulin (IR) and IGF-1 (IGF1R) tyrosine kinase receptors. IGFs also bind the cation-independent mannose-6-phosphatase IGF-2 receptor (IGF2R; Foulstone et al., 2005). The biological actions of IGFs are primarily mediated by binding to the IGF1R, a heterotetramer with extracellular IGF binding domains and intracellular tyrosine kinase domains. The carboxy-terminal domain has docking sites for intracellular substrates (IRS1-4/SHC; Laviola et al., 2007) that, in

turn, bind and activate a network of intracellular signaling molecules. Factor-receptor interactions are modulated by binding proteins (IGFBPs) and associated proteases. In plasma, IGFBPs carry IGFs and these regulate their half-life, distribution and biological actions. IGFBPs control the bioavailability of IGF-1 to its receptors by competing with receptors for free factors (Firth and Baxter, 2002).

The growth hormone (GH) is a peptide hormone secreted by somatotroph cells of the pituitary gland. GH stimulates the growth of all body tissues, thus its level rises progressively during childhood and peaks at puberty. IGF-1 is secreted by the liver as a result of stimulation by GH, which binds to its receptor, GHR, inducing homodimerization and initiating signal transduction through receptor-associated Janus kinase (JAK) 2 (Herrington et al., 2000). Activation of signal transducers and transcription activators of STAT5b family members is critical for regulating liver IGF-1 gene expression (Davey et al., 2001), as well as for the transcriptional regulation of other IGF system genes, including those coding for IGFBP3 and IGFALS. ALS is a secreted hepatic protein that can be found freely circulating or forming a ternary complex with IGF-1 and IGFBP-3, thus prolonging the half-life of IGF-1 and decreasing its availability to tissues (Boisclair et al., 2001).

At the cell surface, IGF-1 binds to high affinity IGF1R, inducing autophosphorylation and allowing docking of the receptor substrates IRS-1 to IRS-4, Grb2-associated binder 1 (Gab-1) and the Src homology 2 domain containing protein (SHC). Activated docking proteins subsequently recruit cytoplasmic components of downstream signaling pathways, including the MAPK (RAF-MEK-ERK1/2 and p38) and the PI3K-AKT pathways, and transduce the IGF signaling (Siddle, 2011). Depending on the cellular context, IGF-1 regulates different processes. For example, IGF-1 modulates gene expression in chondrocytes (Yang et al., 2017), protein synthesis in osteoblasts (Guo et al., 2017), cell cycle in enterocytes (Van Landeghem et al., 2015), metabolism in adipocytes (Chang et al., 2016), survival in cochlear hair cells (Yamahara et al., 2017) and autophagy in otic neural precusors (Aburto et al., 2012b). IGF1R can also translocate to the nucleus, activate transcription and regulate gene expression (Sehat et al., 2010).

Analysis of downstream signaling in the deaf *Igf1*$^{-/-}$ mouse has further contributed toward understanding its cochlear actions and demonstrated that an IGF-1 deficit is associated with activation of the p38 MAPK pathway (related to the cellular response to stress). RAF-ERK1/2 and AKT activity (cell proliferation and survival) are also impaired (Sanchez-Calderon et al., 2010) but the overall autophagic flux is unaffected (de Iriarte Rodríguez et al., 2015b; Magariños et al., 2017). Further analysis of IGF-1 signaling has been carried out by studying null mice for downstream targets such as IRS1 (Tang et al., 2017), IRS2, PTP1B (Murillo-Cuesta et al., 2012), GRF1 (Fernández-Medarde et al., 2009), and CRAF (De Iriarte Rodriguez et al., 2015a). Cochlear comparative transcriptomics have unveiled the role of FoxM1 and FoxP3 forkhead box transcription factors, and myocyte enhancer factor-2 (MEF-2) in inner ear development as well as IGF-1 cochlear actions (Sanchez-Calderon et al., 2010). The impact of IGF-1 deficiency depends on the tissue and organs studied. In the *Igf1*$^{-/-}$ mouse bone, cell survival and AKT signaling are gravely affected, but RAF kinase-mediated proliferation is not (Rodríguez-de La Rosa et al., 2014). In the mouse retina, however, IGF-1 deficit causes impairment of the autophagic flux, leading to increased inflammation, apoptosis and age-associated blindness (Rodriguez-de La Rosa et al., 2012; Arroba et al., 2016). Finally, analysis of the *Igf1*$^{-/-}$ vestibule has confirmed the role of p38 and added new players such as p53 and microRNAs (Rodríguez-de la Rosa et al., 2015).

In summary, IGF-1 has a role in brain development and maturation (Dyer et al., 2016). Later in life, bioactive IGF-1 circulating levels are reduced, a trend that has been associated with human frailty and cognitive decline (Vestergaard et al., 2014). IGF-1 deficiency leads to increased inflammation and to the failure of intracellular cell renewal mechanisms. This is critical in the inner ear because, as discussed below, none of the main cell types essential for hearing regenerate (Mittal et al., 2017).

INNER EAR DEVELOPMENT, ADULT ANATOMY, AND AGE-ASSOCIATED DEGENERATION

The inner ear develops from the otic ectodermal placode that invaginates to form the otic vesicle. This transitory embryonic structure contains the information required to generate most cell types of the adult inner ear, including the sensory cells and the auditory-vestibular neurons (Kelly and Chen, 2009; Magariños et al., 2012, 2014; Burns et al., 2015). Development of the inner ear is tightly regulated by intrinsic and extrinsic factors (Sanchez-Calderon et al., 2007; Gálvez et al., 2017). Among these factors, IGF-1 promotes proliferation and survival of otic progenitor cells, supports neurogenesis and facilitates late differentiation in species from fish to humans (Ayaso et al., 2002; Schlueter et al., 2007; Zou et al., 2009; Aburto et al., 2012a; Varela-Nieto et al., 2013; Tafra et al., 2014). IGF-1 plays a key role in brain development and maintenance of stem cells (Nieto-Estévez et al., 2016).

The adult inner ear comprises the cochlea and the vestibular organ, which are responsible for hearing and equilibrium, respectively. The organ of Corti in the cochlea contains highly specialized hair cells (inner -IHC- and outer -OHC-), which transform mechanical sounds into electrochemical signals that are conveyed to the brain by the VII vestibulocochlear cranial nerve (Stephenson, 2012). Sound induces the movement of hair cell stereocilia, causing the opening of ion channels, an influx of K^+ ions and depolarization. Depolarization of IHC results in glutamate release and synapse with 10–30 afferent auditory bipolar neurons. Meanwhile, OHCs amplify the incoming sound stimulation and enhance frequency selectivity of the cochlear response (Fettiplace and Kim, 2014; Reichenbach and Hudspeth, 2014). The organ of Corti is connected to the brain by two types of neurons in the spiral ganglion (SGN; Coate and Kelley, 2013). Type I and type II neurons innervate IHC and OHC respectively. Of these two kinds of neurons, type I is the most abundant (95%). The SGN

TABLE 1 | Reported mutations of the *IGF1* gene.

	(Woods et al., 1996)	(Batey et al., 2014)	(Walenkamp et al., 2005)	(Netchine et al., 2009)	(Shaheen et al., 2014)	(Fuqua et al., 2012)	(Van Duyvenvoorde et al., 2010, 2011)
Mutation type	Homozygous Deletion	Heterozygous Deletion	Homozygous Missense mutation	Homozygous Missense mutation	Homozygous Missense mutation	Heterozygous Splicing mutation	Heterozygous Insertion
Mutational analysis	181-bp deletion of *IGF1* gene (ex 4–5)	262-kb deletion of chr 12 (*IGF1* gene)	Val44Met c.274G?A, p.V44M	Arg36Gln c.251G?A, p.R36Q (ex 4)	Arg98Trp c.292C?T, p.R98W	Splicing excision ex 4 c.402+1G?C, p.N74Rfs*8	Stop codon ex 3 c.243-246dupCAGC, p.S83Qfs*13
Clinical data	♂ 15.8 yr Intrauterine growth restriction Postnatal growth failure Microcephaly Micrognathia Clinodactyly Mild myopia Cognitive delay	♂ 2.3–8.4 yr Intrauterine growth restriction Postnatal growth failure Microcephaly Micrognathia Clinodactyly Cognitive delay	♂ 55 yr Intrauterine growth restriction Progressive postnatal growth failure Microcephaly Dysmorphic features Severe cognitive delay Deaf-mutism	♂ 11 mo-9 yr Intrauterine growth restriction Progressive postnatal growth failure Microcephaly Non-dysmorphic features Clinodactyly Mild cognitive delay	♀ 2.8 yr (sibling-1) ♂ 5 yr (sibling-2) Primordial dwarfism (severe prenatal and postnatal growth deficiency)	♂ 8.8 yr Severe postnatal growth failure Normal physical examination Normal cognitive development	♀ 8.2 yr (sibling-1) Slow motor development Poor growth Delayed bone age ♂ 6.2 yr (sibling-2) Poor growth Delayed bone maturation
Consanguinity	Yes	No	Yes	Yes	Yes	No	No
Birth weight (kg)	1.4 (−3.9SD)	2.7 (−1.5SDS)	1.4 (−3.9SDS)	2.3 (−2.4SDS)	Sib-1: 1.6 (−3.5SD) Sib-2: ND	3.0 (−1.5SDS)	Sib-1: 2.3 (−2.9SDS) Sib-2: 3.3 (-1.2SDS)
Birth length (cm)	37.8 (−5.4SD)	47.6 (−1.2SDS)	39 (-4.3SDS)	44 (−3.7SDS)	ND	47 (-0.6SDS)	Sib-1: 44 (−3.8SDS) Sib-2: 50 (−1.0SDS)
Growth weight (kg)	15.8 yr: 23 (−6.5SD)	2.3 yr: 8.8 (−3.8SDS) 5.3 yr: 14.4 (−2.5SDS) 8.4 yr: 21.9 (−1.5SDS)	ND	11 mo: 5.3 (−5.0SDS) 2.8 yr: 7.0 (−7.0SDS)	2.8 yr, sib-1: 8.2 (−4.1SD) 5 yr, sib-2: 9 (−4.9SD)	8.8 yr: 21 (−2.1 SDS)	ND
Growth height (cm)	15.8 yr: 119.1 (−6.9SD)	2.3 yr: 77.5 (−3.1SDS) 5.3 yr: 96.9 (−2.9SDS) 8.4 yr: 114.9 (−2.7SDS)	55 yr: 117.8 (−8.5 SDS)	11 mo: 64 (−3.7SDS) 2.8 yr: 76 (−4.9SDS)	2.8 yr, sib-1: 81 (−3.2SD) 5 yr, sib-2: 89 (−4.3SD)	8.8 yr: 109 (−4.0 SDS)	8.2 yr, sib-1: 108.9 (−4.1 SDS) 6.2 yr, sib-2: 98.7 (−4.6 SDS)
Hearing impairment	15.8 yr: severe bilateral HL	Normal hearing	55 yr: severe bilateral HL	9 yr: normal hearing	ND	Normal hearing	ND
IGF-1 levels (ng/mL)	Undetectable 15 yr: ?3	Low-normal 2.3 yr: 43.7; 5.3 yr: 58.5 8.4 yr: 100	Very high 55 yr: 606 (+7.3SDS)	Low 2.7 yr: 11 (before GH treatment)	ND	Low-normal 9.3 yr: 115 (−2.2SDS) (before GH treatment)	Low 8.2 yr, sib-1: 76 (−2.3SDS) 6.2 yr, sib-2: 35 (−2.6SDS)
IGFBP-3 levels (mg/L)	Normal 15 yr: 3.3	Normal 5.3 yr: 4.3 8.4 yr: 5.4	Normal 55 yr: 1.98 (+0.1SDS)	Normal to high (after GH treatment)	ND	Normal 9.3 yr: 2.4 (−1.2SDS) (before GH treatment)	Normal 8.2 yr, sib-1: 3.6 (1.2SDS) 6.2 yr, sib-2: 2.1 (0.1SDS)

(Continued)

TABLE 1 | Continued

	(Woods et al., 1996)	(Batey et al., 2014)	(Walenkamp et al., 2005)	(Netchine et al., 2009)	(Shaheen et al., 2014)	(Fuqua et al., 2012)	(Van Duyvenvoorde et al., 2010, 2011)
ALS levels (mg/L)	Normal	Normal 5.3 yr: 10	High 55 yr: 28.9 (+3.4SDS)	Normal to high (after GH treatment)	ND	Normal to high 9.3 yr: 13 (before GH treatment)	Normal 8.2 yr, sib-1: 20.1 (1.1SDS) 6.2 yr, sib-2: 11.5 (-0.4SDS)
IGF-1 affinity for IGF1R	ND	ND	Extremely low 90-fold lower	Partially reduced 3.9-fold lower	ND	ND	No affinity

Overview of homozygous and heterozygous mutations described in the human IGF1 gene. HL, hearing loss; mo, month; ND, not determined; SD, standard deviation; SDS, standard deviation score; sib, sibling; yr, year. Hearing loss was observed in patients with undetectable or very high IGF-1 levels (2 out 7 cases).

axons form the cochlear branch of the vestibulocochlear nerve and connect the peripheral spiral ganglia with the cochlear nuclei at the brainstem. Sound information progresses in a complex multisynaptic, parallel, and ascendant pathway from the cochlea through the brainstem nuclei to the auditory cortex, preserving the tonotopic organization (Tsukano et al., 2017).

Expression levels of IGF-1 and its high affinity receptor, IGF1R, are elevated during late cochlear development, decline significantly after birth, but baseline expression is maintained throughout the organism's life (Murillo-Cuesta et al., 2011; Okano et al., 2011). In the mouse cochlea, IGF-1 is clearly detected in spiral ganglion neurons and stria vascularis, and its expression is modulated with aging (Riva et al., 2007). IGF binding proteins are also expressed in the developing ear and throughout life (Okano and Kelley, 2013). Finally, IGF system elements are expressed in the vestibular system over a similar time course (Degerman et al., 2013; Rodríguez-de la Rosa et al., 2015).

During aging, peripheral and central auditory structures degenerate, leading to ARHL (Fetoni et al., 2011). The primary pathological alterations observed in mouse models include progressive degeneration and loss of HC and SGN (Bao and Ohlemiller, 2010; Bowl and Dawson, 2015), as well as changes in the central auditory pathway. Typically, presbycusis debuts with loss of OHC, mainly in basal cochlear regions (high frequencies), and extends toward the apex and IHC (Ohlemiller and Gagnon, 2004). OHC defects result in a moderate increase of the hearing threshold, whereas defects in IHC or the auditory neurons can lead to profound deafness (Ouda et al., 2015). Swelling of the afferent nerve terminals and a decrease in the density of their associated ribbon synapses causes a synaptopathy that is sometimes the primary defect (Wan and Corfas, 2015).

The stria vascularis is a three-cell layer structure within the cochlea that maintains the K+ concentration and the endocochlear potential (Magariños et al., 2012). During aging, the stria vascularis shows disorganization and atrophy, with loss of marginal cells and progressive merging of strial capillaries (Ohlemiller, 2009). In addition, thinning, degeneration of fibrocytes and loss of capillaries are observed in the spiral ligament (Hequembourg and Liberman, 2001). In human cohorts, decreased age-related-IGF-1 bioavailability correlates with progression of hearing impairment (Lassale et al., 2017), as will be discussed in depth below. Studies carried out with *Igf1*$^{-/-}$ and *Igf1*$^{-/+}$ mice confirmed this trend and showed acceleration in the damage of the neural structures and stria vascularis (Riquelme et al., 2010).

The molecular mechanisms underlying ARHL that have been described include redox imbalance, accumulation of mitochondrial DNA damage, and excitotoxicity, leading to apoptotic and necrotic cell death (Menardo et al., 2012; Wong and Ryan, 2015). Interestingly, experimental models indicate that anti-oxidant therapy and control of micronutrients could prevent or ameliorate ARHL (Fetoni et al., 2009; Guastini et al., 2011; Ding et al., 2016). These mechanisms are similar to those involved in drug- and noise-induced hearing loss (Frisina et al., 2016; Kalinec et al., 2017).

TABLE 2 | GH/IGF-1 axis and deafness.

Gene	HGDM® mutations (number)	General phenotype	Deafness mutation	Clinical cases	Auditory phenotype	References
GHRHR	Missense (26) Splicing (10) Regulatory (4) Small del (4) Small ins (2) Gross del (1)	Severe dwarfism Central obesity ↑ LDL cholesterol levels ↑ Systolic blood pressure ↓Cranial volume, frontal bossing ↓Depth of skull, ↓facial height, Laryngopharyngeal reflux Laryngeal constriction ↓↓ GH and IGF-1 serum levels	Homozygous splice mutation (c.5711 G.A)	26 (13 ♂, 13 ♀) 47.6 ± 15.1 yr	↑ prevalence of dizziness Early mild high-tone SNHL ↓Stapedius reflex ↓ Transient evoked otoacoustic emissions	Prado-Barreto et al., 2014
GHR	Missense (56) Splicing (21) Small del (4) Small ins (4) Gross del (9) Complex (1)	Laron syndrome Dwarfism Obesity Hypogenitalism Hypoglycemia at birth and in early infancy	GH-R-W-15X (exon 2) GH-R-R217X (exon 7) GH-R-3,5,6 exon del GH-R-43X/Norm (exon 4)	5 untreated (2 ♂, 3 ♀), 49.2 ± 4.8 yr 1 with delayed rhIGF-1 treatment (♂, 15 yr)	Hearing impairment (50% low-tone SNHL; 16.6% high-tone SNHL; 25% combined high-/low-tone SNHL; 8.3% mixed HL)	Attias et al., 2012
PTPN11	Missense (111) Splicing (4) Small del (7) Small ins (1) Small indels (3) Gross del (1) Gross ins (3) Complex (2) Repeats (1)	Noonan syndrome (craniofacial dysmorphic features, short stature, congenital heart defects, including pulmonary stenosis) Leopard syndrome lentigines; Electrocardiogram conduction abnormalities; ocular hypertelorism; pulmonary stenosis; abnormalities of the genitalia; retardation of growth SNHL (15–25% of patients with Leopard syndrome)	Heterozygous missense mutation (c.1381G>A)	5 yr ♀	Bilateral profound SNHL Enlarged vestibular aqueducts	Chu et al., 2013
			Missense mutation (c.836A>G, Tyr279Cys)	17 yr ♂	Severe bilateral SNHL	Martínez-Quintana and Rodríguez-González, 2012
			Missense mutation in exon 7 (836A3G; Tyr279Cys)	16 yr ♂	Severe bilateral SNHL	Kim et al., 2011
			Mutation c.1510A > G Mutation c. 124A > G Mutation c. 922A > G Mutation c. 124A > G Mutation c. 836A > G	Neonatal ♂ Neonatal ♂ Neonatal ♀ 1 yr ♀ Neonatal ♀	Severe congenital HL Severe congenital HL Severe congenital HL and multiple ear infections. Progressive SNHL, otitis media Severe congenital HL	Van Nierop et al., 2017
			c.124A>G c.179 G>C, c.181G>A, c.182A>G c.186A>G, c.236A>G c.417G>C c.794G>A, c.922A>G, c.923A>G c.1504T>A, c.1510A>G	Both sexes Variable age	Temporary hearing impairment (sensorineural, Conductive, mixed) External ear anomalies (81%)	van Trier et al., 2015

(Continued)

TABLE 2 | Continued

Gene	HGDM® mutations (number)	General phenotype	Deafness mutation	Clinical cases	Auditory phenotype	References
IGF1R	Missense (36) Splicing (1) Regulatory (1) Small del (1) Small ins (4) Gross del (12) Gross ins (5) Complex (2)	Moderate- severe growth failure Mild intellectual impairment Microcephaly Dysmorphic features Cardiac malformations Disturbed glucose tolerance Failure to thrive	Deletion p.A711_E714del Complete deletion (15q26.3, exons 1–21) Partial deletion (exons 3–21)	Two siblings, 11 yr ♀and 7 yr ♂ 3 yr ♀ 2 yr ♂	Deafness Bilateral HL Recurrent ear infections	Maystadt et al., 2015 Ester et al., 2009

Reported cases of hearing impairment in patients with mutations in members of the GH/IGF-1 axis, except for IGF-1. Del, deletion; HL, hearing loss; Ins, insertion; SNHL, sensorineural hearing loss; yr, year.

The central auditory system shows alterations that could be secondary to the diminished input from the damaged periphery. These include modifications in the expression of calcium binding proteins (parvalbumin, calbindin and calretinin, and glutamate-decarboxylase), atrophy of the gray and white matter and changes in the content of some metabolites (Ouda et al., 2015). However, a minimal age-related decline in the total number of neurons in central auditory structures has been reported. IGF-1 expression is modulated by cochlear damage in the auditory central pathway (Alvarado et al., 2007) and its deficit also impacts neurotransmission in the cochlear nucleus (Fuentes-Santamaria et al., 2013, 2016).

To summarize, IGF system elements are expressed in the inner ear and central auditory pathway, and their expression is regulated by damage and age in different species. The importance of this system in hearing loss has been further proven by the study of human mutations and human cohorts as described below.

ASSOCIATION OF THE IGF SYSTEM WITH HUMAN GENETIC DEAFNESS AND AGE-RELATED HEARING LOSS

ARHL is a multifactorial process that results in cochlear damage over the span of a life. Noise, ototoxic agents, trauma, vascular insults, metabolic changes, hormones, diet, immune system, and genetic predisposition are all contributing factors (Gates and Mills, 2005; Fetoni et al., 2011). Given the increase in the average age of the population as well as in noxious environmental agents, the impact of ARHL is continuously growing.

The relationship between nutrition and ARHL is an emerging interdisciplinary field and recent evidence points to vitamin imbalances and high fat diets as risk factors (Partearroyo et al., 2017). In addition, the genetic study of ARHL is an expanding field that has rendered several gene candidates thus far (Salminen and Kaarniranta, 2010; Op De Beeck et al., 2011; Fransen et al., 2015; Koffler et al., 2015).

Mutations in human genes coding for IGF-1, IGF1R, and other members of the GH/IGF-1 system cause rare diseases (**Tables 1**, **2**), which normally have an early onset. Patients show growth retardation and frequent microcephaly. Interestingly, only when IGF-1 actions are totally impaired do the affected patients show syndromic hearing loss (**Table 1**). Indeed, of the 7 mutations described in young humans, hearing phenotype of 2 patients have not been reported (Van Duyvenvoorde et al., 2010, 2011; Shaheen et al., 2014) and 3 show low to normal IGF-1 levels, which show reduced affinity for IGF1R binding in the case tested, and normal hearing (Netchine et al., 2009; Fuqua et al., 2012; Batey et al., 2014). No data have been published on the evolution of hearing loss associated with aging that these patients present. Finally, 2 patients showed severe hearing loss which was associated in one case with total absence of circulating IGF-1 (Woods et al., 1996) and in the other with an extremely low binding affinity (Walenkamp et al., 2005). Accordingly, haploinsufficient IGF-1 availability causes growth retardation and has been associated with short adult stature and hearing loss in other genetic syndromes such as Noonan's or Turner'

syndromes (Barrenäs et al., 2000, 2005b; Welch and Dawes, 2007; El Bouchikhi et al., 2016).

Human mutations in the gene coding for the high affinity receptor IGF1R are even less frequent and normally found in heterozygosis (**Table 2**; Abuzzahab et al., 2003; Ester et al., 2009; Klammt et al., 2011; Fang et al., 2012; Gannagé-Yared et al., 2013; Prontera et al., 2015). Reduced bioactive IGF-1 levels caused by mutations in related genes, such as those coding for GH and the GHR, are also associated with hearing loss (**Table 1**; Giordano et al., 2015; Muus et al., 2017). In general, the authors have not done a thorough study of sensory functions, including hearing. However, it is worth noting that Laron's syndrome patients have been studied in depth and show early onset of ARHL (Attias et al., 2012). Laron's syndrome is an autosomal recessive human disorder characterized by mutations in the GHR that cause insensitivity to GH stimuli and, in turn, extremely low IGF-1 synthesis in the liver. Patients have a short stature, among other characteristics, and normal hearing at young ages but develop early onset ARHL, which could be prevented by IGF-1 treatment. Still, much work is needed to fully understand the relationship between genes associated with short stature (Baron et al., 2015; Wit et al., 2016) and hearing loss.

Finally, epidemiologic studies of aging cohorts have shown a relationship between bioactive IGF-1 and hearing loss (Lassale et al., 2017). Furthermore, there is increasing evidence linking these two factors with cognitive decline and onset of neurodegenerative diseases (Dik et al., 2003; Watanabe et al., 2005; Fortunato et al., 2016; Lassale et al., 2017; Wrigley et al., 2017), dementia (Peracino, 2014; Su et al., 2017), depression (Van Varsseveld et al., 2015; Kim et al., 2017), short stature (Barrenäs et al., 2005a; Crowe et al., 2011), cardiovascular pathologies (Burgers et al., 2011; Tan et al., 2017), and aging (Bainbridge and Wallhagen, 2014; Vestergaard et al., 2014).

These data lead us to pose the following questions: (i) which cochlear processes are IGF-1-dependent and dramatically impaired during aging? And (ii) is there a potential for prevention and repair interventions based on IGF-1 targets? Insight into the first question has been obtained from the study of the *Igf1*$^{-/-}$ mouse that shows profound syndromic bilateral sensorineural hearing loss (Cediel et al., 2006). The main cellular alterations reported were delayed postnatal development, a significant decrease in the number and size of auditory neurons, aberrant innervation patterns, increased neural apoptosis, deficits in myelination and increased efficacy of glutamatergic synapses (Camarero et al., 2001, 2002; Sanchez-Calderon et al., 2007; Fuentes-Santamaria et al., 2016). *Igf1*$^{-/-}$ mice develop further cellular degeneration with aging. As bioactive IGF-1 levels decrease, the heterozygous mice also show an accelerated hearing loss secondary to degeneration of the SGN (Riquelme et al., 2010). Our findings support the idea that IGF-1 levels may hold predictive value for the stratification of ARHL and, secondary to hearing loss, for cognitive decline. Regarding the aforementioned mechanisms, IGF-1 is a neurotrophic factor with anti-inflammatory actions. It is anti-apoptotic and favors cell renewal.

In this context, IGF-1 emerges as a potential protector of the inner ear. Studies in animal models have shown that local application of recombinant human IGF-1 (rhIGF-I) protects the cochlea from functional and histologic losses induced by aminoglycoside ototoxicity and noise exposure (Iwai et al., 2006; Yamahara et al., 2015). IGF-1 rescued hair cells from apoptosis by downregulating pro-apoptotic gene expression and regulating glucose transporters (Yamahara et al., 2015). Similarly, IGF-1 and substance P protect vestibular hair cells against neomycin ototoxicity (Yoshida et al., 2015). Recombinant IGF-1 therapy has been approved to increase linear growth, and therefore height, in humans, spurring an increasing interest in the potential use of IGF-1 for the treatment of hearing loss (Yamahara et al., 2015). IGF-1 potential has been studied in patients with sudden sensorineural hearing loss who were resistant to treatment with systemic glucocorticoids (Nakagawa et al., 2012, 2014). A recent study with 120 patients concluded that treatment with topical IGF-1 therapy had significant effects on hearing recovery depending on their age (<60 years) and early initiation of salvage treatment (Nakagawa et al., 2016). Prolonged activation of IGF1R by treatment with IGF-1 or analogs might have undesired secondary effects, thus their potential use to delay aging maybe limited. However available data highlight the interest of exploring IGF-1 downstream targets as drug candidates for ARHL.

AUTHOR CONTRIBUTIONS

LR-dlR, LL, MC, SM-C, IV-N: drafted the work and wrote the manuscript; LR-dlR, SM-C, and IV-N: revised the manuscript. All authors approved the manuscript in its final form.

FUNDING

This work was supported by the European Commission FP7-PEOPLE-2013-IAPP TARGEAR, Spanish MINECO/European Social Funds (FEDER) SAF2014-53979-R and CIBERER-ISCiii MODCELANI_CRISPR ER16P5AC761, and FENOCRISPR ER17P5AC7612 to IV-N. LR-dlR and SM-C are supported by FEDER/CIBERER contracts.

ACKNOWLEDGMENTS

We warmly thank our present and former colleagues in the Neurobiology of Hearing group for sharing information and for useful discussions.

REFERENCES

Aburto, M. R., Magariños, M., Leon, Y., Varela-Nieto, I., and Sanchez-Calderon, H. (2012a). AKT signaling mediates IGF-I survival actions on otic neural progenitors. *PLoS ONE* 7:e30790. doi: 10.1371/journal.pone.0030790

Aburto, M. R., Sánchez-Calderón, H., Hurlé, J. M., Varela-Nieto, I., and Magariños, M. (2012b). Early otic development depends on autophagy for apoptotic cell clearance and neural differentiation. *Cell Death Dis.* 3:e394. doi: 10.1038/cddis.2012.132

Abuzzahab, M. J., Schneider, A., Goddard, A., Grigorescu, F., Lautier, C., Keller, E., et al. (2003). IGF-I receptor mutations resulting in intrauterine

and postnatal growth retardation. *N. Engl. J. Med.* 349, 2211–2222. doi: 10.1056/NEJMoa010107

Alvarado, J. C., Fuentes-Santamaria, V., Franklin, S. R., Brunso-Bechtold, J. K., and Henkel, C. K. (2007). Synaptophysin and insulin-like growth factor-1 immunostaining in the central nucleus of the inferior colliculus in adult ferrets following unilateral cochlear removal: a densitometric analysis. *Synapse* 61, 288–302. doi: 10.1002/syn.20373

Arroba, A. I., Rodríguez-De La Rosa, L., Murillo-Cuesta, S., Vaquero-Villanueva, L., Hurlé, J. M., Varela-Nieto, I., et al. (2016). Autophagy resolves early retinal inflammation in Igf1-deficient mice. *Dis. Model. Mech.* 9, 965–974. doi: 10.1242/dmm.026344

Attias, J., Zarchi, O., Nageris, B. I., and Laron, Z. (2012). Cochlear hearing loss in patients with Laron syndrome. *Eur. Arch. Otorhinolaryngol.* 269, 461–466. doi: 10.1007/s00405-011-1668-x

Ayaso, E., Nolan, C. M., and Byrnes, L. (2002). Zebrafish insulin-like growth factor-I receptor: molecular cloning and developmental expression. *Mol. Cell. Endocrinol.* 191, 137–148. doi: 10.1016/S0303-7207(02)00083-7

Bainbridge, K. E., and Wallhagen, M. I. (2014). Hearing loss in an aging American population: extent, impact, and management. *Annu. Rev. Pub. Heal.* 35, 139–152. doi: 10.1146/annurev-publhealth-032013-182510

Bao, J., and Ohlemiller, K. K. (2010). Age-related loss of spiral ganglion neurons. *Hear. Res.* 264, 93–97. doi: 10.1016/j.heares.2009.10.009

Baron, J., Sävendahl, L., De Luca, F., Dauber, A., Phillip, M., Wit, J. M., et al. (2015). Short and tall stature: a new paradigm emerges. *Nat. Rev. Endocrinol.* 11, 735–746. doi: 10.1038/nrendo.2015.165

Barrenäs, M., Landin-Wilhelmsen, K., and Hanson, C. (2000). Ear and hearing in relation to genotype and growth in Turner syndrome. *Hear. Res.* 144, 21–28. doi: 10.1016/S0378-5955(00)00040-X

Barrenäs, M. L., Bratthall, A., and Dahlgren, J. (2005a). The association between short stature and sensorineural hearing loss. *Hear. Res.* 205, 123–130. doi: 10.1016/j.heares.2005.03.019

Barrenäs, M. L., Jonsson, B., Tuvemo, T., Hellstrom, P. A., and Lundgren, M. (2005b). High risk of sensorineural hearing loss in men born small for gestational age with and without obesity or height catch-up growth: a prospective longitudinal register study on birth size in 245,000 Swedish conscripts. *J. Clin. Endocrinol. Metab.* 90, 4452–4456. doi: 10.1210/jc.2005-0385

Batey, L., Moon, J. E., Yu, Y., Wu, B., Hirschhorn, J. N., Shen, Y., et al. (2014). A novel deletion of IGF1 in a patient with idiopathic short stature provides insight Into IGF1 haploinsufficiency. *J. Clin. Endocrinol. Metab.* 99, E153–E159. doi: 10.1210/jc.2013-3106

Boisclair, Y. R., Rhoads, R. P., Ueki, I., Wang, J., and Ooi, G. T. (2001). The acid-labile subunit (ALS) of the 150 kDa IGF-binding protein complex: an important but forgotten component of the circulating IGF system. *J. Endocrinol.* 170, 63–70. doi: 10.1677/joe.0.1700063

Bowl, M. R., and Dawson, S. J. (2015). The mouse as a model for age-related hearing loss - a mini-review. *Gerontology* 61, 149–157. doi: 10.1159/000368399

Burgers, A. M., Biermasz, N. R., Schoones, J. W., Pereira, A. M., Renehan, A. G., Zwahlen, M., et al. (2011). Meta-analysis and dose-response metaregression: circulating insulin-like growth factor I (IGF-I) and mortality. *J. Clin. Endocrinol. Metab.* 96, 2912–2920. doi: 10.1210/jc.2011-1377

Burns, J. C., Kelly, M. C., Hoa, M., Morell, R. J., and Kelley, M. W. (2015). Single-cell RNA-Seq resolves cellular complexity in sensory organs from the neonatal inner ear. *Nat. Commun.* 6:8557. doi: 10.1038/ncomms9557

Camarero, G., Avendano, C., Fernandez-Moreno, C., Villar, A., Contreras, J., de Pablo, F., et al. (2001). Delayed inner ear maturation and neuronal loss in postnatal Igf-1-deficient mice. *J. Neurosci.* 21, 7630–7641.

Camarero, G., Villar, M. A., Contreras, J., Fernández-Moreno, C., Pichel, J. G., Avendaño, C., et al. (2002). Cochlear abnormalities in insulin-like growth factor-1 mouse mutants. *Hear. Res.* 170, 2–11. doi: 10.1016/S0378-5955(02)00447-1

Cediel, R., Riquelme, R., Contreras, J., Díaz, A., and Varela-Nieto, I. (2006). Sensorineural hearing loss in insulin-like growth factor I-null mice: a new model of human deafness. *Eur. J. Neurosci.* 23, 587–590. doi: 10.1111/j.1460-9568.2005.04584.x

Chang, H. R., Kim, H. J., Xu, X., and Ferrante, A. W. Jr. (2016). Macrophage and adipocyte IGF1 maintain adipose tissue homeostasis during metabolic stresses. *Obesity* 24, 172–183. doi: 10.1002/oby.21354

Chu, H. S., Chung, H. S., Ko, M. H., Kim, H. J., Ki, C. S., Chung, W. H., et al. (2013). Syndromic hearing loss in association with PTPN11-related disorder: the experience of cochlear implantation in a child with LEOPARD syndrome. *Clin. Exp. Otorhinolaryngol.* 6, 99–102. doi: 10.3342/ceo.2013.6.2.99

Coate, T. M., and Kelley, M. W. (2013). Making connections in the inner ear: recent insights into the development of spiral ganglion neurons and their connectivity with sensory hair cells. *Semin. Cell Dev. Biol.* 24, 460–469. doi: 10.1016/j.semcdb.2013.04.003

Crowe, F. L., Key, T. J., Allen, N. E., Appleby, P. N., Overvad, K., Grønbæk, H., et al. (2011). A cross-sectional analysis of the associations between adult height, BMI and serum concentrations of IGF-I and IGFBP-1-2 and-3 in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Ann. Hum. Biol.* 38, 194–202. doi: 10.3109/03014460.2010.507221

Davey, H. W., Xie, T., Mclachlan, M. J., Wilkins, R. J., Waxman, D. J., and Grattan, D. R. (2001). STAT5b is required for GH-induced liver IGF-I gene expression. *Endocrinology* 142, 3836–3841. doi: 10.1210/endo.142.9.8400

Degerman, E., Rauch, U., Lindberg, S., Caye-Thomasen, P., Hultgårdh, A., and Magnusson, M. (2013). Expression of insulin signalling components in the sensory epithelium of the human saccule. *Cell Tissue. Res.* 352, 469–478. doi: 10.1007/s00441-013-1614-x

de Iriarte Rodríguez, R., Magariños, M., Pfeiffer, V., Rapp, U. R., and Varela-Nieto, I. (2015a). C-Raf deficiency leads to hearing loss and increased noise susceptibility. *Cell. Mol. Life Sci.* 72, 3983–3998. doi: 10.1007/s00018-015-1919-x

de Iriarte Rodríguez, R., Pulido, S., Rodríguez-de la Rosa, L., Magariños, M., and Varela-Nieto, I. (2015b). Age-regulated function of autophagy in the mouse inner ear. *Hear. Res.* 330, 39–50. doi: 10.1016/j.heares.2015.07.020.

Dik, M. G., Pluijm, S. M., Jonker, C., Deeg, D. J., Lomecky, M. Z., and Lips, P. (2003). Insulin-like growth factor I (IGF-I) and cognitive decline in older persons. *Neurobiol. Aging* 24, 573–581. doi: 10.1016/S0197-4580(02)00136-7

Ding, D., Jiang, H., Chen, G. D., Longo-Guess, C., Muthaiah, V. P., Tian, C., et al. (2016). N-acetyl-cysteine prevents age-related hearing loss and the progressive loss of inner hair cells in gamma-glutamyl transferase 1 deficient mice. *Aging* 8, 730–750. doi: 10.18632/aging.100927

Dyer, A. H., Vahdatpour, C., Sanfeliu, A., and Tropea, D. (2016). The role of insulin-like growth factor 1 (IGF-1) in brain development, maturation and neuroplasticity. *Neuroscience* 325, 89–99. doi: 10.1016/j.neuroscience.2016.03.056

El Bouchikhi, I., Belhassan, K., Moufid, F. Z., Iraqui Houssaini, M., Bouguenouch, L., Samri, I., et al. (2016). Noonan syndrome-causing genes: molecular update and an assessment of the mutation rate. *Int. J. Pediatr. Adol. Med.* 3, 133–142. doi: 10.1016/j.ijpam.2016.06.003

Ester, W. A., van Duyvenvoorde, H. A., de Wit, C. C., Broekman, A. J., Ruivenkamp, C. A., Govaerts, L. C., et al. (2009). Two short children born small for gestational age with insulin-like growth factor 1 receptor haploinsufficiency illustrate the heterogeneity of its phenotype. *J. Clin. Endocrinol. Metab.* 94, 4717–4727. doi: 10.1210/jc.2008-1502

Fang, P., Cho, Y. H., Derr, M. A., Rosenfeld, R. G., Hwa, V., and Cowell, C. T. (2012). Severe short stature caused by novel compound heterozygous mutations of the insulin-like growth factor 1 receptor (IGF1R). *J. Clin. Endocrinol. Metab.* 97, E243–E247. doi: 10.1210/jc.2011-2142

Fernández-Medarde, A., Barhoum, R., Riquelme, R., Porteros, A., Núñez, A., de Luis, A., et al. (2009). RasGRF1 disruption causes retinal photoreception defects and associated transcriptomic alterations. *J. Neurochem.* 110, 641–652. doi: 10.1111/j.1471-4159.2009.06162.x

Fetoni, A. R., Piacentini, R., Fiorita, A., Paludetti, G., and Troiani, D. (2009). Water-soluble Coenzyme Q10 formulation (Q-ter) promotes outer hair cell survival in a guinea pig model of noise induced hearing loss (NIHL). *Brain Res.* 1257, 108–116. doi: 10.1016/j.brainres.2008.12.027

Fetoni, A. R., Picciotti, P. M., Paludetti, G., and Troiani, D. (2011). Pathogenesis of presbycusis in animal models: a review. *Exp. Gerontol.* 46, 413–425. doi: 10.1016/j.exger.2010.12.003

Fettiplace, R., and Kim, K. X. (2014). The physiology of mechanoelectrical transduction channels in hearing. *Physiol. Rev.* 94, 951–986. doi: 10.1152/physrev.00038.2013

Firth, S. M., and Baxter, R. C. (2002). Cellular actions of the insulin-like growth factor binding proteins. *Endocr. Rev.* 23, 824–854. doi: 10.1210/er.2001-0033

Fortunato, S., Forli, F., Guglielmi, V., De Corso, E., Paludetti, G., Berrettini, S., et al. (2016). A review of new insights on the association between hearing loss and cognitive decline in ageing. *Acta Otorhinolaryngol. Ital.* 36, 155–166. doi: 10.14639/0392-100X-993

Foulstone, E., Prince, S., Zaccheo, O., Burns, J. L., Harper, J., Jacobs, C., et al. (2005). Insulin-like growth factor ligands, receptors, and binding proteins in cancer. *J. Pathol.* 205, 145–153. doi: 10.1002/path.1712

Fransen, E., Bonneux, S., Corneveaux, J. J., Schrauwen, I., Di Berardino, F., White, C. H., et al. (2015). Genome-wide association analysis demonstrates the highly polygenic character of age-related hearing impairment. *Eur. J. Hum. Genet.* 23, 110–115. doi: 10.1038/ejhg.2014.56

Frisina, R. D., Ding, B., Zhu, X., and Walton, J. P. (2016). Age-related hearing loss: prevention of threshold declines, cell loss and apoptosis in spiral ganglion neurons. *Aging* 8, 2081–2099. doi: 10.18632/aging.101045

Fuentes-Santamaria, V., Alvarado, J. C., Gabaldon-Ull, M. C., and Manuel Juiz, J. (2013). Upregulation of insulin-like growth factor and interleukin 1β occurs in neurons but not in glial cells in the cochlear nucleus following cochlear ablation. *J. Comp. Neurol.* 521, 3478–3499. doi: 10.1002/cne.23362

Fuentes-Santamaria, V., Alvarado, J. C., Rodriguez-De La Rosa, L., Murillo-Cuesta, S., Contreras, J., Juiz, J. M., et al. (2016). IGF-1 deficiency causes atrophic changes associated with upregulation of VGluT1 and downregulation of MEF2 transcription factors in the mouse cochlear nuclei. *Brain Struct. Funct.* 221, 709–734. doi: 10.1007/s00429-014-0934-2

Fuqua, J. S., Derr, M., Rosenfeld, R. G., and Hwa, V. (2012). Identification of a novel heterozygous IGF1 splicing mutation in a large kindred with familial short stature. *Horm. Res. Paediatr.* 78, 59–66. doi: 10.1159/000337249

Gálvez, H., Abelló, G., and Giraldez, F. (2017). Signaling and transcription factors during inner ear development: the generation of hair cells and otic neurons. *Front. Cell Dev. Biol.* 5:21. doi: 10.3389/fcell.2017.00021

Gannagé-Yared, M. H., Klammt, J., Chouery, E., Corbani, S., Mégarbané, H., Abou Ghoch, J., et al. (2013). Homozygous mutation of the IGF1 receptor gene in a patient with severe pre- and postnatal growth failure and congenital malformations. *Eur. J. Endocrinol.* 168, K1–K7. doi: 10.1530/EJE-12-0701

Gates, G. A., and Mills, J. H. (2005). Presbycusis. *Lancet* 366, 1111–1120. doi: 10.1016/S0140-6736(05)67423-5

Giordano, M., Gertosio, C., Pagani, S., Meazza, C., Fusco, I., Bozzola, E., et al. (2015). A 5.8 Mb interstitial deletion on chromosome Xq21.1 in a boy with intellectual disability, cleft palate, hearing impairment and combined growth hormone deficiency. *BMC Med. Genet.* 16:74. doi: 10.1186/s12881-015-0220-z

Guastini, L., Mora, R., Dellepiane, M., Santomauro, V., Giorgio, M., and Salami, A. (2011). Water-soluble coenzyme Q10 formulation in presbycusis: long-term effects. *Acta Otolaryngol.* 131, 512–517. doi: 10.3109/00016489.2010.539261

Guo, Y., Tang, C. Y., Man, X. F., Tang, H. N., Tang, J., Zhou, C. L., et al. (2017). Insulin-like growth factor-1 promotes osteogenic differentiation and collagen I alpha 2 synthesis via induction of mRNA-binding protein LARP6 expression. *Dev. Growth Differ.* 59, 94–103. doi: 10.1111/dgd.12342

Hequembourg, S., and Liberman, M. C. (2001). Spiral ligament pathology: a major aspect of age-related cochlear degeneration in C57BL/6 mice. *J. Assoc. Res. Otolaryngol.* 2, 118–129. doi: 10.1007/s101620010075

Herrington, J., Smit, L. S., Schwartz, J., and Carter-Su, C. (2000). The role of STAT proteins in growth hormone signaling. *Oncogene* 19, 2585–2597. doi: 10.1038/sj.onc.1203526

Iwai, K., Nakagawa, T., Endo, T., Matsuoka, Y., Kita, T., Kim, T. S., et al. (2006). Cochlear protection by local insulin-like growth factor-1 application using biodegradable hydrogel. *Laryngoscope* 116, 529–533. doi: 10.1097/01.mlg.0000200791.77819.eb

Kalinec, G. M., Lomberk, G., Urrutia, R. A., and Kalinec, F. (2017). Resolution of cochlear inflammation: novel target for preventing or ameliorating drug-, noise- and age-related hearing loss. *Front. Cell. Neurosci.* 11:192. doi: 10.3389/fncel.2017.00192

Kelly, M. C., and Chen, P. (2009). Development of form and function in the mammalian cochlea. *Curr. Opin. Neurobiol.* 19, 395–401. doi: 10.1016/j.conb.2009.07.010

Kim, J., Kim, M. R., Kim, H. J., Lee, K. A., and Lee, M. G. (2011). LEOPARD syndrome with PTPN11 gene mutation showing six cardinal symptoms of LEOPARD. *Ann. Dermatol.* 23, 232–235. doi: 10.5021/ad.2011.23.2.232

Kim, S. Y., Kim, H. J., Park, E. K., Joe, J., Sim, S., and Choi, H. G. (2017). Severe hearing impairment and risk of depression: a national cohort study. *PLoS ONE* 12:e0179973. doi: 10.1371/journal.pone.0179973

Klammt, J., Kiess, W., and Pfäffle, R. (2011). IGF1R mutations as cause of SGA. *Best Pract. Res. Clin. Endocrinol. Metab.* 25, 191–206. doi: 10.1016/j.beem.2010.09.012

Koffler, T., Ushakov, K., and Avraham, K. B. (2015). Genetics of hearing loss: syndromic. *Otolaryngol. Clin. North Am.* 48, 1041–1061. doi: 10.1016/j.otc.2015.07.007

Lassale, C., Batty, G. D., Steptoe, A., and Zaninotto, P. (2017). Insulin-like Growth Factor 1 in relation to future hearing impairment: findings from the English Longitudinal Study of Ageing. *Sci. Rep.* 7, 4212. doi: 10.1038/s41598-017-04526-7

Laviola, L., Natalicchio, A., and Giorgino, F. (2007). The IGF-I signaling pathway. *Curr. Pharm. Des.* 13, 663–669. doi: 10.2174/138161207780249146

Magariños, M., Contreras, J., Aburto, M. R., and Varela-Nieto, I. (2012). Early development of the vertebrate inner ear. *Anat. Rec.* 295, 1775–1790. doi: 10.1002/ar.22575

Magariños, M., Contreras, J., and Varela-Nieto, I. (2014). "Early development of the vertebrate inner ear," in *Development of Auditory and Vestibular Systems*, eds. R. Romand and I. Varela-Nieto (Kidlington: Academic Press), 1–30.

Magariños, M., Pulido, S., Aburto, M. R., de Iriarte Rodríguez, R., and Varela-Nieto, I. (2017). Autophagy in the vertebrate inner ear. *Front. Cell Dev. Biol.* 5:56. doi: 10.3389/fcell.2017.00056

Martínez-Quintana, E., and Rodríguez-González, F. (2012). LEOPARD Syndrome Caused by Tyr279Cys Mutation in the PTPN11 Gene. *Mol. Syndromol.* 2, 251–253. doi: 10.1159/000335995

Maystadt, I., Andrew, S.F., De Schepper, J., Wauters, N., Benoît, V., Joset, P., et al. (2015). "Novel Homozygous IGF1 Receptor (IGF1R) mutation, p.A711_E714del, in two siblings with severe growth failure, congenital malformations, deafness and insulin insensitivity," in *Endocrine Society's 97th Annual Meeting and Expo* (San Diego).

Menardo, J., Tang, Y., Ladrech, S., Lenoir, M., Casas, F., Michel, C., et al. (2012). Oxidative stress, inflammation, and autophagic stress as the key mechanisms of premature age-related hearing loss in SAMP8 mouse Cochlea. *Antioxid. Redox Signal.* 16, 263–274. doi: 10.1089/ars.2011.4037

Mittal, R., Nguyen, D., Patel, A. P., Debs, L. H., Mittal, J., Yan, D., et al. (2017). Recent advancements in the regeneration of auditory hair cells and hearing restoration. *Front. Mol. Neurosci.* 10:236. doi: 10.3389/fnmol.2017.00236

Murillo-Cuesta, S., Camarero, G., González-Rodríguez, A., Rodríguez de La Rosa, L., Burks, D. J., Avendano, C., et al. (2012). Insulin receptor substrate 2 (IRS2)-deficient mice show sensorineural hearing loss that is delayed by concomitant protein tyrosine phosphatase 1B (PTP1B) loss of function. *Mol. Med.* 18, 260–269. doi: 10.2119/molmed.2011.00328

Murillo-Cuesta, S., Rodriguez-De La Rosa, L., Cediel, R., Lassaletta, L., and Varela-Nieto, I. (2011). The role of insulin-like growth factor-I in the physiopathology of hearing. *Front. Mol. Neurosci.* 4:11. doi: 10.3389/fnmol.2011.00011

Muus, J. S., Weir, F. W., Kreicher, K. L., Bowlby, D. A., Discolo, C. M., and Meyer, T. A. (2017). Hearing loss in children with growth hormone deficiency. *Int. J. Pediatr. Otorhinolaryngol.* 100, 107–113. doi: 10.1016/j.ijporl.2017.06.037

Nakagawa, T., Kumakawa, K., Usami, S., Hato, N., Tabuchi, K., Takahashi, M., et al. (2014). A randomized controlled clinical trial of topical insulin-like growth factor-1 therapy for sudden deafness refractory to systemic corticosteroid treatment. *BMC Med.* 12:219. doi: 10.1186/s12916-014-0219-x

Nakagawa, T., Ogino-Nishimura, E., Hiraumi, H., Sakamoto, T., Yamamoto, N., and Ito, J. (2012). Audiometric outcomes of topical IGF1 treatment for sudden deafness refractory to systemic steroids. *Otol. Neurotol.* 33, 941–946. doi: 10.1097/MAO.0b013e31825f251a

Nakagawa, T., Yamamoto, M., Kumakawa, K., Usami, S., Hato, N., Tabuchi, K., et al. (2016). Prognostic impact of salvage treatment on hearing recovery in patients with sudden sensorineural hearing loss refractory to systemic corticosteroids: a retrospective observational study. *Auris Nasus Larynx* 43, 489–494. doi: 10.1016/j.anl.2015.12.004

Netchine, I., Azzi, S., Houang, M., Seurin, D., Perin, L., Ricort, J. M., et al. (2009). Partial primary deficiency of insulin-like growth factor (IGF)-I activity associated with IGF1 mutation demonstrates its critical role in growth and brain development. *J. Clin. Endocrinol. Metab.* 94, 3913–3921. doi: 10.1210/jc.2009-0452

Nieto-Estévez, V., Oueslati-Morales, C. O., Li, L., Pickel, J., Morales, A. V., and Vicario-Abejón, C. (2016). Brain insulin-like growth factor-i directs the transition from stem cells to mature neurons during postnatal/adult hippocampal neurogenesis. *Stem Cells* 34, 2194–2209. doi: 10.1002/stem.2397

Ohlemiller, K. K. (2009). Mechanisms and genes in human strial presbycusis from animal models. *Brain Res.* 1277, 70–83. doi: 10.1016/j.brainres.2009.02.079

Ohlemiller, K. K., and Gagnon, P. M. (2004). Cellular correlates of progressive hearing loss in 129S6/SvEv mice. *J. Comp. Neurol.* 469, 377–390. doi: 10.1002/cne.11011

Okano, T., and Kelley, M. W. (2013). Expression of insulin-like growth factor binding proteins during mouse cochlear development. *Dev. Dyn.* 242, 1210–1221. doi: 10.1002/dvdy.24005

Okano, T., Xuan, S., and Kelley, M. W. (2011). Insulin-like growth factor signaling regulates the timing of sensory cell differentiation in the mouse cochlea. *J. Neurosci.* 31, 18104–18118. doi: 10.1523/JNEUROSCI.3619-11.2011

Op De Beeck, K., Schacht, J., and Van Camp, G. (2011). Apoptosis in acquired and genetic hearing impairment: the programmed death of the hair cell. *Hear. Res.* 281, 18–27. doi: 10.1016/j.heares.2011.07.002

Ouda, L., Profant, O., and Syka, J. (2015). Age-related changes in the central auditory system. *Cell Tissue Res.* 361, 337–358. doi: 10.1007/s00441-014-2107-2

Partearroyo, T., Vallecillo, N., Pajares, M. A., Varela-Moreiras, G., and Varela-Nieto, I. (2017). Cochlear homocysteine metabolism at the crossroad of nutrition and sensorineural hearing loss. *Front. Mol. Neurosci.* 10:107. doi: 10.3389/fnmol.2017.00107

Peracino, A. (2014). Hearing loss and dementia in the aging population. *Audiol. Neurootol.* 19(Suppl. 1), 6–9. doi: 10.1159/000371595

Prado-Barreto, V. M., Salvatori, R., Santos Júnior, R. C., Brandão-Martins, M. B., Correa, E. A., Garcez, F. B., et al. (2014). Hearing status in adult individuals with lifetime, untreated isolated growth hormone deficiency. *Otolaryngol. Head Neck Surg.* 150, 464–471. doi: 10.1177/0194599813517987

Prontera, P., Micale, L., Verrotti, A., Napolioni, V., Stangoni, G., and Merla, G. (2015). A new homozygous iGF1R variant defines a clinically recognizable incomplete dominant form of SHORT syndrome. *Hum. Mutat.* 36, 1043–1047. doi: 10.1002/humu.22853

Reichenbach, T., and Hudspeth, A. J. (2014). The physics of hearing: fluid mechanics and the active process of the inner ear. *Rep. Prog. Phys.* 77:076601. doi: 10.1088/0034-4885/77/7/076601

Riquelme, R., Cediel, R., Contreras, J., la Rosa Lourdes, R. D., Murillo-Cuesta, S., Hernandez-Sanchez, C., et al. (2010). A comparative study of age-related hearing loss in wild type and insulin-like growth factor I deficient mice. *Front. Neuroanat.* 4:27. doi: 10.3389/fnana.2010.00027

Riva, C., Donadieu, E., Magnan, J., and Lavieille, J. P. (2007). Age-related hearing loss in CD/1 mice is associated to ROS formation and HIF target proteins up-regulation in the cochlea. *Exp. Gerontol.* 42, 327–336. doi: 10.1016/j.exger.2006.10.014

Rodriguez-de La Rosa, L., Fernandez-Sanchez, L., Germain, F., Murillo-Cuesta, S., Varela-Nieto, I., de la Villa, P., et al. (2012). Age-related functional and structural retinal modifications in the Igf1-/- null mouse. *Neurobiol. Dis.* 46, 476–485. doi: 10.1016/j.nbd.2012.02.013

Rodríguez-de La Rosa, L., López-Herradón, A., Portal-Núñez, S., Murillo-Cuesta, S., Lozano, D., Cediel, R., et al. (2014). Treatment with N- and C-terminal peptides of parathyroid hormone-related protein partly compensate the skeletal abnormalities in IGF-I deficient mice. *PLoS ONE*9:e87536. doi: 10.1371/journal.pone.0087536

Rodríguez-de la Rosa, L., Sánchez-Calderón, H., Contreras, J., Murillo-Cuesta, S., Falagan, S., Avendaño, C., et al. (2015). Comparative gene expression study of the vestibular organ of the Igf1 deficient mouse using whole-transcript arrays. *Hear. Res.* 330, 62–77. doi: 10.1016/j.heares.2015.08.016

Salminen, A., and Kaarniranta, K. (2010). Insulin/IGF-1 paradox of aging: regulation via AKT/IKK/NF-kappaB signaling. *Cell. Signal.* 22, 573–577. doi: 10.1016/j.cellsig.2009.10.006

Sanchez-Calderon, H., Milo, M., Leon, Y., and Varela-Nieto, I. (2007). A network of growth and transcription factors controls neuronal differentation and survival in the developing ear. *Int. J. Dev. Biol.* 51, 557–570. doi: 10.1387/ijdb.072373hs

Sanchez-Calderon, H., Rodriguez-De la Rosa, L., Milo, M., Pichel, J. G., Holley, M., and Varela-Nieto, I. (2010). RNA microarray analysis in prenatal mouse cochlea reveals novel IGF-I target genes: implication of MEF2 and FOXM1 transcription factors. *PLoS ONE* 5:e8699. doi: 10.1371/journal.pone.0008699

Schlueter, P. J., Peng, G., Westerfield, M., and Duan, C. (2007). Insulin-like growth factor signaling regulates zebrafish embryonic growth and development by promoting cell survival and cell cycle progression. *Cell Death Differ.* 14, 1095–1105. doi: 10.1038/sj.cdd.4402109

Sehat, B., Tofigh, A., Lin, Y., Trocmé, E., Liljedahl, U., Lagergren, J., et al. (2010). SUMOylation mediates the nuclear translocation and signaling of the IGF-1 receptor. *Sci. Signal.* 3:ra10. doi: 10.1126/scisignal.2000628

Shaheen, R., Faqeih, E., Ansari, S., Abdel-Salam, G., Al-Hassnan, Z. N., Al-Shidi, T., et al. (2014). Genomic analysis of primordial dwarfism reveals novel disease genes. *Genome Res.* 24, 291–299. doi: 10.1101/gr.160572.113

Siddle, K. (2011). Signalling by insulin and IGF receptors: supporting acts and new players. *J. Mol. Endocrinol.* 47, R1–R10. doi: 10.1530/JME-11-0022

Stephenson, L. (2012). Structure and innervation of the cochlea and organ of corti. *J. Vis. Commun. Med.* 35, 159. doi: 10.3109/08039488.2012.747176

Su, P., Hsu, C. C., Lin, H. C., Huang, W. S., Yang, T. L., Hsu, W. T., et al. (2017). Age-related hearing loss and dementia: a 10-year national population-based study. *Eur. Arch. Otorhinolaryngol.* 274, 2327–2334. doi: 10.1007/s00405-017-4471-5

Tafra, R., Brakus, S. M., Vukojevic, K., Kablar, B., Colovic, Z., and Saraga-Babic, M. (2014). Interplay of proliferation and proapoptotic and antiapoptotic factors is revealed in the early human inner ear development. *Otol. Neurotol.* 35, 695–703. doi: 10.1097/MAO.0000000000000210

Tan, H. E., Lan, N. S. R., Knuiman, M. W., Divitini, M. L., Swanepoel, D. W., Hunter, M., et al. (2017). Associations between cardiovascular disease and its risk factors with hearing loss-A cross-sectional analysis. *Clin. Otolaryngol.* doi: 10.1111/coa.12936

Tang, C. Y., Man, X. F., Guo, Y., Tang, H. N., Tang, J., Zhou, C. L., et al. (2017). IRS-2 partially compensates for the insulin signal defects in IRS-1-/- mice mediated by miR-33. *Mol. Cells* 40, 123–132. doi: 10.14348/molcells.2017.2228

Tsukano, H., Horie, M., Ohga, S., Takahashi, K., Kubota, Y., Hishida, R., et al. (2017). Reconsidering tonotopic maps in the auditory cortex and lemniscal auditory thalamus in mice. *Front. Neural Circuits* 11:14. doi: 10.3389/fncir.2017.00014

Van Duyvenvoorde, H. A., Van Doorn, J., Koenig, J., Gauguin, L., Oostdijk, W., Wade, J. D., et al. (2011). The severe short stature in two siblings with a heterozygous IGF1 mutation is not caused by a dominant negative effect of the putative truncated protein. *Growth Horm. IGF Res.* 21, 44–50. doi: 10.1016/j.ghir.2010.12.004

van Duyvenvoorde, H. A., van Setten, P. A., Walenkamp, M. J., van Doorn, J., Koenig, J., Gauguin, L., et al. (2010). Short stature associated with a novel heterozygous mutation in the insulin-like growth factor 1 gene. *J. Clin. Endocrinol. Metab.* 95, E363–E367. doi: 10.1210/jc.2010-0511

Van Landeghem, L., Santoro, M. A., Mah, A. T., Krebs, A. E., Dehmer, J. J., Mcnaughton, K. K., et al. (2015). IGF1 stimulates crypt expansion via differential activation of 2 intestinal stem cell populations. *FASEB J.* 29, 2828–2842. doi: 10.1096/fj.14-264010

Van Nierop, J. W. I., Van Trier, D. C., Van Der Burgt, I., Draaisma, J. M. T., Mylanus, E. A. M., Snik, A. F., et al. (2017). Cochlear implantation and clinical features in patients with Noonan syndrome and Noonan syndrome with multiple lentigines caused by a mutation in PTPN11. *Int. J. Pediatr. Otorhinolaryngol.* 97, 228–234. doi: 10.1016/j.ijporl.2017.04.024

van Trier, D. C., van Nierop, J., Draaisma, J. M., van Der Burgt, I., Kunst, H., Croonen, E. A., et al. (2015). External ear anomalies and hearing impairment in Noonan Syndrome. *Int. J. Pediatr. Otorhinolaryngol.* 79, 874–878. doi: 10.1016/j.ijporl.2015.03.021

van Varsseveld, N. C., van Bunderen, C. C., Sohl, E., Comijs, H. C., Penninx, B. W., Lips, P., et al. (2015). Serum insulin-like growth factor 1 and late-life depression: a population-based study. *Psychoneuroendocrinology* 54, 31–40. doi: 10.1016/j.psyneuen.2015.01.014

Varela-Nieto, I., Murillo-Cuesta, S., Rodríguez-de la Rosa, L., Lassatetta, L., and Contreras, J. (2013). IGF-I deficiency and hearing loss: molecular clues and clinical implications. *Pediatr. Endocrinol. Rev.* 10, 460–472.

Vestergaard, P. F., Hansen, M., Frystyk, J., Espelund, U., Christiansen, J. S., Jørgensen, J. O., et al. (2014). Serum levels of bioactive IGF1 and physiological markers of ageing in healthy adults. *Eur. J. Endocrinol.* 170, 229–236. doi: 10.1530/EJE-13-0661

Walenkamp, M. J., Karperien, M., Pereira, A. M., Hilhorst-Hofstee, Y., van Doorn, J., Chen, J. W., et al. (2005). Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. *J. Clin. Endocrinol. Metab.* 90, 2855–2864. doi: 10.1210/jc.2004-1254

Wan, G., and Corfas, G. (2015). No longer falling on deaf ears: mechanisms of degeneration and regeneration of cochlear ribbon synapses. *Hear. Res.* 329, 1–10. doi: 10.1016/j.heares.2015.04.008

Watanabe, T., Miyazaki, A., Katagiri, T., Yamamoto, H., Idei, T., and Iguchi, T. (2005). Relationship between serum insulin-like growth factor-1 levels and Alzheimer's disease and vascular dementia. *J. Am. Geriatr. Soc.* 53, 1748–1753. doi: 10.1111/j.1532-5415.2005.53524.x

Welch, D., and Dawes, P. J. (2007). Childhood hearing is associated with growth rates in infancy and adolescence. *Pediatr. Res.* 62, 495–498. doi: 10.1203/PDR.0b013e3181425869

Wit, J. M., Oostdijk, W., Losekoot, M., van Duyvenvoorde, H. A., Ruivenkamp, C. A., and Kant, S. G. (2016). Mechanisms in endocrinology: novel genetic causes of short stature. *Eur. J. Endocrinol.* 174, R145–R173. doi: 10.1530/EJE-15-0937

Wong, A. C., and Ryan, A. F. (2015). Mechanisms of sensorineural cell damage, death and survival in the cochlea. *Front. Aging Neurosci.* 7:58. doi: 10.3389/fnagi.2015.00058

Woods, K. A., Camacho-Hübner, C., Savage, M. O., and Clark, A. J. (1996). Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N. Engl. J. Med.* 335, 1363–1367. doi: 10.1056/NEJM199610313351805

Wrigley, S., Arafa, D., and Tropea, D. (2017). Insulin-like growth factor 1: at the crossroads of brain development and aging. *Front. Cell. Neurosci.* 11:14. doi: 10.3389/fncel.2017.00014

Yamahara, K., Nakagawa, T., Ito, J., Kinoshita, K., Omori, K., and Yamamoto, N. (2017). Netrin 1 mediates protective effects exerted by insulin-like growth factor 1 on cochlear hair cells. *Neuropharmacology* 119, 26–39. doi: 10.1016/j.neuropharm.2017.03.032

Yamahara, K., Yamamoto, N., Nakagawa, T., and Ito, J. (2015). Insulin-like growth factor 1: a novel treatment for the protection or regeneration of cochlear hair cells. *Hear. Res.* 330, 2–9. doi: 10.1016/j.heares.2015.04.009

Yang, Z. Q., Zhang, H. L., Duan, C. C., Geng, S., Wang, K., Yu, H. F., et al. (2017). IGF1 regulates RUNX1 expression via IRS1/2: implications for antler chondrocyte differentiation. *Cell Cycle* 16, 522–532. doi: 10.1080/15384101.2016.1274471

Yoshida, S., Sugahara, K., Hashimoto, M., Hirose, Y., Shimogori, H., and Yamashita, H. (2015). The minimum peptides of IGF-1 and substance P protect vestibular hair cells against neomycin ototoxicity. *Acta Otolaryngol.* 135, 411–415. doi: 10.3109/00016489.2014.979438

Zou, S., Kamei, H., Modi, Z., and Duan, C. (2009). Zebrafish IGF genes: gene duplication, conservation and divergence, and novel roles in midline and notochord development. *PLoS ONE* 4:e7026. doi: 10.1371/journal.pone.0007026

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

MINI REVIEW
published: 03 November 2017
doi: 10.3389/fnagi.2017.00365

Insulin-Like Growth Factor-1 and Neuroinflammation

Jose L. Labandeira-Garcia[1,2], Maria A. Costa-Besada[1,2], Carmen M. Labandeira[3], Begoña Villar-Cheda[1,2] and Ana I. Rodríguez-Perez[1,2]*

[1]Laboratory of Neuroanatomy and Experimental Neurology, Department of Morphological Sciences, CIMUS, University of Santiago de Compostela, Santiago de Compostela, Spain, [2]Networking Research Center on Neurodegenerative Diseases (CIBERNED), Madrid, Spain, [3]Department of Clinical Neurology, Hospital Alvaro Cunqueiro, University Hospital Complex, Vigo, Spain

Insulin-like growth factor-1 (IGF-1) effects on aging and neurodegeneration is still controversial. However, it is widely admitted that IGF-1 is involved in the neuroinflammatory response. In peripheral tissues, several studies showed that IGF-1 inhibited the expression of inflammatory markers, although other studies concluded that IGF-1 has proinflammatory functions. Furthermore, proinflammatory cytokines such as TNF-α impaired IGF-1 signaling. In the brain, there are controversial results on effects of IGF-1 in neuroinflammation. In addition to direct protective effects on neurons, several studies revealed anti-inflammatory effects of IGF-1 acting on astrocytes and microglia, and that IGF-1 may also inhibit blood brain barrier permeability. Altogether suggests that the aging-related decrease in IGF-1 levels may contribute to the aging-related pro-inflammatory state. IGF-1 inhibits the astrocytic response to inflammatory stimuli, and modulates microglial phenotype (IGF-1 promotes the microglial M2 and inhibits of M1 phenotype). Furthermore, IGF-1 is mitogenic for microglia. IGF-1 and estrogen interact to modulate the neuroinflammatory response and microglial and astrocytic phenotypes. Brain renin-angiotensin and IGF-1 systems also interact to modulate neuroinflammation. Induction of microglial IGF-1 by angiotensin, and possibly by other pro-inflammatory inducers, plays a major role in the repression of the M1 microglial neurotoxic phenotype and the enhancement of the transition to an M2 microglial repair/regenerative phenotype. This mechanism is impaired in aged brains. Aging-related decrease in IGF-1 may contribute to the loss of capacity of microglia to undergo M2 activation. Fine tuning of IGF-1 levels may be critical for regulating the neuroinflammatory response, and IGF-1 may be involved in inflammation in a context-dependent mode.

Keywords: aging, angiotensin, astrocytes, estrogen, IGF-1, insulin, microglia, neurodegeneration

OPEN ACCESS

Edited by:
Luis Miguel Garcia-Segura, Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
Maria Jose Bellini, Argentinian National Council of Research, Argentinia
Gloria Patricia Cardona Gomez, Universidad de Antioquia, Colombia

****Correspondence:***
Jose L. Labandeira-Garcia
joseluis.labandeira@usc.es

Received: *09 September 2017*
Accepted: *23 October 2017*
Published: *03 November 2017*

Citation:
Labandeira-Garcia JL, Costa-Besada MA, Labandeira CM, Villar-Cheda B and Rodríguez-Perez AI (2017) Insulin-Like Growth Factor-1 and Neuroinflammation. Front. Aging Neurosci. 9:365. doi: 10.3389/fnagi.2017.00365

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) is a protein produced in several organs, such as gonads, muscle, bones, liver, gut and brain and is also present in plasma. IGF-1 signals primarily via IGF-1 receptors (IGF-1R), but IGF-1 can act also through the insulin receptor. IGF-1 is actively transported to the central nervous system (CNS) from plasma through the choroid plexus (Carro et al., 2000; Santi et al., 2017), and it is also locally produced in the brain by neurons and glial cells (Quesada et al., 2007; Suh et al., 2013; Rodriguez-Perez et al., 2016).

IGF-1 has multiple effects in the CNS, regulating early brain development, myelination, synapse formation, adult neurogenesis, production of neurotransmitters and cognition (Nieto-Estévez et al., 2016; Wrigley et al., 2017). Furthermore, it is usually considered that IGF-1 is a potent neuroprotective compound (Carro et al., 2003; Tien et al., 2017), and that this is, at least partially, due to inhibition of neuroinflammation (Sukhanov et al., 2007; Park et al., 2011). Consistent with this, a decrease in IGF-1 signaling has been related with neurodegeneration, depressive disorders and other brain diseases, in which IGF-1 has been suggested as a possible therapy (Torres Aleman, 2012; Guan et al., 2013). Several decades ago, circulating GH and IGF-1 have been shown to decrease with aging. However, the possible relationship between IGF-1 effects and aging is still controversial, and opposite concepts can be found in the literature. Both the increase in IGF-1 levels and the inhibition of the IG-1R signal appear to induce beneficial effects in the CNS, and exert either aging or anti-aging effects (Cohen and Dillin, 2008; Fernandez and Torres-Alemán, 2012; Sonntag et al., 2012).

This apparent contradiction has been related to different explanations. Mild decrease in IGF-1 signal may lead to mild metabolic changes that induce protective defenses against more intense and deleterious conditions associated to aging, finally leading to an increase in lifespan (Fernandez and Torres-Alemán, 2012; Sonntag et al., 2012). IGF-1 decrease may lead to increased resistance to oxidative stress (OS), mild mitochondrial dysfunction leading to hormesis (Troulinaki and Bano, 2012), or increased resistance to proteotoxicity (Cohen et al., 2009; George et al., 2017). It has also been proposed that deletion of IGF-1R may counteract possible deleterious IGF-1R signaling independently of IGF-1 (Torres Aleman, 2012). Inhibition of IGF-1-related tumor development may also result in increased longevity (Novosyadlyy and Leroith, 2012). We suggest that development of compensatory mechanisms against mild dysregulation of the neuroinflammatory response may also be involved. Actions of IGF-1 may be context-dependent (Fernandez and Torres-Alemán, 2012), and IGF-1 may be involved in inflammation in a context-dependent mode, which may explain controversial results on the role of IGF-1 in neuroinflammation.

In summary, it is known that IGF-1 plays a major role in regulation of brain cells in health and disease conditions, although this role is controversial and has not been totally clarified. However, it is widely admitted that IGF-1 levels increase in response to brain injury, and IGF-1 is involved in the neuroinflammatory response to injury. Since neuroinflammation plays a major role in brain aging and neurodegeneration, clarification of the role of IGF-1 in the neuroinflammatory process may shed light on the above mentioned controversy.

IGF-1 AND NEUROINFLAMMATION

In peripheral tissues, several studies have shown that IGF-1 regulates macrophagic functions, inhibits expression of pro-inflammatory cytokines and decreases disease progression (Sukhanov et al., 2007; Hijikawa et al., 2008). However, other studies concluded that IGF-1 has proinflammatory functions, as it was observed that IGF-1 increased chemotactic migration and TNF-α expression in macrophages (Renier et al., 1996). Conversely, proinflammatory cytokines such as TNF-α impaired IGF-1 signaling (Hotamisligil et al., 1993). Controversial results on effects of IGF-1 in neuroinflammation have also been reported. IGF-1 (Park et al., 2011) and IGF-1 gene transfer (Hung et al., 2007; Dodge et al., 2008) inhibited neuroinflammatory responses. However, other studies observed that inhibition of IGF-1R signaling decreased neuroinflammation and neuronal death in Alzheimer's disease (AD) mice models: IGF1R-deficient mice were more resistant to amyloid-β oligomer-induced proteotoxicity, showing notably less activated astrocytes and less microgliosis (Cohen et al., 2009; George et al., 2017). As previously observed in peripheral tissues, TNF-α inhibited IGF-1 signaling in neurons (Venters et al., 1999).

The mechanisms responsible for the above mentioned effects are unclear. The anti-inflammatory properties of IGF-1 may be related to regulation of infiltration of inflammatory cells into tissues (Motani et al., 1996), including CNS, rather than a direct effect on the inflammatory cells (i.e., IGF-1 may act on the blood brain barrier; BBB). Consistent with this, IGF-1 ameliorated the breakdown of the BBB in a traumatic lesion of the spinal cord (Sharma, 2005), and in a model of multiple sclerosis (Liu et al., 1995). However, other studies showed that, IGF-1 enhanced BBB permeability and leukocyte infiltration (Pang et al., 2010): co-administration of IGF-1 with LPS further enhanced BBB permeability induced by LPS alone, while no change of BBB integrity was found in rats treated with IGF-1 alone. It was suggested that the effects of IGF-1 may depend on pathological conditions, and that in an acute inflammation, IGF-1 may have detrimental effects. However, several *in vitro* experiments revealed direct anti-inflammatory effects of IGF-1 on astrocytes and microglia (Bluthé et al., 2006; Palin et al., 2007). A simultaneous effect on the BBB and glial cells was also suggested (Bake et al., 2014).

On the basis of the anti-inflammatory effects of IGF-1, it has been suggested that development of resistance to IGF-1 may contribute to neuroinflammation and progression of major brain diseases. Furthermore, neuroinflammation may contribute to disease progression by decreasing levels of neuroprotective molecules such as IGF-1. Therefore, aging-related decrease in IGF-1 levels may contribute to the aging-related pro-inflammatory state and vice versa. However, the molecular mechanisms involved in the above mentioned controversial effects of IGF-1 have not been clarified. Investigation of the specific role of IGF-1 in neurons, astrocytes and microglia in different experimental contexts may shed light on the controversy (**Figure 1**).

IGF-1 AND NEURONS

Our recent studies and others (Zhou et al., 1999; Rodriguez-Perez et al., 2016) have shown the presence of IGF-1 and IGF-1R in neurons, astrocytes and microglia. IGF-1 is

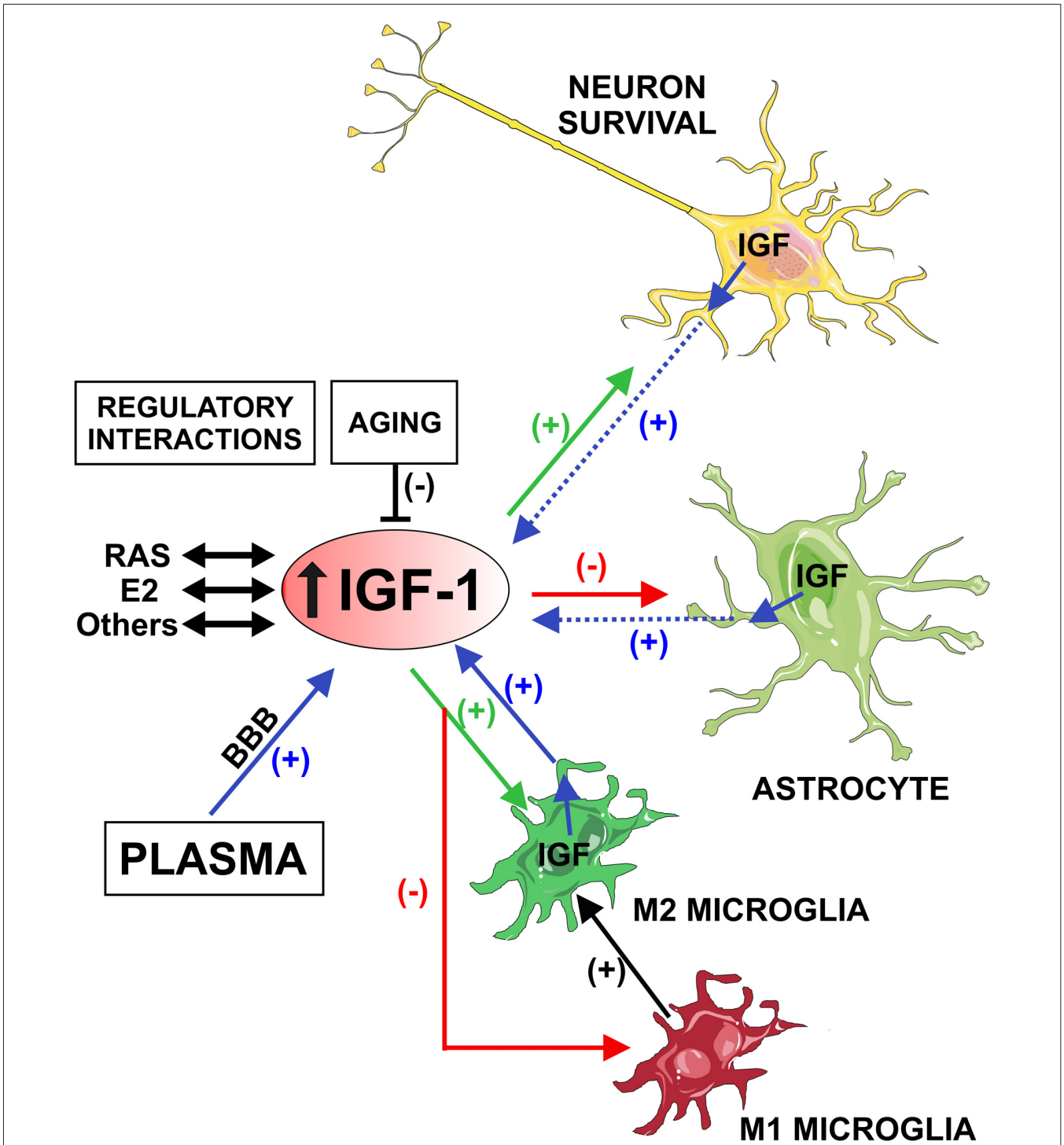


FIGURE 1 | Schematic model showing the possible role of Insulin-like growth factor-1 (IGF-1) in neuroinflammation. IGF-1 is actively transported from plasma and locally produced in the brain by neurons and glial cells (blue arrows). Microglial cells are a major source of IGF-1(blue arrow) in comparison with astrocytes and neurons (dashed blue arrows). IGF-1 receptors are predominantly expressed in neurons and astrocytes, which appear to be targeted by IGF-1 in lesioned regions. IGF-1 promotes neuronal survival and the M2 microglial repair/regenerative phenotype (green arrows), and inhibits the astrocytic response to inflammatory stimuli and the M1 microglial phenotype (red arrows). Therefore, IGF-1 induces repression of the M1 microglial neurotoxic phenotype and enhancement of the transition to M2 (black arrow). Aging-related decrease in IGF-1 may contribute to the loss of capacity of microglia to undergo M2 activation, leading to an aging-related pro-inflammatory state. Brain IGF-1, estrogen and angiotensin interact to modulate the neuroinflammatory response. However, these regulatory mechanisms are impaired in aged brains. Abbreviations: BBB, blood-brain barrier; E2, estrogen; RAS, renin-angiotensin system. Figure was produced using Servier Medical Art (http://www.servier.com).

synthesized by both neurons and glial cells, although its role is different in each cell type. IGF-1 protects neurons from neurotoxins in the presence of glia, which may be related to direct IGF-1 regulation of the glial inflammatory response (Nadjar et al., 2009). However, IGF-1 may also modulate neuroinflammation indirectly through effects on neurons, which modulate the neuroinflammatory response. IGF-1 directly protects neurons in pure neuronal cultures (Offen et al., 2001; Rodriguez-Perez et al., 2016). The mechanisms responsible for the direct neuronal protection of IGF-1 have not been clarified. However, effects on mitochondrial function (Puche et al., 2008), inhibition of OS, Sirtuin-1 activation (Vinciguerra et al., 2009; Tran et al., 2014) and other possible mechanisms have been suggested (Fernandez and Torres-Alemán, 2012; Torres Aleman, 2012; Werner and Leroith, 2014).

IGF-1 AND ASTROCYTES

IGF-1 is also synthesized by astrocytes, and several studies indicate that IGF-1 plays a major role in modulating the astrocytic activity. IGF-1 regulates astrocytic glucose control and CNS glucose metabolism (Hernandez-Garzón et al., 2016). Furthermore, astrocytes contribute to regulation of IGF-1R expression in neurons (Costantini et al., 2010). Astrocytic IGF-1 protects neurons against oxidative damage (Genis et al., 2014) and traumatic brain injury (Madathil et al., 2013). IGF-1 administration or IGF-1 gene therapy inhibited expression of toll-like receptor 4, and reduced the astrocytic response to inflammatory stimuli (Bellini et al., 2011) and the expression of inflammatory mediators such as TNF-α, IL-1β and iNOS (Park et al., 2011). However, it was also found that IGF1R-deficient mice showed less amyloid-β oligomer-induced activation of astrocytes and less microgliosis (Cohen et al., 2009; George et al., 2017). Furthermore, astrocytes modulate the inflammatory response both directly and indirectly by releasing mediators that control the microglial response (Dominguez-Meijide et al., 2017).

IGF-1 AND MICROGLIA

Microglia act as resident macrophages in the CNS and major mediators of neuroinflammatory responses (Prinz and Priller, 2014). Classically, microglia present two functional states (i.e., resting and activated microglia). In a healthy state, neurons release immunosuppressive signals, which induce the classical inactivated state in the surrounding microglial cells (Harrison et al., 1998). However, it is now considered that the classical microglial activation comprises a group of "activated" states, and that microglia, in response to their environment, can adopt multiple phenotypes and functions to control CNS homeostasis (Ransohoff, 2016; Labandeira-Garcia et al., 2017; Nissen, 2017). Appropriate progression of the microglial response from the so-called proinflammatory/M1 to the so-called immunoregulatory/M2 phenotype is required for an efficient repair of brain injuries. A dysregulation of this process, leading to continued release reactive nitrogen species (RNS), reactive oxygen species (ROS) and inflammatory cytokines, results in progression neuronal death and brain diseases (Kettenmann et al., 2011; Labandeira-Garcia et al., 2017).

Microglial cells are a major source of IGF-1, which is upregulated during the inflammatory process. IGF-1R are predominantly expressed in neurons and astrocytes, which appear to be targeted by IGF-1 in lesioned regions (Butovsky et al., 2006; Suh et al., 2013) to promote neuronal survival (Arroba et al., 2011; Ueno et al., 2013; **Figure 1**). The immunoregulatory/M2 phenotype is typically promoted by cytokines such as IL-4 or IL-13. However, a number of data suggest that IGF-1 may also modulate the microglial phenotype: an increase in IGF-1 levels promotes the M2 phenotype (Lee et al., 2013), and treatment with IL-4 increases IGF-1 release by microglial cells (Ferger et al., 2010). IGF-1 also inhibits microglial ROS and markers of M1 phenotype such as TNF-α (Grinberg et al., 2013). Consistent with this, microglia showed a marked upregulation of IGF-1 levels and down-regulation of IL-6 in a transgenic mouse model of amyotrophic lateral sclerosis, which was related to modulation of a beneficial inflammatory response to neuronal damage (Chiu et al., 2008). Similarly, in rat models of ischemic lesion, IGF-1 mRNA was overexpressed in astrocytes and microglia surrounding surviving neurons (Beilharz et al., 1998), and elimination of proliferating microglia enhanced infarct volume and reduced levels of IGF-1 induced by the ischemic lesion (Lalancette-Hébert et al., 2007). Furthermore, microglia may be responsible for the neuroprotective effects of peripheral IGF-1 transported into the CNS, since treatment of microglia with IGF-1 was mitogenic (O'Donnell et al., 2002), and proliferation of microglia is usually considered a major regulator of the neuroinflammatory response, and a source of neuroprotective molecules such as IGF-1 (Lalancette-Hébert et al., 2007). Apparently controversial results were also observed: activation of human microglia with LPS reduced IGF-1 levels, which suggested that chronic neuroinflammation and increased inflammatory cytokines may lead to neurodegeneration by inhibiting the release of microglia-derived neuronal trophic factors, such as IGF-1 (Suh et al., 2013). However, a sudden increase in IGF levels may induce feedback signals decreasing responsiveness of the IGF-1R and IGF signaling, which may be different to the aging and neurodegenerative context (Suh et al., 2008).

Interestingly, aging (Lee et al., 2013) and mitochondrial dysfunctions (Ferger et al., 2010) inhibited IL-4-induced M2 phenotype and IL-4-induced increase in IGF-1 levels. This is consistent with recent studies suggesting that mitochondrial metabolism may modulate microglial polarization (Orihuela et al., 2016).

INTERACTION BETWEEN IGF-1 AND ESTROGEN IN NEUROINFLAMMATION

Sex hormones induce trophic effects on neurons and glia, enhance neuron survival and modulate several CNS functions (Rettberg et al., 2014). As in the case of IGF-1, estrogens are

transported to the brain through the BBB and, in addition, the CNS can synthesize some endogenous estrogens (Azcoitia et al., 2011). Several studies have shown the presence of estrogen receptors (ERs) in neurons, astrocytes and microglia (Rettberg et al., 2014; Rodriguez-Perez et al., 2015). The mechanism responsible for estrogen-induced neuroprotection is still unclear. Direct anti-apoptotic (Brendel et al., 2013) and trophic (Campos et al., 2012) effects on neurons have been observed. However, a number of recent studies have revealed that regulation of the neuroinflammatory response constitutes a major mechanism involved in estrogen neuroprotective effects (Morale et al., 2006; Vegeto et al., 2008).

Several studies have shown that neurons and glial cells co-express ERs and IGF-1R (Cardona-Gómez et al., 2000; Quesada et al., 2007), and that estrogen and IGF-1 interact in the CNS for regulation of developmental and synaptic plasticity events and adult neurogenesis (Garcia-Segura et al., 2010). Furthermore, IGF-1 may mediate neuroprotection induced by estrogen. In aged ovariectomized rats, JB-1 (IGF-1R inhibitor) inhibited the beneficial effects of estrogen administration, suggesting that the IGF-1R signaling may mediate the effects of estrogen (Witty et al., 2013). Similarly, in a rat model of stroke, JB-1 blocked the estrogen neuroprotective effects (Selvamani and Sohrabji, 2010; Sohrabji, 2015). Several possible molecular mechanisms have been involved in the IGF-1/estrogen interaction, such as cross-regulation of ER and IGF-1R expression, IGF-1 regulation of ER-induced transcription, or regulation of IGF-1R signaling by estrogen (Garcia-Segura et al., 2010). Estrogen and IGF-1 may also stimulate common signaling pathways such as MAP kinases, PI3kinase/AKT ERK1 and ERK2 (Singh et al., 1999; Sohrabji, 2015).

Administration of either IGF-1 or estrogen can modify the inflammatory response and microglial and astrocytic phenotypes. However, they may also induce synergistic effects on neuroinflammation by still unclear mechanisms. Brain lesions stimulate production of IGF-1 and estrogen by reactive astrocytes, and increase ER and IGF-1R expression in activated glial cells (Garcia-Estrada et al., 1992; Blurton-Jones and Tuszynski, 2001; García-Ovejero et al., 2002; Hwang et al., 2004). Similar to IGF-1, estrogen has complex effects on the inflammatory response (Bellini et al., 2011; Rodriguez-Perez et al., 2015, 2016). Both estrogen and IGF-1 produced by activated glial cells may act directly on the same glial cells and on the surrounding neurons, modulating both the neuroinflammatory response and neuronal survival. Furthermore, as indicated above for IGF-1, effects of estrogen on neuroinflammation may be in part related to regulation of the BBB, since estrogen-deficient conditions such as menopause or reproductive senescence increase permeability of BBB (Bake and Sohrabji, 2004).

Consistent with the important interactions between estrogen and brain IGF-1 system, gender differences have been observed in several responses mediated by IGF-1 such as effects of exercise (Munive et al., 2016). Furthermore, in a series of studies using cell cultures, young rodents and menopausal rats (Rodriguez-Perez et al., 2010, 2011, 2012, 2015, 2016; Labandeira-Garcia et al., 2016), we have revealed important interactions and mutual regulation between estrogen and brain renin-angiotensin system (RAS). Interestingly, we also observed mutual regulation between RAS and IGF-1 (see below).

INTERACTION BETWEEN IGF-1 AND RAS IN NEUROINFLAMMATION

As in the case of IGF-1, the brain RAS has been associated with longevity, neuroinflammation and aging-related neuronal vulnerability to degeneration (Labandeira-Garcia et al., 2011, 2013, 2017; Labandeira-García et al., 2014). Angiotensin II (AII) is classically considered the main effector peptide of the RAS. AII signaling is mediated via AII type 1 and 2 receptors (AT1R and AT2R). AT2R induce effects that counteract those induced by AT1R stimulation (McCarthy et al., 2013). Overstimulation of the local/paracrine/tissue RAS, through AT1R, leads to OS due to NADPH oxidase overactivation, and triggers inflammatory responses. Consistent with this, RAS overactivation reduces longevity and induces aging-related degeneration in several tissues (Benigni et al., 2009, 2013; de Cavanagh et al., 2015).

Mutual regulation between RAS and IGF-1 was observed in peripheral cells, particularly in vascular smooth muscle cells (Ma et al., 2006; Jia et al., 2011) and cardiomyocytes (Leri et al., 1999; Kajstura et al., 2001). In a recent study, we have investigated possible interactions between both systems in the brain and in the neuroinflammatory process in particular (Rodriguez-Perez et al., 2016), and the results may shed light on the role of IGF-1 in the neuroinflammatory response. We observed reciprocal regulation between RAS and IGF-1: IGF-1 administration decreased RAS activity in neurons and glial cells (i.e., IGF-1 decreased AT1R, increased AT2R and reduced angiotensinogen/angiotensin levels). Inversely, AT1R activation increased IGF-1 and IGF-1R levels in microglia, while AT2R stimulation reduced IGF-1 and IGF-1R expression. AII administration promoted the microglial M1 phenotype via AT1R, which was blocked by activation of AT2R. Consistent with the above-mentioned interactions, the AII-induced enhancement of M1 phenotype markers was inhibited by administration of IGF-1. This suggests that induction of microglial IGF-1 production by AII, and possibly by other OS and pro-inflammatory inducers, plays a major role in the repression the M1 microglial neurotoxic phenotype and the enhancement of the transition to an M2 microglial repair/regenerative phenotype.

According to previous observations in plasma, and other brain regions and tissues (Bartke et al., 2003; Brown-Borg, 2015), we observed a reduction in IGF-1 levels in the substantia nigra of aged rats in comparison with young controls. In young animals and cultures, AT1R stimulation induced an increase in IGF-1 levels (see above). Since AII/AT1R activity is enhanced in the nigra of aged rats (Villar-Cheda et al., 2012, 2014), a counterregulatory increase rather than a decrease in IGF-1 levels may be expected. This suggests an aging-related loss of the IGF-1-mediated counterregulatory mechanism, which may lead to the pro-oxidative and pro-inflammatory state observed aged brains

(Villar-Cheda et al., 2012; Lee et al., 2013). Low levels of IGF-1 may contribute to the loss of capacity of microglia to undergo M2 activation in the aged brain.

In addition to IGF-1-induced neuronal protection by modulation of the microglial inflammatory response, IGF-1 mediate a direct effect on neurons (see above), which may lead to indirect (i.e., neuron-mediated) modulation of the glial inflammatory response. This is consistent with our recent studies on the intraneuronal or intracrine RAS, in which we observed that activation of nuclear AT1R induces several intraneuronal protective mechanisms that may counteract the deleterious effects of activation of plasmatic membrane pro-oxidative AT1R (Valenzuela et al., 2016; Villar-Cheda et al., 2017). This protective response includes an increase in transcription of IGF-1. Interestingly, this intracrine protective response was impaired in nuclei isolated from aged brains (Villar-Cheda et al., 2017).

CONCLUSION

Experimental manipulation of IGF-1 and IGF-1R led to some controversial findings, possibly because a fine tuning of IGF-1 levels is necessary for each specific situation. This may be critical for regulating the neuroinflammatory response, as well as other IGF-1 functions and brain health. Furthermore, IGF-1 may be involved in inflammation in a context-dependent mode. Views of IGF-1 as beneficial or detrimental appear over-simplistic. Future studies taking into account different experimental contexts and progression in the knowledge of the microglial responses and phenotypes will help to solve current controversies.

AUTHOR CONTRIBUTIONS

All authors have contributed to this work and approved its final version for submission. JLL-G developed the idea for this review and wrote the manuscript. AIR-P prepared the figure and was involved in literature review and revision of the manuscript. MAC-B, CML and BV-C were involved in literature review and preparation of the manuscript.

ACKNOWLEDGMENTS

The work reported here was supported by Spanish Ministry of Science and Innovation (BFU2015-70523), Spanish Ministry of Health, Instituto de Salud Carlos III (RD12/0019/0020, RD16/0011/0016 and CIBERNED), Galician Government, Consellería de Cultura, Educación e Ordenación Universitaria, Xunta de Galicia (XUGA GRC2014/002; CIMUS accreditation 2016-2019, ED431G/05) and European Regional Development Fund (FEDER).

REFERENCES

Arroba, A. I., Alvarez-Lindo, N., Van Rooijen, N., and de la Rosa, E. J. (2011). Microglia-mediated IGF-I neuroprotection in the rd10 mouse model of retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 52, 9124–9130. doi: 10.1167/iovs.11-7736

Azcoitia, I., Yague, J. G., and Garcia-Segura, L. M. (2011). Estradiol synthesis within the human brain. *Neuroscience* 191, 139–147. doi: 10.1016/j.neuroscience.2011.02.012

Bake, S., Selvamani, A., Cherry, J., and Sohrabji, F. (2014). Blood brain barrier and neuroinflammation are critical targets of IGF-1-mediated neuroprotection in stroke for middle-aged female rats. *PLoS One* 9:e91427. doi: 10.1371/journal.pone.0091427

Bake, S., and Sohrabji, F. (2004). 17β-estradiol differentially regulates blood-brain barrier permeability in young and aging female rats. *Endocrinology* 145, 5471–5475. doi: 10.1210/en.2004-0984

Bartke, A., Chandrashekar, V., Dominici, F., Turyn, D., Kinney, B., Steger, R., et al. (2003). Insulin-like growth factor 1 (IGF-1) and aging: controversies and new insights. *Biogerontology* 4, 1–8. doi: 10.1023/A:1022448532248

Beilharz, E. J., Russo, V. C., Butler, G., Baker, N. L., Connor, B., Sirimanne, E. S., et al. (1998). Co-ordinated and cellular specific induction of the components of the IGF/IGFBP axis in the rat brain following hypoxic-ischemic injury. *Mol. Brain Res.* 59, 119–134. doi: 10.1016/s0169-328x(98)00122-3

Bellini, M. J., Hereñú, C. B., Goya, R. G., and Garcia-Segura, L. M. (2011). Insulin-like growth factor-I gene delivery to astrocytes reduces their inflammatory response to lipopolysaccharide. *J. Neuroinflammation* 8:21. doi: 10.1186/1742-2094-8-21

Benigni, A., Corna, D., Zoja, C., Sonzogni, A., Latini, R., Salio, M., et al. (2009). Disruption of the Ang II type 1 receptor promotes longevity in mice. *J. Clin. Invest.* 119, 524–530. doi: 10.1172/JCI36703

Benigni, A., Orisio, S., Noris, M., Iatropoulos, P., Castaldi, D., Kamide, K., et al. (2013). Variations of the angiotensin II type 1 receptor gene are associated with extreme human longevity. *Age* 35, 993–1005. doi: 10.1007/s11357-012-9408-8

Blurton-Jones, M., and Tuszynski, M. H. (2001). Reactive astrocytes express estrogen receptors in the injured primate brain. *J. Comp. Neurol.* 433, 115–123. doi: 10.1002/cne.1129

Bluthé, R. M., Kelley, K. W., and Dantzer, R. (2006). Effects of insulin-like growth factor-I on cytokine-induced sickness behavior in mice. *Brain Behav. Immun.* 20, 57–63. doi: 10.1016/j.bbi.2005.02.003

Brendel, A., Felzen, V., Morawe, T., Manthey, D., and Behl, C. (2013). Differential regulation of apoptosis-associated genes by estrogen receptor α in human neuroblastoma cells. *Restor. Neurol. Neurosci.* 31, 199–211. doi: 10.3233/RNN-120272

Brown-Borg, H. M. (2015). The somatotropic axis and longevity in mice. *Am. J. Physiol. Endocrinol. Metab.* 309, E503–E510. doi: 10.1152/ajpendo.00262.2015

Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A. E., Pluchino, S., et al. (2006). Microglia activated by IL-4 or IFN-γ differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Mol. Cell. Neurosci.* 31, 149–160. doi: 10.1016/j.mcn.2005.10.006

Campos, F. L., Cristovão, A. C., Rocha, S. M., Fonseca, C. P., and Baltazar, G. (2012). GDNF contributes to oestrogen-mediated protection of midbrain dopaminergic neurones. *J. Neuroendocrinol.* 24, 1386–1397. doi: 10.1111/j.1365-2826.2012.02348.x

Cardona-Gómez, G. P., Doncarlos, L., and Garcia-Segura, L. M. (2000). Insulin-like growth factor I receptors and estrogen receptors colocalize in female rat brain. *Neuroscience* 99, 751–760. doi: 10.1016/s0306-4522(00)00228-1

Carro, E., Nuñez, A., Busiguina, S., and Torres-Aleman, I. (2000). Circulating insulin-like growth factor I mediates effects of exercise on the brain. *J. Neurosci.* 20, 2926–2933.

Carro, E., Trejo, J. L., Núñez, A., and Torres-Aleman, I. (2003). Brain repair and neuroprotection by serum insulin-like growth factor I. *Mol. Neurobiol.* 27, 153–162. doi: 10.1385/mn:27:2:153

Chiu, I. M., Chen, A., Zheng, Y., Kosaras, B., Tsiftsoglou, S. A., Vartanian, T. K., et al. (2008). T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS. *Proc. Natl. Acad. Sci. U S A* 105, 17913–17918. doi: 10.1073/pnas.0804610105

Cohen, E., and Dillin, A. (2008). The insulin paradox: aging, proteotoxicity and neurodegeneration. *Nat. Rev. Neurosci.* 9, 759–767. doi: 10.1038/nrn2474

Cohen, E., Paulsson, J. F., Blinder, P., Burstyn-Cohen, T., Du, D., Estepa, G., et al. (2009). Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. *Cell* 139, 1157–1169. doi: 10.1016/j.cell.2009.11.014

Costantini, C., Lorenzetto, E., Cellini, B., Buffelli, M., Rossi, F., and Della-Bianca, V. (2010). Astrocytes regulate the expression of insulin-like growth factor 1 receptor (IGF1-R) in primary cortical neurons during *in vitro* senescence. *J. Mol. Neurosci.* 40, 342–352. doi: 10.1007/s12031-009-9305-5

de Cavanagh, E. M., Inserra, F., and Ferder, L. (2015). Angiotensin II blockade: how its molecular targets may signal to mitochondria and slow aging. Coincidences with calorie restriction and mTOR inhibition. *Am. J. Physiol. Heart Circ. Physiol.* 309, H15–H44. doi: 10.1152/ajpheart.00459.2014

Dodge, J. C., Haidet, A. M., Yang, W., Passini, M. A., Hester, M., Clarke, J., et al. (2008). Delivery of AAV-IGF-1 to the CNS extends survival in ALS mice through modification of aberrant glial cell activity. *Mol. Ther.* 16, 1056–1064. doi: 10.1038/mt.2008.60

Dominguez-Meijide, A., Rodriguez-Perez, A. I., Diaz-Ruiz, C., Guerra, M. J., and Labandeira-García, J. L. (2017). Dopamine modulates astroglial and microglial activity via glial renin-angiotensin system in cultures. *Brain Behav. Immun.* 62, 277–290. doi: 10.1016/j.bbi.2017.02.013

Ferger, A. I., Campanelli, L., Reimer, V., Muth, K. N., Merdian, I., Ludolph, A. C., et al. (2010). Effects of mitochondrial dysfunction on the immunological properties of microglia. *J. Neuroinflammation* 7:45. doi: 10.1186/1742-2094-7-45

Fernandez, A. M., and Torres-Alemán, I. (2012). The many faces of insulin-like peptide signalling in the brain. *Nat. Rev. Neurosci.* 13, 225–239. doi: 10.1038/nrn3209

Garcia-Estrada, J., Garcia-Segura, L. M., and Torres-Aleman, I. (1992). Expression of insulin-like growth factor I by astrocytes in response to injury. *Brain Res.* 592, 343–347. doi: 10.1016/0006-8993(92)91695-b

García-Ovejero, D., Veiga, S., García-Segura, L. M., and Doncarlos, L. L. (2002). Glial expression of estrogen androgen receptors after rat brain injury. *J. Comp. Neurol.* 450, 256–271. doi: 10.1002/cne.10325

Garcia-Segura, L. M., Arévalo, M. A., and Azcoitia, I. (2010). Interactions of estradiol and insulin-like growth factor-I signalling in the nervous system: new advances. *Prog. Brain Res.* 181, 251–272. doi: 10.1016/s0079-6123(08)81014-x

Genis, L., Dávila, D., Fernandez, S., Pozo-Rodrigálvarez, A., Martinez-Murillo, R., and Torres-Aleman, I. (2014). Astrocytes require insulin-like growth factor I to protect neurons against oxidative injury. *F1000Res.* 3:28. doi: 10.12688/f1000research.3-28.v2

George, C., Gontier, G., Lacube, P., François, J. C., Holzenberger, M., and Aid, S. (2017). The Alzheimer's disease transcriptome mimics the neuroprotective signature of IGF-1 receptor-deficient neurons. *Brain* 140, 2012–2027. doi: 10.1093/brain/awx132

Grinberg, Y. Y., Dibbern, M. E., Levasseur, V. A., and Kraig, R. P. (2013). Insulin-like growth factor-1 abrogates microglial oxidative stress and TNF-α responses to spreading depression. *J. Neurochem.* 126, 662–672. doi: 10.1111/jnc.12267

Guan, J., Mathai, S., Liang, H. P., and Gunn, A. J. (2013). Insulin-like growth factor-1 and its derivatives: potential pharmaceutical application for treating neurological conditions. *Recent Pat. CNS Drug Discov.* 8, 142–160. doi: 10.2174/1574889811308020004

Harrison, J. K., Jiang, Y., Chen, S., Xia, Y., Maciejewski, D., McNamara, R. K., et al. (1998). Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. *Proc. Natl. Acad. Sci. U S A* 95, 10896–10901. doi: 10.1073/pnas.95.18.10896

Hernandez-Garzón, E., Fernandez, A. M., Perez-Alvarez, A., Genis, L., Bascuñana, P., Fernandez de la Rosa, R., et al. (2016). The insulin-like growth factor I receptor regulates glucose transport by astrocytes. *Glia* 64, 1962–1971. doi: 10.1002/glia.23035

Hijikawa, T., Kaibori, M., Uchida, Y., Yamada, M., Matsui, K., Ozaki, T., et al. (2008). Insulin-like growth factor 1 prevents liver injury through the inhibition of TNF-α and iNOS induction in D-galactosamine and LPS-treated rats. *Shock* 29, 740–747. doi: 10.1097/shk.0b013e31815d0780

Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science* 259, 87–91. doi: 10.1126/science.7678183

Hung, K. S., Tsai, S. H., Lee, T. C., Lin, J. W., Chang, C. K., and Chiu, W. T. (2007). Gene transfer of insulin-like growth factor-I providing neuroprotection after spinal cord injury in rats. *J. Neurosurg. Spine* 6, 35–46. doi: 10.3171/spi.2007.6.1.35

Hwang, I. K., Yoo, K. Y., Park, S. K., An, S. J., Lee, J. Y., Choi, S. Y., et al. (2004). Expression and changes of endogenous insulin-like growth factor-1 in neurons and glia in the gerbil hippocampus and dentate gyrus after ischemic insult. *Neurochem. Int.* 45, 149–156. doi: 10.1016/j.neuint.2003.10.006

Jia, G., Aggarwal, A., Yohannes, A., Gangahar, D. M., and Agrawal, D. K. (2011). Cross-talk between angiotensin II and IGF-1-induced connexin 43 expression in human saphenous vein smooth muscle cells. *J. Cell. Mol. Med.* 15, 1695–1702. doi: 10.1111/j.1582-4934.2010.01161.x

Kajstura, J., Fiordaliso, F., Andreoli, A. M., Li, B., Chimenti, S., Medow, M. S., et al. (2001). IGF-1 overexpression inhibits the development of diabetic cardiomyopathy and angiotensin II-mediated oxidative stress. *Diabetes* 50, 1414–1424. doi: 10.2337/diabetes.50.6.1414

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553. doi: 10.1152/physrev.00011.2010

Labandeira-García, J. L., Garrido-Gil, P., Rodriguez-Pallares, J., Valenzuela, R., Borrajo, A., and Rodriguez-Perez, A. I. (2014). Brain renin-angiotensin system and dopaminergic cell vulnerability. *Front. Neuroanat.* 8:67. doi: 10.3389/fnana.2014.00067

Labandeira-Garcia, J. L., Rodriguez-Pallares, J., Dominguez-Meijide, A., Valenzuela, R., Villar-Cheda, B., and Rodriguez-Perez, A. I. (2013). Dopamine-angiotensin interactions in the basal ganglia and their relevance for Parkinson's disease. *Mov. Disord.* 28, 1337–1342. doi: 10.1002/mds.25614

Labandeira-Garcia, J. L., Rodriguez-Pallares, J., Villar-Cheda, B., Rodríguez-Perez, A. I., Garrido-Gil, P., and Guerra, M. J. (2011). Aging, Angiotensin system and dopaminergic degeneration in the substantia nigra. *Aging Dis.* 2, 257–274.

Labandeira-Garcia, J. L., Rodriguez-Perez, A. I., Garrido-Gil, P., Rodriguez-Pallares, J., Lanciego, J. L., and Guerra, M. J. (2017). Brain renin-angiotensin system and microglial polarization: implications for aging and neurodegeneration. *Front. Aging Neurosci.* 9:129. doi: 10.3389/fnagi.2017.00129

Labandeira-Garcia, J. L., Rodriguez-Perez, A. I., Valenzuela, R., Costa-Besada, M. A., and Guerra, M. J. (2016). Menopause and Parkinson's disease. Interaction between estrogens and brain renin-angiotensin system in dopaminergic degeneration. *Front. Neuroendocrinol.* 43, 44–59. doi: 10.1016/j.yfrne.2016.09.003

Lalancette-Hébert, M., Gowing, G., Simard, A., Weng, Y. C., and Kriz, J. (2007). Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. *J. Neurosci.* 27, 2596–2605. doi: 10.1523/jneurosci.5360-06.2007

Lee, D. C., Ruiz, C. R., Lebson, L., Selenica, M. L., Rizer, J., Hunt, J. B. Jr., et al. (2013). Aging enhances classical activation but mitigates alternative activation in the central nervous system. *Neurobiol. Aging* 34, 1610–1620. doi: 10.1016/j.neurobiolaging.2012.12.014

Leri, A., Liu, Y., Wang, X., Kajstura, J., Malhotra, A., Meggs, L. G., et al. (1999). Overexpression of insulin-like growth factor-1 attenuates the myocyte renin-angiotensin system in transgenic mice. *Circ. Res.* 84, 752–762. doi: 10.1161/01.res.84.7.752

Liu, X., Yao, D. L., and Webster, H. (1995). Insulin-like growth factor I treatment reduces clinical deficits and lesion severity in acute demyelinating experimental autoimmune encephalomyelitis. *Mult. Scler.* 1, 2–9. doi: 10.1177/135245859500100102

Ma, Y., Zhang, L., Peng, T., Cheng, J., Taneja, S., Zhang, J., et al. (2006). Angiotensin II stimulates transcription of insulin-like growth factor I receptor in vascular smooth muscle cells: role of nuclear factor-κB. *Endocrinology* 147, 1256–1263. doi: 10.1210/en.2005-0888

Madathil, S. K., Carlson, S. W., Brelsfoard, J. M., Ye, P., D'Ercole, A. J., and Saatman, K. E. (2013). Astrocyte-specific overexpression of insulin-like growth factor-1 protects hippocampal neurons and reduces behavioral deficits following traumatic brain injury in mice. *PLoS One* 8:e67204. doi: 10.1371/journal.pone.0067204

McCarthy, C. A., Widdop, R. E., Denton, K. M., and Jones, E. S. (2013). Update on the angiotensin AT_2 receptor. *Curr. Hypertens. Rep.* 15, 25–30. doi: 10.1007/s11906-012-0321-4

Morale, M. C., Serra, P. A., L'episcopo, F., Tirolo, C., Caniglia, S., Testa, N., et al. (2006). Estrogen, neuroinflammation and neuroprotection in Parkinson's disease: glia dictates resistance versus vulnerability to neurodegeneration. *Neuroscience* 138, 869–878. doi: 10.1016/j.neuroscience.2005.07.060

Motani, A., Forster, L., Tull, S., Anggard, E. E., and Ferns, G. A. (1996). Insulin-like growth factor-I modulates monocyte adhesion to EAhy 926 endothelial cells. *Int. J. Exp. Pathol.* 77, 31–35. doi: 10.1046/j.1365-2613.1996.960098.x

Munive, V., Santi, A., and Torres-Aleman, I. (2016). A concerted action of estradiol and insulin like growth factor I underlies sex differences in mood regulation by exercise. *Sci. Rep.* 6:25969. doi: 10.1038/srep25969

Nadjar, A., Berton, O., Guo, S., Leneuve, P., Dovero, S., Diguet, E., et al. (2009). IGF-1 signaling reduces neuro-inflammatory response and sensitivity of neurons to MPTP. *Neurobiol. Aging* 30, 2021–2030. doi: 10.1016/j.neurobiolaging.2008.02.009

Nieto-Estévez, V., Defterali, Ç., and Vicario-Abejón, C. (2016). IGF-I: a key growth factor that regulates neurogenesis and synaptogenesis from embryonic to adult stages of the brain. *Front. Neurosci.* 10:52. doi: 10.3389/fnins.2016.00052

Nissen, J. C. (2017). Microglial function across the spectrum of age and gender. *Int. J. Mol. Sci* 18:E561. doi: 10.3390/ijms18030561

Novosyadlyy, R., and Leroith, D. (2012). Insulin-like growth factors and insulin: at the crossroad between tumor development and longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* 67, 640–651. doi: 10.1093/gerona/gls065

O'Donnell, S. L., Frederick, T. J., Krady, J. K., Vannucci, S. J., and Wood, T. L. (2002). IGF-I and microglia/macrophage proliferation in the ischemic mouse brain. *Glia* 39, 85–97. doi: 10.1002/glia.10081

Offen, D., Shtaif, B., Hadad, D., Weizman, A., Melamed, E., and Gil-Ad, I. (2001). Protective effect of insulin-like-growth-factor-1 against dopamine-induced neurotoxicity in human and rodent neuronal cultures: possible implications for Parkinson's disease. *Neurosci. Lett.* 316, 129–132. doi: 10.1016/s0304-3940(01)02344-8

Orihuela, R., McPherson, C. A., and Harry, G. J. (2016). Microglial M1/M2 polarization and metabolic states. *Br. J. Pharmacol.* 173, 649–665. doi: 10.1111/bph.13139

Palin, K., Bluthé, R. M., McCusker, R. H., Moos, F., Dantzer, R., and Kelley, K. W. (2007). TNFα-induced sickness behavior in mice with functional 55 kD TNF receptors is blocked by central IGF-I. *J. Neuroimmunol.* 187, 55–60. doi: 10.1016/j.jneuroim.2007.04.011

Pang, Y., Zheng, B., Campbell, L. R., Fan, L. W., Cai, Z., and Rhodes, P. G. (2010). IGF-1 can either protect against or increase LPS-induced damage in the developing rat brain. *Pediatr. Res.* 67, 579–584. doi: 10.1203/PDR.0b013e3181dc240f

Park, S. E., Dantzer, R., Kelley, K. W., and McCusker, R. H. (2011). Central administration of insulin-like growth factor-I decreases depressive-like behavior and brain cytokine expression in mice. *J. Neuroinflammation* 8:12. doi: 10.1186/1742-2094-8-12

Prinz, M., and Priller, J. (2014). Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. *Nat. Rev. Neurosci.* 15, 300–312. doi: 10.1038/nrn3722

Puche, J. E., García-Fernández, M., Muntané, J., Rioja, J., González-Barán, S., and Castilla Cortazar, I. (2008). Low doses of insulin-like growth factor-I induce mitochondrial protection in aging rats. *Endocrinology* 149, 2620–2627. doi: 10.1210/en.2007-1563

Quesada, A., Romeo, H. E., and Micevych, P. (2007). Distribution and localization patterns of estrogen receptor-β and insulin-like growth factor-1 receptors in neurons and glial cells of the female rat substantia nigra: localization of ERβ and IGF-1R in substantia nigra. *J. Comp. Neurol.* 503, 198–208. doi: 10.1002/cne.21358

Ransohoff, R. M. (2016). A polarizing question: do M1 and M2 microglia exist? *Nat. Neurosci.* 19, 987–991. doi: 10.1038/nn.4338

Renier, G., Clement, I., Desfaits, A. C., and Lambert, A. (1996). Direct stimulatory effect of insulin-like growth factor-I on monocyte and macrophage tumor necrosis factor-α production. *Endocrinology* 137, 4611–4618. doi: 10.1210/en.137.11.4611

Rettberg, J. R., Yao, J., and Brinton, R. D. (2014). Estrogen: a master regulator of bioenergetic systems in the brain and body. *Front. Neuroendocrinol.* 35, 8–30. doi: 10.1016/j.yfrne.2013.08.001

Rodriguez-Perez, A. I., Borrajo, A., Diaz-Ruiz, C., Garrido-Gil, P., and Labandeira-Garcia, J. L. (2016). Crosstalk between insulin-like growth factor-1 and angiotensin-II in dopaminergic neurons and glial cells: role in neuroinflammation and aging. *Oncotarget* 7, 30049–30067. doi: 10.18632/oncotarget.9174

Rodriguez-Perez, A. I., Borrajo, A., Valenzuela, R., Lanciego, J. L., and Labandeira-Garcia, J. L. (2015). Critical period for dopaminergic neuroprotection by hormonal replacement in menopausal rats. *Neurobiol. Aging* 36, 1194–1208. doi: 10.1016/j.neurobiolaging.2014.10.028

Rodriguez-Perez, A. I., Valenzuela, R., Joglar, B., Garrido-Gil, P., Guerra, M. J., and Labandeira-Garcia, J. L. (2011). Renin angiotensin system and gender differences in dopaminergic degeneration. *Mol. Neurodegener.* 6:58. doi: 10.1186/1750-1326-6-58

Rodriguez-Perez, A. I., Valenzuela, R., Villar-Cheda, B., Guerra, M. J., and Labandeira-Garcia, J. L. (2012). Dopaminergic neuroprotection of hormonal replacement therapy in young and aged menopausal rats: role of the brain angiotensin system. *Brain* 135, 124–138. doi: 10.1093/brain/awr320

Rodriguez-Perez, A. I., Valenzuela, R., Villar-Cheda, B., Guerra, M. J., Lanciego, J. L., and Labandeira-Garcia, J. L. (2010). Estrogen and angiotensin interaction in the substantia nigra. Relevance to postmenopausal Parkinson's disease. *Exp. Neurol.* 224, 517–526. doi: 10.1016/j.expneurol.2010.05.015

Santi, A., Genis, L., and Torres Aleman, I. (2017). A coordinated action of blood-borne and brain insulin-like growth factor I in the response to traumatic brain injury. *Cereb. Cortex* doi: 10.1093/cercor/bhx106 [Epub ahead of print].

Selvamani, A., and Sohrabji, F. (2010). The neurotoxic effects of estrogen on ischemic stroke in older female rats is associated with age-dependent loss of insulin-like growth factor-1. *J. Neurosci.* 30, 6852–6861. doi: 10.1523/JNEUROSCI.0761-10.2010

Sharma, H. S. (2005). Neuroprotective effects of neurotrophins and melanocortins in spinal cord injury: an experimental study in the rat using pharmacological and morphological approaches. *Ann. N Y Acad. Sci.* 1053, 407–421. doi: 10.1196/annals.1344.036

Singh, M., Sétáló, G. Jr., Guan, X., Warren, M., and Toran-Allerand, C. D. (1999). Estrogen-induced activation of mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. *J. Neurosci.* 19, 1179–1188.

Sohrabji, F. (2015). Estrogen-IGF-1 interactions in neuroprotection: ischemic stroke as a case study. *Front. Neuroendocrinol.* 36, 1–14. doi: 10.1016/j.yfrne.2014.05.003

Sonntag, W. E., Csiszar, A., deCabo, R., Ferrucci, L., and Ungvari, Z. (2012). Diverse roles of growth hormone and insulin-like growth factor-1 in mammalian aging: progress and controversies. *J. Gerontol. A Biol. Sci. Med. Sci.* 67, 587–598. doi: 10.1093/gerona/gls115

Suh, Y., Atzmon, G., Cho, M. O., Hwang, D., Liu, B., Leahy, D. J., et al. (2008). Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc. Natl. Acad. Sci. U S A* 105, 3438–3442. doi: 10.1073/pnas.0705467105

Suh, H. S., Zhao, M. L., Derico, L., Choi, N., and Lee, S. C. (2013). Insulin-like growth factor 1 and 2 (IGF1, IGF2) expression in human microglia: differential regulation by inflammatory mediators. *J. Neuroinflammation* 10:37. doi: 10.1186/1742-2094-10-37

Sukhanov, S., Higashi, Y., Shai, S. Y., Vaughn, C., Mohler, J., Li, Y., et al. (2007). IGF-1 reduces inflammatory responses, suppresses oxidative stress, and decreases atherosclerosis progression in ApoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 27, 2684–2690. doi: 10.1161/atvbaha.107.156257

Tien, L. T., Lee, Y. J., Pang, Y., Lu, S., Lee, J. W., Tseng, C. H., et al. (2017). Neuroprotective effects of intranasal IGF-1 against neonatal lipopolysaccharide-induced neurobehavioral deficits and neuronal inflammation in the substantia nigra and locus coeruleus of juvenile rats. *Dev. Neurosci.* doi: 10.1159/000477898 [Epub ahead of print].

Torres Aleman, I. (2012). Insulin-like growth factor-1 and central neurodegenerative diseases. *Endocrinol. Metab. Clin. North. Am.* 41, 395–408, vii. doi: 10.1016/j.ecl.2012.04.016

Tran, D., Bergholz, J., Zhang, H., He, H., Wang, Y., Zhang, Y., et al. (2014). Insulin-like growth factor-1 regulates the SIRT1–p53 pathway in cellular senescence. *Aging Cell* 13, 669–678. doi: 10.1111/acel.12219

Troulinaki, K., and Bano, D. (2012). Mitochondrial deficiency: a double-edged sword for aging and neurodegeneration. *Front. Genet.* 3:244. doi: 10.3389/fgene.2012.00244

Ueno, M., Fujita, Y., Tanaka, T., Nakamura, Y., Kikuta, J., Ishii, M., et al. (2013). Layer V cortical neurons require microglial support for survival during postnatal development. *Nat. Neurosci.* 16, 543–551. doi: 10.1038/nn.3358

Valenzuela, R., Costa-Besada, M. A., Iglesias-Gonzalez, J., Perez-Costas, E., Villar-Cheda, B., Garrido-Gil, P., et al. (2016). Mitochondrial angiotensin receptors in dopaminergic neurons. Role in cell protection and aging-related vulnerability to neurodegeneration. *Cell Death Dis.* 7:e2427. doi: 10.1038/cddis.2016.327

Vegeto, E., Benedusi, V., and Maggi, A. (2008). Estrogen anti-inflammatory activity in brain: a therapeutic opportunity for menopause and neurodegenerative diseases. *Front. Neuroendocrinol.* 29, 507–519. doi: 10.1016/j.yfrne.2008.04.001

Venters, H. D., Tang, Q., Liu, Q., VanHoy, R. W., Dantzer, R., and Kelley, K. W. (1999). A new mechanism of neurodegeneration: a proinflammatory cytokine inhibits receptor signaling by a survival peptide. *Proc. Natl. Acad. Sci. U S A* 96, 9879–9884. doi: 10.1073/pnas.96.17.9879

Villar-Cheda, B., Costa-Besada, M. A., Valenzuela, R., Perez-Costas, E., Melendez-Ferro, M., and Labandeira-Garcia, J. L. (2017). The intracellular angiotensin system buffers deleterious effects of the extracellular paracrine system. *Cell Death Dis.* 8:e3044. doi: 10.1038/cddis.2017.439

Villar-Cheda, B., Dominguez-Meijide, A., Valenzuela, R., Granado, N., Moratalla, R., and Labandeira-Garcia, J. L. (2014). Aging-related dysregulation of dopamine and angiotensin receptor interaction. *Neurobiol. Aging* 35, 1726–1738. doi: 10.1016/j.neurobiolaging.2014.01.017

Villar-Cheda, B., Valenzuela, R., Rodriguez-Perez, A. I., Guerra, M. J., and Labandeira-Garcia, J. L. (2012). Aging-related changes in the nigral angiotensin system enhances proinflammatory and pro-oxidative markers and 6-OHDA-induced dopaminergic degeneration. *Neurobiol. Aging* 33, 204.e1–204.e11. doi: 10.1016/j.neurobiolaging.2010.08.006

Vinciguerra, M., Santini, M. P., Claycomb, W. C., Ladurner, A. G., and Rosenthal, N. (2009). Local IGF-1 isoform protects cardiomyocytes from hypertrophic and oxidative stresses via SirT1 activity. *Aging (Albany NY)* 2, 43–62. doi: 10.18632/aging.100107

Werner, H., and Leroith, D. (2014). Insulin and insulin-like growth factor receptors in the brain: physiological and pathological aspects. *Eur. Neuropsychopharmacol.* 24, 1947–1953. doi: 10.1016/j.euroneuro.2014.01.020

Witty, C. F., Gardella, L. P., Perez, M. C., and Daniel, J. M. (2013). Short-term estradiol administration in aging ovariectomized rats provides lasting benefits for memory and the hippocampus: a role for insulin-like growth factor-I. *Endocrinology* 154, 842–852. doi: 10.1210/en.2012-1698

Wrigley, S., Arafa, D., and Tropea, D. (2017). Insulin-like growth factor 1: at the crossroads of brain development and aging. *Front. Cell Neurosci.* 11:14. doi: 10.3389/fncel.2017.00014

Zhou, X., Herman, J. P., and Paden, C. M. (1999). Evidence that IGF-I acts as an autocrine/paracrine growth factor in the magnocellular neurosecretory system: neuronal synthesis and induction of axonal sprouting. *Exp. Neurol.* 159, 419–432. doi: 10.1006/exnr.1999.7189

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

REVIEW
published: 27 April 2018
doi: 10.3389/fnagi.2018.00124

Mitochondria, Estrogen and Female Brain Aging

Imane Lejri[1,2], *Amandine Grimm*[1,2] *and Anne Eckert*[1,2]*

[1]Neurobiology Lab for Brain Aging and Mental Health, Transfaculty Research Platform Molecular and Cognitive Neuroscience, University of Basel, Basel, Switzerland, [2]Psychiatric University Clinics, University of Basel, Basel, Switzerland

Mitochondria play an essential role in the generation of steroid hormones including the female sex hormones. These hormones are, in turn, able to modulate mitochondrial activities. Mitochondria possess crucial roles in cell maintenance, survival and well-being, because they are the main source of energy as well as of reactive oxygen species (ROS) within the cell. The impairment of these important organelles is one of the central features of aging. In women's health, estrogen plays an important role during adulthood not only in the estrous cycle, but also in the brain via neuroprotective, neurotrophic and antioxidant modes of action. The hypestrogenic state in the peri- as well as in the prolonged postmenopause might increase the vulnerability of elderly women to brain degeneration and age-related pathologies. However, the underlying mechanisms that affect these processes are not well elucidated. Understanding the relationship between estrogen and mitochondria might therefore provide better insights into the female aging process. Thus, in this review, we first describe mitochondrial dysfunction in the aging brain. Second, we discuss the estrogen-dependent actions on the mitochondrial activity, including recent evidence of the estrogen—brain-derived neurotrophic factor and estrogen—sirtuin 3 (SIRT3) pathways, as well as their potential implications during female aging.

Keywords: neurodegeneration, female brain aging, mitochondria, estrogen, protection, bioenergetics, SIRT3, BDNF

OPEN ACCESS

Edited by:
Luis Miguel Garcia-Segura, Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
George E. Barreto, Pontifical Xavierian University, Colombia
Ricardo Gredilla, Complutense University of Madrid, Spain

***Correspondence:**
Anne Eckert
anne.eckert@upkbs.ch

Received: *14 December 2017*
Accepted: *11 April 2018*
Published: *27 April 2018*

Citation:
Lejri I, Grimm A and Eckert A (2018) Mitochondria, Estrogen and Female Brain Aging. Front. Aging Neurosci. 10:124. doi: 10.3389/fnagi.2018.00124

INTRODUCTION

Mitochondria play crucial roles in different aspects of cellular physiology, including calcium homeostasis, metabolism, apoptosis and ATP production (Rizzuto et al., 2012). At the electron transport chain (ETC), especially at the respiratory complexes I and III, mitochondria produce reactive oxygen species (ROS; Quinlan et al., 2013). Mitochondrial respiration via ETC generates a flux of electrons that is coupled to a gradient of protons. During the oxidative phosphorylation (OXPHOS), electrons are neutralized to water upon reaction with oxygen at complex IV (cytochrome *c* oxidase (COX)), while the gradient of protons is necessary for the ATP synthesis via complex V (ATP synthase; Velarde, 2014).

Mitochondria are also essential sites for steroid hormone biosynthesis including estrogen, the main female steroid hormone (Vest and Pike, 2013). Estrogen is involved in many physiological functions such as modulation of the effects of the trophic factors in the brain, enhancement of the cerebral blood flow, and prevention of atrophy of cholinergic neurons (Castellani et al., 2010). The estrogen family (E2) includes the hormones estrone, estradiol (17β-estradiol) and estriol, all of them playing a role in the estrous cycle. Regarding to the estrogenic effect, estradiol is the

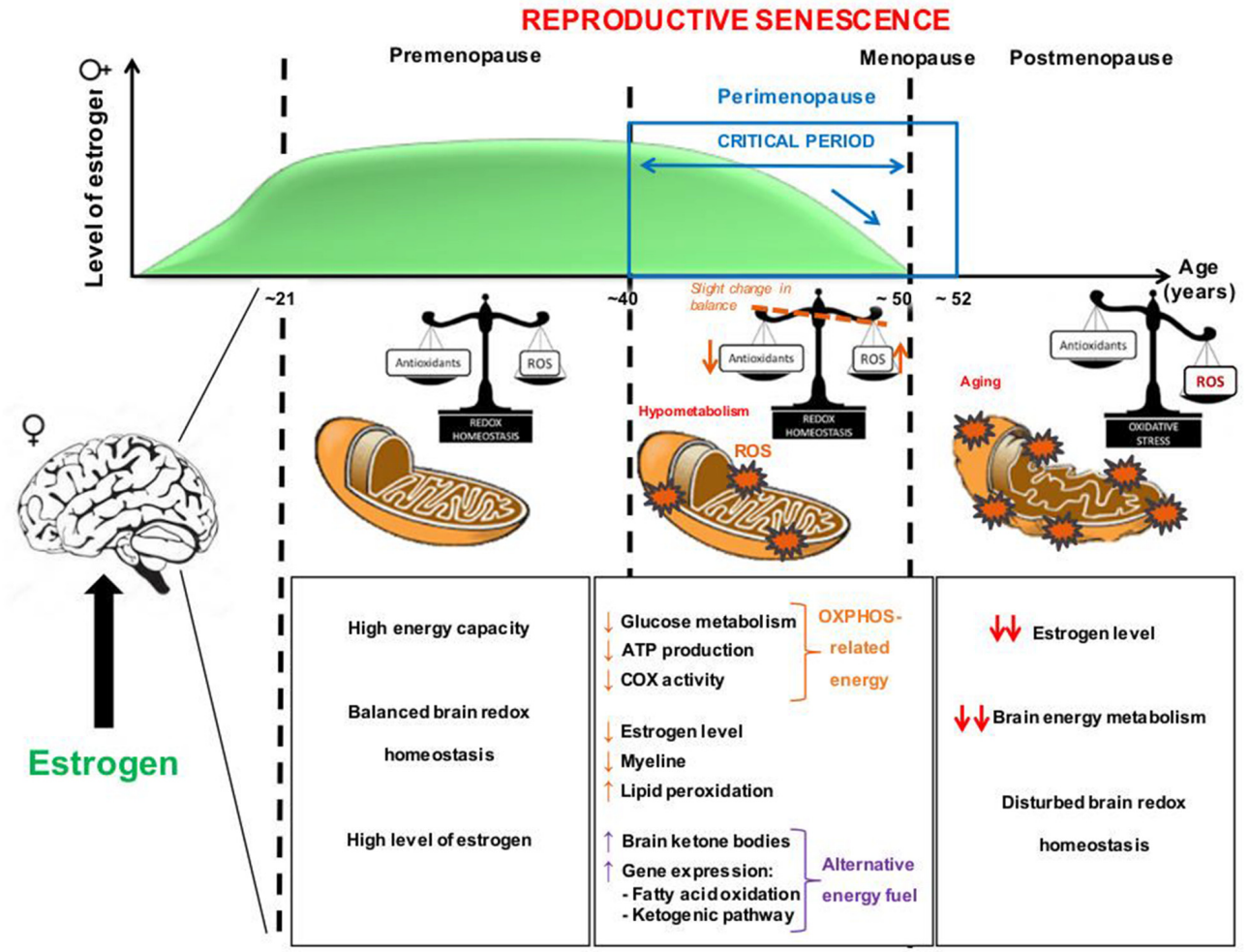


FIGURE 1 | Potential sequence of pathological events occurring at the mitochondrial level during aging and «the critical time period» of decline in estrogen (E2) in female brain. During the premenopause, high levels of estrogen are paralleled by a normal mitochondrial activity with a balanced redox homeostasis and a high brain energy metabolism. At the beginning of the reproductive senescence, or perimenopause, a decrease of estrogen levels is accompanied with an increase of oxidative stress (ROS levels) and, consequently, hypometabolism. During aging, alterations of the mitochondrial membrane lipid profile are also reported. Cells possess compensatory mechanisms, as antioxidant defenses, to keep the system in balance. In case the compensatory system is exhausted, metabolic impairments may occur, such as a decrease of ATP levels, glucose hypometabolism and mitochondrial respiration (e.g., cytochrome c oxidase (COX) activity) as well as myelin impairments. In parallel, an increased expression of genes involved in fatty acid oxidation and the ketogenic pathway were reported as an alternative fuel for cells. The "critical period" (represented by the blue arrow) indicates a fast drop of estrogen levels in a relatively short time period, suggesting a period of women's life where they are more vulnerable and more likely to develop age-related brain disorders such as Alzheimer's disease (AD). Estrogen replacement therapies (ERT) may be beneficial before women reach a critical threshold of cellular damage during perimenopause and early after menopause. ROS, reactive oxygen species.

predominant form of estrogen and ten times as potent as estrone and about 80 times as potent as estriol. During reproductive senescence, a hormonal deficit occurs in menopausal women, characterized by a sudden decline in circulating estrogen level (**Figure 1**; Vest and Pike, 2013). Menopause officially marks the end of female reproduction. Postmenopause represents the years after the menopause while the transitional stage leading from reproductive years to permanent infertility that occurs immediately before menopause is termed perimenopause (Dalal and Agarwal, 2015). Perimenopause or "menopause transition" can begin 8–10 years before menopause, when the ovaries gradually produce less estrogen. It usually starts in the fourth decade of woman's life. In the last 1–2 years of perimenopause, the drop in estrogen accelerates (**Figure 1**; Dalal and Agarwal, 2015). As a result of a lower level of estrogen, postmenopausal women seem to be at higher risk for a number of diseases, such as osteoporosis, heart disease and dementia (Dalal and Agarwal, 2015).

In males, estradiol is known to be an active metabolic product of testosterone. The serum levels of estradiol in males are about 14–55 pg/ml and are comparable to those of postmenopausal women <35 pg/ml. Testosterone freely enters the brain and

might be converted to estradiol by local aromatase enzymes before acting at the cellular level (Gillies and McArthur, 2010). In the central nervous system (CNS) of men and women, specific estrogen receptors (ER) have been shown to localize in mitochondria in the frontal lobe and the hippocampus, confirming an implication of estrogen in controlling memory processes and cognitive functions via energy supply (Genazzani et al., 2007). Further studies have shown that estrogen plays an important neuroprotective role during the aging process, in particular through their beneficial impact upon mitochondrial metabolism (Grimm et al., 2012). Moreover, females live longer than males. This sex difference in life longevity can be attributed in part to the antioxidant effect of estrogen and the up-regulation of life longevity-related genes (Viña et al., 2013).

Normal cellular function can be disrupted by mitochondrial impairment which represents a key feature of the aging process (Balaban et al., 2005; Wanagat et al., 2010). The brain is our most energy-consuming organ and the age-dependent dysregulation in cerebral redox homeostasis and bioenergetics might therefore be able to trigger the development of neurodegenerative disorders.

MITOCHONDRIAL IMPAIRMENTS IN BRAIN AGING: INSIGHT INTO THE ROLE OF ESTROGEN

Aging is characterized by a progressive decline in physiological functions, often accompanied by neurodegenerative diseases and mitochondrial dysfunction is one of the main factors contributing to the aging process (**Figure 2**; Cui et al., 2012).

During aging, energy production in the brain is reduced and, in parallel, the redox status is switched from an antioxidant to pro-oxidant state, partly due to the mitochondrial production of O^{2-} and H_2O_2 (Yin et al., 2014). Globally, a reduction in mitochondrial functions, including the activity of ETC complexes, the level of the antioxidant glutathione (GSH) as well as the antioxidant defense enzymes such as superoxide dismutase (SOD), was observed with increasing age (**Figure 2**; Leuner et al., 2012; Grimm et al., 2016a).

We recently reviewed the age-dependent modifications in redox homeostasis and brain bioenergetics, both hallmarks of normal brain aging, with a specific focus on sex differences (Grimm et al., 2016b). We highlighted the tight relationship between the age-related decrease in sex steroid levels and the age-related increase in brain oxidative insults. Especially, women experience a drastic loss of estrogen at the menopause that may be correlated with a decrease of antioxidant defenses (GSH levels) and increased oxidation in the brain (**Figure 1**; Mandal et al., 2012; Rekkas et al., 2014; Grimm et al., 2016b). Strikingly, women are more armed against oxidative stress before the menopause due to their elevated antioxidant defense mechanisms compared to men (Viña and Borrás, 2010). Healthy young females (mean age: 26 years) showed higher GSH levels in the frontal and parietal cortex compared to young men (Mandal et al., 2012), with a progressive decrease during aging from young to old women (mean age: 56 years). One of the relevant sources of ROS, the monoamine oxidase A (MAO-A) was found to be higher in the brain of perimenopausal women (41–51 years old) compared to young women (21–40 years old; Rekkas et al., 2014). In perimenopausal women who underwent bilateral oophorectomy, the serum estrogen levels were decreased and an increase of oxidative stress was observed. This effect was prevented by a treatment with estrogen (Bellanti et al., 2013).

These human data are supported by numerous animal studies (reviewed in Grimm et al., 2012, 2016b). Namely, Yao et al. (2010) showed that in the brain of perimenopausal mice during reproductive senescence, the drop of estrogen and progesterone (the second female sex hormone) concentrations was accompanied by an increased expression of genes implicated in fatty acid oxidation and the ketogenic pathway, especially at an age between 9 months and 12 months (**Figure 1**). Further investigations revealed that, in response to a glucose deprivation, the fatty acid metabolism was activated in the perimenopausal mouse brain. This increase was coupled with myelin degeneration as well as a rise of brain ketone bodies that were used as an alternative energy fuel (**Figure 1**; Yao et al., 2010; Klosinski et al., 2015). Thus, these findings highlighted the role of estrogen on the brain redox balance and energy metabolism at the menopause (**Figure 1**).

A growing body of evidence supports the mitochondrial theory of aging. The decreased functionality of complex I is often cited as the most likely site of an ETC impairment (**Figure 2**; Sandhu and Kaur, 2003; Petrosillo et al., 2009; Sun et al., 2016). Indeed, complex I activity is decreased across the brain in aged mice (Pollard et al., 2016), together with a decrease of COX (complex IV) activity and an increase of ROS production (**Figure 2**; reviewed in Grimm and Eckert, 2017). Finally, modifications in the fatty acid profile of mitochondrial membranes that are more prone to peroxidation are induced by ovariectomy (OVX), and is a feature also reported in aging (Pamplona, 2008).

Mitochondrial DNA (mtDNA) is close to ROS produced by complex I and complex III and seems to be more susceptible to oxidative damage than nuclear DNA (nDNA; Turrens, 2003; Fang et al., 2016). During normal aging, increased levels of oxidative modifications are reported in the brain as well as mutations in mtDNA (Melov, 2004; Vermulst et al., 2007). Of note, no difference in mtDNA oxidative stress markers was detected between 10 months old male and female mice in the liver or the muscle (Sanz et al., 2007). However, since mtDNA is exclusively maternally inherited, female mitochondria appear to be better equipped to prevent mtDNA damage and mutation to reduce the risk of producing inheritable metabolic disorders (reviewed in Demarest and McCarthy, 2015). In line, estrogen supplementation was able to improve the transcription levels of several DNA repair enzymes involved notably in base excision repair pathway in the dorsal raphe of OVX old female macaques (Bethea et al., 2016).

Mitochondrial dynamics play an important role in maintaining a healthy organelle population (Grimm and Eckert, 2017; Schmitt et al., 2018). Impairments in this quality control system lead to the accumulation of defective

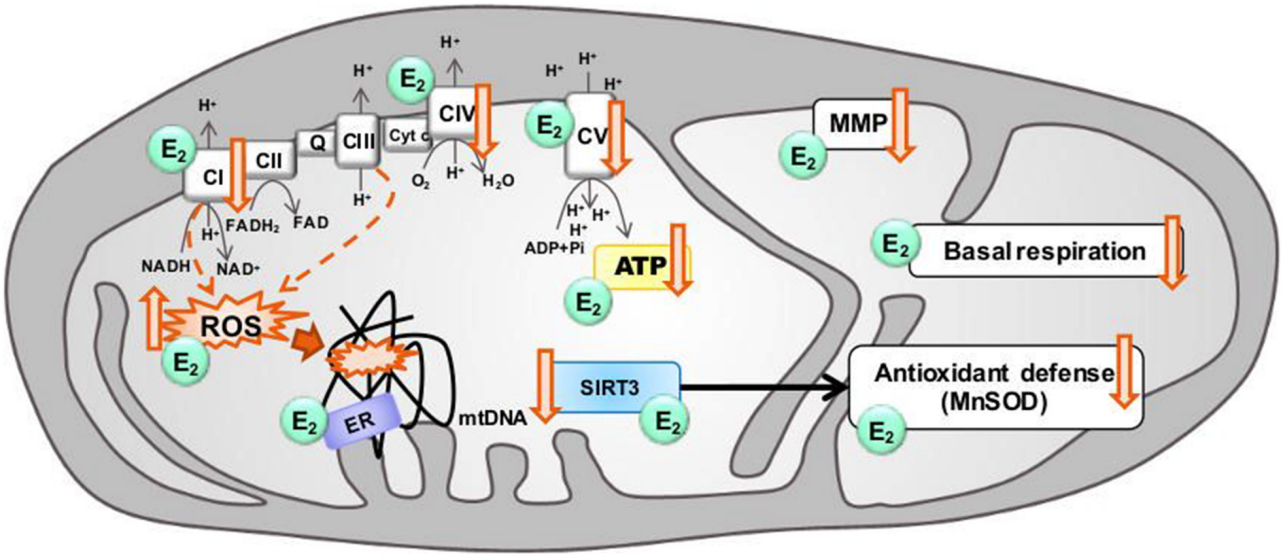


FIGURE 2 | Modulation of mitochondrial function in aging by estrogen. Aging is associated with electron transport chain (ETC) impairments leading to decreased ATP levels, basal respiration and mitochondrial membrane potential (MMP), together with a decrease of antioxidant defenses, and an increase of ROS production by complex I and complex III (dashed arrows) as well as mitochondrial DNA (mtDNA) oxidative damages. Estrogen (E2) has been shown, to increase ETC activity, stabilize the MMP, prevent the ROS production, and ameliorate the basal respiration and the production of ATP levels. Sirtuin 3 (SIRT3) and estrogen may converge on a common pathway to rescue mitochondrial activity in aging by increasing antioxidant defense activity. Estrogen and SIRT3 are suggested to rescue age-related impairments. ↓: age-related decrease, ↑: age-related increase.

mitochondria, as well as inefficient mitochondrial transport and distribution. Strikingly, only few studies were focused on mitochondrial fusion/fission activity in the brain during aging (see Grimm and Eckert, 2017). Alterations in the expression of fusion/fission proteins were shown in mice synaptosomal mitochondria, with namely an increase in dynamin-related protein 1 (Drp1) expression, a fission protein, between 5 months and 12 months of age, and a decrease from 12 months to 24 months (Stauch et al., 2014). In parallel, the expression of mitochondrial fusion proteins, including mitofusins 1/2 (Mfn1/2) and optic atrophy 1 (Opa1), decreased from 5 months to 12 months and increased from 12 months to 24 months, suggesting that synaptosomal mitochondria were shifted to a pro-fusion state in aged animals. Additionally, a pro-fusion effect of estrogen (increased mRNA and protein level of Mfn1/2) was shown in MCF-7 breast cancer cell line (Sastre-Serra et al., 2012). However, to our current knowledge, estrogen effects on mitochondrial dynamics in the brain remain still under investigated. An age-related decline in mitophagy (elimination of damaged mitochondria by autophagy) and mitochondrial biogenesis have also been previously reported (reviewed in Grimm and Eckert, 2017), but the precise reason for this decrease remains elusive, especially in the brain. It has been suggested that one of the potential mechanisms regulating mitochondrial biogenesis occurs via hormonal control such as estrogen (reviewed in Scheller and Sekeris, 2003; Chen et al., 2009).

Thus, mitochondrial dysfunction seems to play a central role in brain aging, leading to decreased bioenergetics (glucose metabolism, mitochondrial respiration and ATP synthesis) and increased oxidation (increase of ROS and decrease of antioxidant defenses). High levels of estrogen, especially that of estradiol, before the menopause may be involved in the high energy capacity as well as the control of redox balance in female brain (**Figure 1**). Then, the drastic drop of estrogen levels may disturb this finely controlled homeostasis, leading to the above-mentioned impairments (see also Grimm et al., 2016b). The **Figure 2** summarizes where estrogen can potentially act to prevent or rescue age-related mitochondria impairments (reviewed in Grimm et al., 2012, 2016b see also section: "Estrogen and Bioenergetics in Female Brain: Implications for Estrogen Replacement Therapy").

ESTROGEN IN THE CENTRAL NERVOUS SYSTEM

Mitochondria, Steroid Synthesis and Estrogen

Steroid hormones can be synthesized within the nervous system independently of peripheral steroid glands and are called neurosteroids (Corpéchot et al., 1981). Mitochondria play a crucial role during the first step of the steroid hormone biosynthesis which is the production of pregnenolone, the common precursor of steroids and neurosteroids (Velarde, 2014). The limiting step during steroidogenesis is the transport of cholesterol from the outside to the inside of

mitochondria (Papadopoulos and Miller, 2012). This process highlights the paramount role of mitochondria in steroid homeostasis. The interaction between the steroidogenic acute regulatory protein (StAR) and a multi-component molecular complex including an 18 kDa translocator protein (TSPO) regulates the cholesterol transport (Miller, 2013). Once in the mitochondria, cholesterol is converted to pregnenolone by the cytochrome P450 side-chain cleavage enzyme (Miller and Auchus, 2011). Then, pregnenolone can be transferred out of the mitochondria and converted into the different steroids, including estrogen, by specific microsomal P450 enzymes (Miller, 2005).

Estrogen: From Brain Development to Brain Aging

Today, it is recognized that the action of estrogen is not limited to the regulation of endocrine functions and behavior. Estrogen plays predominant roles as pleiotropic factor in the development and function of the CNS. Numerous evidences highlighted the influence of both male and female sex hormones on neural circuit development, sex-specific behaviors and sexual differentiation of mammalian brain from the fetal-neonatal period to puberty and adult life (reviewed in Hines, 2011). For instance, the transition from childhood to adulthood is marked by morphological changes in the brain that are associated with sex hormone levels (Koolschijn et al., 2014). Interestingly, the structure and function of the brains of female and male animals as well as humans have been demonstrated to be markedly different in the number of cells in specific brain areas (Kruijver et al., 2000). This sexual dimorphism in several areas of the brain appears to be dependent on the action of gonadal hormones as variations in the volume of cortical and sub-cortical regions were correlated to the levels of estrogen and testosterone during puberty (Kruijver et al., 2000; Herting et al., 2014).

Interestingly, the group of Shah showed that the conversion of testosterone to estradiol by the enzyme aromatase is necessary during the perinatal period for the expression of masculine sex behavior in the adult, while the absence of testosterone and estrogen is necessary for normal development of female brains (Wu et al., 2009). This points out the importance of brain estrogen synthesis in aromatase-expressing neurons during fetal male life. Although testosterone is important for male development and behavior throughout life, it is now recognized that estrogen plays also important roles in the male brain (reviewed in Gillies and McArthur, 2010).

Estrogen can induce several effects through different pathways (see also next section). The identification of ER outside their classical CNS regions like the hypothalamus and the pituitary gland justifies their role in controlling different brain functions (Genazzani et al., 2007). This organizational effect is firstly acting during the fetal-neonatal period where estrogen modulates neuronal development and the development of neuronal circuits (Kruijver et al., 2001). Brain plasticity begins with the formation of the nervous system during early development and continues through the puberty, reproduction and adult life (Cooke et al., 1998). Estrogen seems to be also important for the maintenance and regulation of the network integrity of brain areas related to cognition, especially the hippocampus and associated structures (Garcia-Segura et al., 1994). In peri- and postmenopause, neurosteroids including estrogen undergo important changes due to the disturbance of gonadal hormone production and many CNS functions associated with hippocampal activities deteriorate, such as memory, cognition and attention (Genazzani et al., 2005).

Estrogen Pathways: Estrogen Receptors, BDNF and Sirtuin 3

Different pathways play a role in the pleiotropic effects of estrogen in the brain. In the following, we discuss the emerging evidence about the role of ER and the estrogen—brain-derived neurotrophic factor (BDNF) as well as estrogen—sirtuin 3 (SIRT3) pathways that converge on the mitochondria.

Estrogen Receptors

Among the sex steroid hormone receptors, ERα and ERβ have been shown to be localized in mitochondria depending on the cell type and they ensure the mitochondrial function. The co-localization of ERβ and mitochondrial markers have been demonstrated in rat primary neurons and a murine hippocampal cell line suggesting a role of mitochondrial ERβ in mediating estrogen effects (Yang et al., 2004; **Figure 2**). Indeed, ERβ is described as the type of ER that is most frequently present in mitochondria in most cell types (Vasconsuelo et al., 2013). ERα seems also to play a role, since estrogen exerted protective effects in human cerebral endothelium by increasing mitochondrial cytochrome *c* protein and mRNA, as well as reducing ROS through ERα but not ERβ receptors (Razmara et al., 2008).

In addition, estrogen response elements (ERE) are present in the mtDNA and allow the binding of steroid receptors. In fact, ER can bind to ERE located in the mtDNA (Chen et al., 2004). This association is known to improve the expression of mitochondrial-encoded genes and the ETC activity (**Figure 2**; Arnold et al., 2012). Moreover, sex steroid hormone receptors have been suggested to bind mitochondrial proteins. Indeed, the association of mitochondrial ERβ with mitochondrial respiratory complex proteins was investigated in a series of co-immunoprecipitation studies showing that ERβ can interact with the respiratory complex V (Alvarez-Delgado et al., 2010; Velarde, 2014). In different brain areas, there is a differential expression of mitochondrial ERα and ERβ variants suggesting that they can participate in several functions in the brain during aging (Alvarez-Delgado et al., 2010). In female rats, mitochondrial ERα and ERβ were detected in the hypothalamus, cortex and hippocampus with no difference between young (3 months old) and aged (18 months old) animals (Alvarez-Delgado et al., 2010).

Sirtuin 3 Pathway

The function of the sirtuin family consisting of seven members is important in different aspects of cellular regulation such

as lipid homeostasis and metabolism (reviewed Houtkooper et al., 2012), apoptosis resistance (reviewed in Pantazi et al., 2013) and oxidative stress management (reviewed in Rajendran et al., 2011). In addition, sirtuins are believed to contribute to the aging process and may play direct roles in longevity regulation (Guarente, 2013). Among the sirtuin members, SIRT3 has received much attention for its role in aging and neurodegenerative diseases and is reported to affect human lifespan (Reviewed in Ansari et al., 2017). SIRT3 is one of the three genomically expressed sirtuins that localize to mitochondria (Onyango et al., 2002; Schwer et al., 2002) as well as the primary mitochondrial protein deacetylase (Lombard et al., 2007). SIRT3 controls energy demand during stress conditions including oxidative stress conditions related to the aging process (**Figure 2**; reviewed in Ansari et al., 2017). In addition, its over-expression prevented neuronal derangements in certain *in vitro* and *in vivo* models of aging (Anamika et al., 2017), whereas its knockout in *Sirt3*−/− mouse embryonic fibroblasts induced abnormal mitochondrial physiology as well as increases in stress-induced superoxide levels and increased genomic instability (Kim et al., 2010). At the mRNA level, *Sirt3* is the third most expressed member of the sirtuin family in the whole brain of the adult rat, except in the cerebellum where its expression was modestly lower (Sidorova-Darmos et al., 2014). During the development, mRNA expression pattern of *Sirt3* was similar in cortex, cerebellum and hippocampus, with *Sirt3* mRNA levels being fairly consistent from embryonic day 18 (E18) until 24 months of age (Sidorova-Darmos et al., 2014). Unfortunately, sex differences were not investigated in the study of Sidorova-Darmos et al. (2014), since the sex was not specifically determined for E18, postnatal days (PN) 2, PN7 or PN21 stages, while only female rats were used for the 3 and 24 month tissue samples, but *Sirt3* mRNA levels were similar at least in liver tissue from male and female mice at the age of 11 months (Yu et al., 2015). Braidy et al. (2015) investigated changes in the protein levels of various sirtuins in the aged female rat brain. Twenty-four month old rats showed lower SIRT3 levels in the hippocampus and frontal cortex, but not occipital or temporal lobe, compared to 3 month old rats.

The co-presence of SIRT3 and the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) implicates its role in mitochondrial biogenesis that provides ATP to support fundamental cellular processes involved in synaptic plasticity such as neurite outgrowth (Kong et al., 2010). Interestingly, the mitochondrial SIRT3 promoter region carries an estrogen-related receptor (ERR)-binding element (ERRE) located 399- to 407-bp downstream (**Figure 3**; Kong et al., 2010). SIRT3 attenuates ROS and protects the cell from ROS both directly through the deacetylation of manganese superoxide dismutase (MnSOD; **Figures 2**, **3**) and indirectly via interaction with transcription factors such as forkhead box O3 (FOXO3a; **Figure 3**) as demonstrated in TCam-2 cells (Panza et al., 2017). Sirt3-induced activated FOXO3a translocates to the nucleus and augments FOXO3a-dependent antioxidant defense mechanisms, through upregulation of PGC-1α and MnSOD (**Figure 3**).

BDNF Pathway

Research has implicated one of the neurotrophic factors, BDNF, to be involved in age-related cognitive decline. A hypothesis has emerged that aging is associated with a decreased BDNF signaling capacity in the CNS (Mattson et al., 2004). In humans, BDNF levels have been shown to decrease in brain regions involved in age-related neurodegenerative diseases: e.g., in Alzheimer's disease (AD) in the frontal cortex (Ferrer et al., 1999), the hippocampus (Phillips et al., 1991), the parietal cortex (Hock et al., 2000) and the entorhinal cortex (Narisawa-Saito et al., 1996). However, age-related changes in brain BDNF levels in elderly humans during normal cognitive aging are under-investigated. In aged monkeys (26, 30 and 32 years), the intensity of BDNF-immunoreactivity has been found to decline in cell bodies and dendrites of the neurons in the hippocampal formation (Hayashi et al., 2001). In humans, BDNF levels in plasma have been found to decrease with increasing age (Lommatzsch et al., 2005; Ziegenhorn et al., 2007; Erickson et al., 2010) and the levels of tropomyosin receptor kinase B (trkB, BNDF receptor) mRNA decreased over the life span (Webster et al., 2006). In accordance with these results, it has been shown that the levels of trkB decrease in the rat hippocampus during aging (Croll et al., 1998; Silhol et al., 2005) and 24-month-old rats exhibited significantly less BDNF protein in three brain regions as compared to 4-month-old rats (Bimonte-Nelson et al., 2008). However, it should be noticed that conflicting data from animal research exist not always confirming whether age-related changes do occur in the brain (Bimonte-Nelson et al., 2008). Compared to females, males had lower BDNF levels in the hippocampus at 20 months emphasizing the important role of sex and sex hormones (Bimonte-Nelson et al., 2008). Therefore, discordant findings between aging studies may also be related to the fact that some studies used males (e.g., Narisawa-Saito and Nawa, 1996; Yurek and Fletcher-Turner, 2001), whereas others used female animals (e.g., Scott et al., 1994). In agreement with these findings, some authors found that elderly females, but not elderly males, with poorer cognitive performance had lower plasma BDNF levels than better performers (e.g., Komulainen et al., 2008) and smaller hippocampal volumes are associated with reduced levels of serum BDNF and poorer memory performance (Erickson et al., 2010). Moreover, genetic studies have identified a single nucleotide polymorphism on the BDNF gene that moderates age-related cognitive decline (Erickson et al., 2008). Of note, a very recent study showed that estrogen tweaks circuit function by interacting with a uniquely human version of the gene that encodes for BDNF, the Val66Met genotype (Wei et al., 2018). The BDNF Val66Met variant and ovarian steroids interactively modulate working memory-related hippocampal function in women suggesting that hormone-gene interaction may underlie sex/individual differences in brain disorders, thus providing a potential explanation for observations of individual differences in the effects of estrogen on hippocampal performance in women (Wei et al., 2018).

In summary, there is evidence suggesting that the BDNF-trkB system, especially in the hippocampus, is sensitive to aging and is

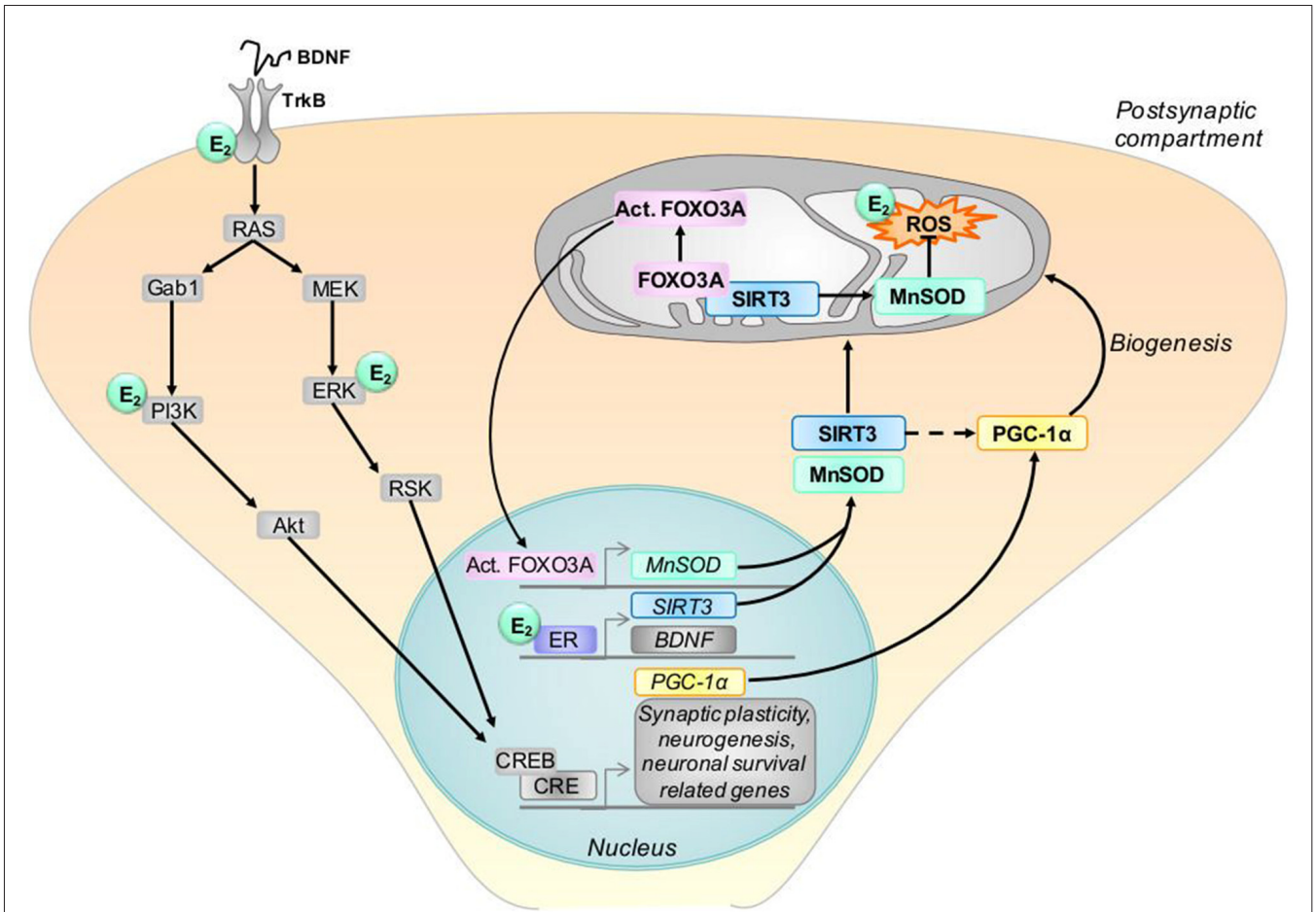


FIGURE 3 | Model of possible interactions between estrogen, BDNF and SIRT3. BDNF protein binds to its tyrosine kinase B receptor (TrkB) which stimulates signaling pathways, including the extracellular signal-regulated kinase (ERK) and the phosphatidylinositol 3-kinase (PI3K) pathways. This leads to the activation of the cAMP response element-binding protein (CREB) and transcription of genes, including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). Estrogen (E2) activates similar signaling pathways as BDNF, and, by binding its receptor (ER), modulates the gene expression of BDNF, TrkB and SIRT3. SIRT3 protein translocates to mitochondria and interacts with Forkhead box O3 (FOXO3A), thereby activating this protein. In turn, activated FOXO3A (Act. FOXO3A) induces the transcription of the manganese superoxide dismutase (MnSOD) which acts as ROS scavengers in mitochondria. In addition, SIRT3 protects the cell from ROS directly through the deacetylation of MnSOD. SIRT3 regulates also the expression of PGC-1α (indicated by the doted arrow) which is involved in mitochondrial biogenesis. In the graph, E2 designates where estrogen may interact with the BDNF and SIRT3 pathways. BDNF: brain derived neurotrophic factor, CRE, cAMP response elements, PGC-1α, proliferator-activated receptor gamma coactivator 1-alpha, SIRT3, sirtuin 3.

modulated by sex and sex hormones respectively. In this context, the drop in estrogen in females during perimenopause might play a role in the cognitive function decline during female aging.

BDNF and estrogen are both important modulators of synaptic plasticity (**Figure 3**; Zárate et al., 2017). Synaptic plasticity, the dynamic regulation of synaptic mechanisms like long-term potentiation (LTP), spine density and form, number and length of dendrites and axons (neuritogenesis) and the number of neurons (neurogenesis and apoptosis) represent major mechanisms by which our brain can adapt to periods of pathologically enhanced or reduced function or to save information at the synaptic level. Mitochondria play a crucial role as they provide the cellular energy for these adaptive responses or initiate apoptosis in case of neuronal damage beyond the possibility of repair. Changes of synaptic function and plasticity play a major role for cognitive deficits in aging and age-related brain disorders (Barnes, 1990; Rosenzweig and Barnes, 2003).

Activation of the high-affinity BDNF receptor TrkB results in phosphorylation of tyrosine residues in the cytoplasmic domain of the receptor and subsequent recruitment and activation of signaling proteins that engage extracellular signal-regulated kinases (ERKs) and the phosphatidylinositol 3-kinase (PI3K)-Akt kinase pathway (**Figure 3**; Cheng et al., 2012).

Notably, it has been shown that estrogen and BDNF receptor (trkB) coexpression leads to convergence of their signaling pathways (**Figure 3**; Scharfman and MacLusky, 2006) and PI3K and MAPK/ERK protein cascades may be activated by estrogen or BDNF-bound membrane receptors (Cover et al., 2014). Both pathways phosphorylate the transcription factor

cAMP response element-binding protein (CREB) which, in turn, induces the expression of PGC-1α resulting in protein transcription, neuronal plasticity and memory formation and consolidation (Marosi and Mattson, 2014). Intra-pathway crosstalk is in addition possible. Moreover, estrogen and BDNF can interact directly, because estrogen induces BDNF gene expression, which in turn acts on trkB to exert its effects (**Figure 3**; Scharfman and MacLusky, 2006). Induction of this kind may occur by an ERE on the BDNF gene or by estrogen-induced increase in neural activity that upregulates BDNF (**Figure 3**; Scharfman and MacLusky, 2006; Zárate et al., 2017). By enhancing the uptake of energy substrates, mitochondrial biogenesis, and protein synthesis capabilities of neurons, estrogen-BDNF signaling plays pivotal roles in the adaptive changes in synapse formation and modification. Thus, the concerted action between BDNF and estrogen is important for the improvement of synaptic function and plasticity in aging.

ESTROGEN AND BIOENERGETICS IN FEMALE BRAIN: IMPLICATIONS FOR ESTROGEN REPLACEMENT THERAPY

Estrogen is an essential regulator of the metabolic system of the female brain and contributes to processes within the entire bioenergetic system including glucose metabolism, glucose transport and ATP production as well as mitochondrial respiration (**Figure 2**; Rettberg et al., 2014). A decline of 15%–25% in metabolic function in the brain was observed after the loss of estrogen by surgical removal of ovaries or reproductive endocrine aging (Loembe et al., 1988; Slebos et al., 1988). Recent research of our group confirmed *in vitro* the effects of estrogen on mitochondrial function (**Figure 2**). We showed that estrogen, such as estradiol and estrone, was able to improve bioenergetics and antioxidant defenses in primary neuronal cultures and in human neuroblastoma cells (SH-SY5Y) by increasing mitochondrial membrane potential (MMP), ATP levels, basal respiration and MnSOD activity (Grimm et al., 2014; **Figure 2**). Besides, a treatment with estrogen was efficient in reducing bioenergetic impairments observed in a cellular model of AD (Grimm et al., 2016a; Lejri et al., 2017). Thus, estrogen represents an attractive therapeutic tool to counteract the mitochondrial impairments in aging and age-related disorders.

Female sex is the major risk factor for AD after advanced age. Preclinical studies demonstrated that the perimenopause to menopause transition is a sex-specific risk factor for AD (Mosconi et al., 2017). In animal studies, estrogenic regulation of cerebral glucose metabolism falters during perimenopause (Mosconi et al., 2017). During aging, a decline of genes required for mitochondrial function and β-amyloid degradation was observed in a rat model recapitulating fundamental characteristics of the human perimenopause (Yin et al., 2015). Impaired synaptic function and emergence of glucose hypometabolism in brain give plausible mechanisms of neurological symptoms of perimenopause and can be predictive of later-life vulnerability to hypometabolic conditions like AD (Yin et al., 2015).

In the metabolic system of the aging female brain, the earliest change is the persistent decline in neuronal glucose transport and metabolism, followed by a drop in mitochondrial function (Ding et al., 2013). In fact, immediately before the transition into reproductive senescence, a hypometabolism in the brain of female mice (at 6–9 months) was demonstrated by a reduction in glucose uptake and hexokinase activity (Ding et al., 2013). These phenomena were also accompanied by inactivation of complex IV activity and pyruvate dehydrogenase leading to an impairment of the mitochondrial energy-conservation system in the brain of female mice (Ding et al., 2013). In parallel to the decline in glucose transport, lactate transport and utilization were also reduced, suggesting that the lactate is not used as an alternative fuel source during the transition to reproductive senescence.

Earlier preclinical findings indicating emergence of bioenergetic deficits in perimenopausal and postmenopausal women were recently validated and suggested that the optimal window of opportunity for therapeutic intervention in women is early in the endocrine aging process (Mosconi et al., 2017). A study bridged basic to clinical science to characterize brain bioenergetics in a cohort of 43, 40–60 year-old clinically and cognitively normal women at different endocrine transition stages including premenopause, perimenopause and postmenopause. Compared to premenopause, both

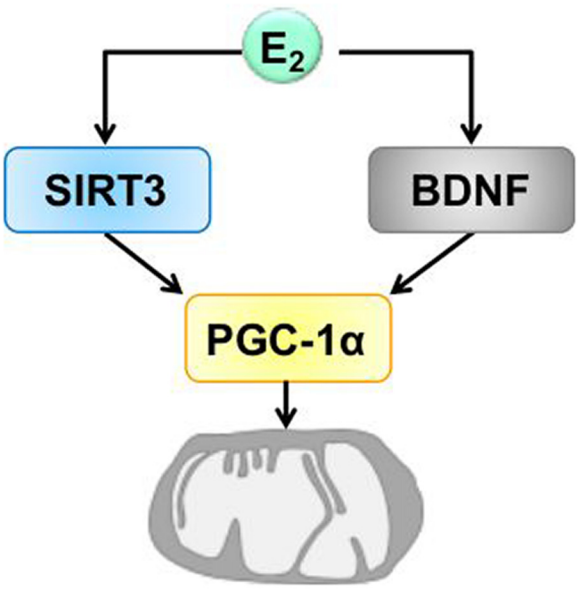


FIGURE 4 | Hypothetic model of the convergence of BDNF and SIRT3 on mitochondria induced by estrogen. Estrogen (E2) induces the expression of BDNF and SIRT3. The action of BDNF and SIRT3 converges on mitochondria by regulating the expression of PGC-1α which is involved in mitochondrial biogenesis that is necessary for synaptic plasticity. BDNF, brain derived neurotrophic factor, PGC-1α, proliferator-activated receptor gamma coactivator 1-alpha, SIRT3, sirtuin 3.

perimenopause and postmenopause groups exhibited a reduction in mitochondrial COX activity which was correlated with a decline of cerebral glucose metabolism in AD-vulnerable regions. A gradient in biomarker abnormalities correlated with immediate and delayed memory scores and was most pronounced in post-menopause, intermediate in perimenopause, and lowest in premenopause (Mosconi et al., 2017). Therefore, estrogen replacement therapies (ERT) may only be beneficial before women accumulate a certain threshold of cellular damage, at the appropriate time of a woman's life, also known as the "critical period" that starts during perimenopause and ends early after menopause (**Figure 1**; Velarde, 2013; Viña et al., 2013; Grimm et al., 2016b). This "critical period" might also explain the failure of the Women's Health Initiative (WHI) study including only postmenopausal aged over 68 years thereby missing the vulnerable time frame (Rossouw et al., 2002; Anderson et al., 2004). The window of opportunity concept supports that estrogen can have beneficial effects only on healthy brain during the years before the menopause compared to some years after the menopause onset where ERT could even present detrimental effect on brain activity (Morrison et al., 2006; Brinton, 2008, 2009; Scott et al., 2012; Rettberg et al., 2014; Miller and Harman, 2017). The effectiveness of ERT is also dependent on the formulation of treatments (e.g., use of synthetic conjugated equine estrogen vs. natural estrogen, or co-treatment with progesterone), the mode of delivery (transdermal or oral) and the regimen (cyclic or continuous; Miller and Harman, 2017).

Importantly, recent evaluation of the role of estrogen showed that hormonal therapy can prevent the deleterious effects of aging in cognition, and decreases the risks of dementia, if initiated early (Girard et al., 2017). In this context, beneficial treatment effects of estrogen might be mediated by the BDNF and SIRT3 pathways and their convergence via PGC-1α on mitochondria (**Figure 4**).

CONCLUSION

Over the last decade, accumulating evidence has suggested a causative link between mitochondrial dysfunction and major phenotypes associated with aging. A number of recent studies link mitochondrial function to signaling pathways that regulate brain plasticity, life span and to the aging process. It is clear that estrogen exerts actions on the mitochondria and that these organelles play an important role in age-related processes. In this context, estrogen/BDNF or estrogen/SIRT3 actions and interactions represent complex and fundamental mechanisms of neuronal plasticity, a process highly depending on energy supply via mitochondrial activity (**Figure 4**). Notably, a growing body of evidence indicates emergence of bioenergetic deficits already in perimenopausal women, suggesting that the optimal window of opportunity should be used for the therapeutic intervention in women during the endocrine aging process.

A full understanding of the molecular mechanisms triggered by estrogen at the mitochondrial level and its effects in aging populations may provide profound and exciting possibilities for the future treatment of age-dependent diseases associated with the deregulation of sexual hormone levels.

AUTHOR CONTRIBUTIONS

The authors had an equal contribution in the writing of the manuscript (IL, AG and AE).

FUNDING

This work was funded by Research Funds from the Psychiatric University Clinics Basel (UPK Forschungs fonds to AE) and the Swiss National Science Foundation (SNF #31003A 149728 to AE).

REFERENCES

Alvarez-Delgado, C., Mendoza-Rodríguez, C. A., Picazo, O., and Cerbón, M. (2010). Different expression of α and β mitochondrial estrogen receptors in the aging rat brain: interaction with respiratory complex V. *Exp. Gerontol.* 45, 580–585. doi: 10.1016/j.exger.2010.01.015

Anamika, Khanna, A., Acharjee, P., Acharjee, A., and Trigun, S. K. (2017). Mitochondrial SIRT3 and neurodegenerative brain disorders. *J. Chem. Neuroanat.* doi: 10.1016/j.jchemneu.2017.11.009 [Epub ahead of print].

Anderson, G. L., Limacher, M., Assaf, A. R., Bassford, T., Beresford, S. A., Black, H., et al. (2004). Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's health initiative randomized controlled trial. *JAMA* 291, 1701–1712. doi: 10.1001/jama.291.14.1701

Ansari, A., Rahman, M. S., Saha, S. K., Saikot, F. K., Deep, A., and Kim, K. H. (2017). Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. *Aging Cell* 16, 4–16. doi: 10.1111/acel.12538

Arnold, S., Victor, M. B., and Beyer, C. (2012). Estrogen and the regulation of mitochondrial structure and function in the brain. *J. Steroid Biochem. Mol. Biol.* 131, 2–9. doi: 10.1016/j.jsbmb.2012.01.012

Balaban, R. S., Nemoto, S., and Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell* 120, 483–495. doi: 10.1016/j.cell.2005.02.001

Barnes, C. A. (1990). Effects of aging on the dynamics of information processing and synaptic weight changes in the mammalian hippocampus. *Prog. Brain Res.* 86, 89–104. doi: 10.1016/s0079-6123(08)63169-6

Bellanti, F., Matteo, M., Rollo, T., De Rosario, F., Greco, P., Vendemiale, G., et al. (2013). Sex hormones modulate circulating antioxidant enzymes: impact of estrogen therapy. *Redox Biol.* 1, 340–346. doi: 10.1016/j.redox.2013.05.003

Bethea, C. L., Kohama, S. G., Reddy, A. P., and Urbanski, H. F. (2016). Ovarian steroids regulate gene expression in the dorsal raphe of old female macaques. *Neurobiol. Aging* 37, 179–191. doi: 10.1016/j.neurobiolaging.2015.10.004

Bimonte-Nelson, H. A., Granholm, A. C., Nelson, M. E., and Moore, A. B. (2008). Patterns of neurotrophin protein levels in male and female Fischer 344 rats from adulthood to senescence: how young is "young" and how old is "old"? *Exp. Aging Res.* 34, 13–26. doi: 10.1080/03610730701761908

Braidy, N., Poljak, A., Grant, R., Jayasena, T., Mansour, H., Chan-Ling, T., et al. (2015). Differential expression of sirtuins in the aging rat brain. *Front. Cell. Neurosci.* 9:167. doi: 10.3389/fncel.2015.00167

Brinton, R. D. (2008). The healthy cell bias of estrogen action: mitochondrial bioenergetics and neurological implications. *Trends Neurosci.* 31, 529–537. doi: 10.1016/j.tins.2008.07.003

Brinton, R. D. (2009). Estrogen-induced plasticity from cells to circuits: predictions for cognitive function. *Trends Pharmacol. Sci.* 30, 212–222. doi: 10.1016/j.tips.2008.12.006

Castellani, R. J., Rolston, R. K., and Smith, M. A. (2010). Alzheimer disease. *Dis. Mon.* 56, 484–546. doi: 10.1016/j.disamonth.2010.06.001

Chen, J. Q., Cammarata, P. R., Baines, C. P., and Yager, J. D. (2009). Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and pharmacological implications. *Biochim. Biophys. Acta* 1793, 1540–1570. doi: 10.1016/j.bbamcr.2009.06.001

Chen, J. Q., Eshete, M., Alworth, W. L., and Yager, J. D. (2004). Binding of MCF-7 cell mitochondrial proteins and recombinant human estrogen receptors α and β to human mitochondrial DNA estrogen response elements. *J. Cell. Biochem.* 93, 358–373. doi: 10.1002/jcb.20178

Cheng, A., Wan, R., Yang, J. L., Kamimura, N., Son, T. G., Ouyang, X., et al. (2012). Involvement of PGC-1α in the formation and maintenance of neuronal dendritic spines. *Nat. Commun.* 3:1250. doi: 10.1038/ncomms2238

Cooke, B., Hegstrom, C. D., Villeneuve, L. S., and Breedlove, S. M. (1998). Sexual differentiation of the vertebrate brain: principles and mechanisms. *Front. Neuroendocrinol.* 19, 323–362. doi: 10.1006/frne.1998.0171

Corpéchot, C., Robel, P., Axelson, M., Sjövall, J., and Baulieu, E. E. (1981). Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. *Proc. Natl. Acad. Sci. U S A* 78, 4704–4707. doi: 10.1073/pnas.78.8.4704

Cover, K. K., Maeng, L. Y., Lebrón-Milad, K., and Milad, M. R. (2014). Mechanisms of estradiol in fear circuitry: implications for sex differences in psychopathology. *Transl. Psychiatry* 4:e422. doi: 10.1038/tp.2014.67

Croll, S. D., Ip, N. Y., Lindsay, R. M., and Wiegand, S. J. (1998). Expression of BDNF and trkB as a function of age and cognitive performance. *Brain Res.* 812, 200–208. doi: 10.1016/s0006-8993(98)00993-7

Cui, H., Kong, Y., and Zhang, H. (2012). Oxidative stress, mitochondrial dysfunction, and aging. *J. Signal Transduct.* 2012:646354. doi: 10.1016/b978-0-12-405933-7.00004-4

Dalal, P. K., and Agarwal, M. (2015). Postmenopausal syndrome. *Indian J. Psychiatry* 57, S222–S232. doi: 10.4103/0019-5545.161483

Demarest, T. G., and McCarthy, M. M. (2015). Sex differences in mitochondrial (dys)function: implications for neuroprotection. *J. Bioenerg. Biomembr.* 47, 173–188. doi: 10.1007/s10863-014-9583-7

Ding, F., Yao, J., Rettberg, J. R., Chen, S., and Brinton, R. D. (2013). Early decline in glucose transport and metabolism precedes shift to ketogenic system in female aging and Alzheimer's mouse brain: implication for bioenergetic intervention. *PLoS One* 8:e79977. doi: 10.1371/journal.pone.0079977

Erickson, K. I., Kim, J. S., Suever, B. L., Voss, M. W., Francis, B. M., and Kramer, A. F. (2008). Genetic contributions to age-related decline in executive function: a 10-year longitudinal study of COMT and BDNF polymorphisms. *Front. Hum. Neurosci.* 2:11. doi: 10.3389/neuro.09.009.2008

Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Heo, S., Mclaren, M., et al. (2010). Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. *J. Neurosci.* 30, 5368–5375. doi: 10.1523/jneurosci.6251-09.2010

Fang, E. F., Scheibye-Knudsen, M., Chua, K. F., Mattson, M. P., Croteau, D. L., and Bohr, V. A. (2016). Nuclear DNA damage signalling to mitochondria in ageing. *Nat. Rev. Mol. Cell Biol.* 17, 308–321. doi: 10.1038/nrm.2016.14

Ferrer, I., Marín, C., Rey, M. J., Ribalta, T., Goutan, E., Blanco, R., et al. (1999). BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *J. Neuropathol. Exp. Neurol.* 58, 729–739. doi: 10.1097/00005072-199907000-00007

Garcia-Segura, L. M., Chowen, J. A., Duenas, M., Torres-Aleman, I., and Naftolin, F. (1994). Gonadal steroids as promoters of neuro-glial plasticity. *Psychoneuroendocrinology* 19, 445–453. doi: 10.1016/0306-4530(94)90031-0

Genazzani, A. R., Bernardi, F., Pluchino, N., Begliuomini, S., Lenzi, E., Casarosa, E., et al. (2005). Endocrinology of menopausal transition and its brain implications. *CNS Spectr.* 10, 449–457. doi: 10.1017/s1092852900023142

Genazzani, A. R., Pluchino, N., Luisi, S., and Luisi, M. (2007). Estrogen, cognition and female ageing. *Hum. Reprod. Update* 13, 175–187. doi: 10.1093/humupd/dml042

Gillies, G. E., and McArthur, S. (2010). Estrogen actions in the brain and the basis for differential action in men and women: a case for sex-specific medicines. *Pharmacol. Rev.* 62, 155–198. doi: 10.1124/pr.109.002071

Girard, R., Météreau, E., Thomas, J., Pugeat, M., Qu, C., and Dreher, J. C. (2017). Hormone therapy at early post-menopause increases cognitive control-related prefrontal activity. *Sci. Rep.* 7:44917. doi: 10.1038/srep44917

Grimm, A., and Eckert, A. (2017). Brain aging and neurodegeneration: from a mitochondrial point of view. *J. Neurochem.* 143, 418–431. doi: 10.1111/jnc.14037

Grimm, A., Friedland, K., and Eckert, A. (2016a). Mitochondrial dysfunction: the missing link between aging and sporadic Alzheimer's disease. *Biogerontology* 17, 281–296. doi: 10.1007/s10522-015-9618-4

Grimm, A., Mensah-Nyagan, A. G., and Eckert, A. (2016b). Alzheimer, mitochondria and gender. *Neurosci. Biobehav. Rev.* 67, 89–101. doi: 10.1016/j.neubiorev.2016.04.012

Grimm, A., Lim, Y. A., Mensah-Nyagan, A. G., Götz, J., and Eckert, A. (2012). Alzheimer's disease, oestrogen and mitochondria: an ambiguous relationship. *Mol. Neurobiol.* 46, 151–160. doi: 10.1007/s12035-012-8281-x

Grimm, A., Schmitt, K., Lang, U. E., Mensah-Nyagan, A. G., and Eckert, A. (2014). Improvement of neuronal bioenergetics by neurosteroids: implications for age-related neurodegenerative disorders. *Biochim. Biophys. Acta* 1842, 2427–2438. doi: 10.1016/j.bbadis.2014.09.013

Guarente, L. (2013). Calorie restriction and sirtuins revisited. *Genes Dev.* 27, 2072–2085. doi: 10.1101/gad.227439.113

Hayashi, M., Mistunaga, F., Ohira, K., and Shimizu, K. (2001). Changes in BDNF-immunoreactive structures in the hippocampal formation of the aged macaque monkey. *Brain Res.* 918, 191–196. doi: 10.1016/s0006-8993(01)03002-5

Herting, M. M., Gautam, P., Spielberg, J. M., Kan, E., Dahl, R. E., and Sowell, E. R. (2014). The role of testosterone and estradiol in brain volume changes across adolescence: a longitudinal structural MRI study. *Hum. Brain Mapp.* 35, 5633–5645. doi: 10.1002/hbm.22575

Hines, M. (2011). Prenatal endocrine influences on sexual orientation and on sexually differentiated childhood behavior. *Front. Neuroendocrinol.* 32, 170–182. doi: 10.1016/j.yfrne.2011.02.006

Hock, C., Heese, K., Hulette, C., Rosenberg, C., and Otten, U. (2000). Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch. Neurol.* 57, 846–851. doi: 10.1001/archneur.57.6.846

Houtkooper, R. H., Pirinen, E., and Auwerx, J. (2012). Sirtuins as regulators of metabolism and healthspan. *Nat. Rev. Mol. Cell Biol.* 13, 225–238. doi: 10.1038/nrm3293

Kim, H. S., Patel, K., Muldoon-Jacobs, K., Bisht, K. S., Aykin-Burns, N., Pennington, J. D., et al. (2010). SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. *Cancer Cell* 17, 41–52. doi: 10.1016/j.ccr.2009.11.023

Klosinski, L. P., Yao, J., Yin, F., Fonteh, A. N., Harrington, M. G., Christensen, T. A., et al. (2015). White matter lipids as a ketogenic fuel supply in aging female brain: implications for Alzheimer's disease. *EBioMedicine* 2, 1888–1904. doi: 10.1016/j.ebiom.2015.11.002

Komulainen, P., Pedersen, M., Hänninen, T., Bruunsgaard, H., Lakka, T. A., Kivipelto, M., et al. (2008). BDNF is a novel marker of cognitive function in ageing women: the DR's EXTRA study. *Neurobiol. Learn. Mem.* 90, 596–603. doi: 10.1016/j.nlm.2008.07.014

Kong, X., Wang, R., Xue, Y., Liu, X., Zhang, H., Chen, Y., et al. (2010). Sirtuin 3, a new target of PGC-1α, plays an important role in the suppression of ROS and mitochondrial biogenesis. *PLoS One* 5:e11707. doi: 10.1371/journal.pone.0011707

Koolschijn, P. C., Peper, J. S., and Crone, E. A. (2014). The influence of sex steroids on structural brain maturation in adolescence. *PLoS One* 9:e83929. doi: 10.1371/journal.pone.0083929

Kruijver, F. P., Fernández-Guasti, A., Fodor, M., Kraan, E. M., and Swaab, D. F. (2001). Sex differences in androgen receptors of the human mamillary bodies are related to endocrine status rather than to sexual orientation or transsexuality. *J. Clin. Endocrinol. Metab.* 86, 818–827. doi: 10.1210/jcem.86.2.7258

Kruijver, F. P., Zhou, J. N., Pool, C. W., Hofman, M. A., Gooren, L. J., and Swaab, D. F. (2000). Male-to-female transsexuals have female neuron numbers in a limbic nucleus. *J. Clin. Endocrinol. Metab.* 85, 2034–2041. doi: 10.1210/jc.85.5.2034

Lejri, I., Grimm, A., Miesch, M., Geoffroy, P., Eckert, A., and Mensah-Nyagan, A. G. (2017). Allopregnanolone and its analog BR 297 rescue neuronal cells from oxidative stress-induced death through bioenergetic improvement.

Biochim. Biophys. Acta 1863, 631–642. doi: 10.1016/j.bbadis.2016.12.007

Leuner, K., Müller, W. E., and Reichert, A. S. (2012). From mitochondrial dysfunction to amyloid β formation: novel insights into the pathogenesis of Alzheimer's disease. *Mol. Neurobiol.* 46, 186–193. doi: 10.1007/s12035-012-8307-4

Loembe, P. M., Bouger, D., and Dukuly, L. (1988). Injuries of the cervical spine. Review of 70 cases treated over a 5-year period at the "Fondation Jeanne Ebori" of Libreville, Gabon (Central Africa). *Neurochirurgie* 34, 258–261.

Lombard, D. B., Alt, F. W., Cheng, H. L., Bunkenborg, J., Streeper, R. S., Mostoslavsky, R., et al. (2007). Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. *Mol. Cell. Biol.* 27, 8807–8814. doi: 10.1128/mcb.01636-07

Lommatzsch, M., Zingler, D., Schuhbaeck, K., Schloetcke, K., Zingler, C., Schuff-Werner, P., et al. (2005). The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiol. Aging* 26, 115–123. doi: 10.1016/j.neurobiolaging.2004.03.002

Mandal, P. K., Tripathi, M., and Sugunan, S. (2012). Brain oxidative stress: detection and mapping of anti-oxidant marker 'Glutathione' in different brain regions of healthy male/female, MCI and Alzheimer patients using non-invasive magnetic resonance spectroscopy. *Biochem. Biophys. Res. Commun.* 417, 43–48. doi: 10.1016/j.bbrc.2011.11.047

Marosi, K., and Mattson, M. P. (2014). BDNF mediates adaptive brain and body responses to energetic challenges. *Trends Endocrinol. Metab.* 25, 89–98. doi: 10.1016/j.tem.2013.10.006

Mattson, M. P., Maudsley, S., and Martin, B. (2004). BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.* 27, 589–594. doi: 10.1016/j.tins.2004.08.001

Melov, S. (2004). Modeling mitochondrial function in aging neurons. *Trends Neurosci.* 27, 601–606. doi: 10.1016/j.tins.2004.08.004

Miller, W. L. (2005). Minireview: regulation of steroidogenesis by electron transfer. *Endocrinology* 146, 2544–2550. doi: 10.1210/en.2005-0096

Miller, W. L. (2013). Steroid hormone synthesis in mitochondria. *Mol. Cell. Endocrinol.* 379, 62–73. doi: 10.1016/j.mce.2013.04.014

Miller, W. L., and Auchus, R. J. (2011). The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev* 32, 81–151. doi: 10.1210/er.2010-0013

Miller, V. M., and Harman, S. M. (2017). An update on hormone therapy in postmenopausal women: mini-review for the basic scientist. *Am. J. Physiol. Heart Circ. Physiol.* 313, H1013–H1021. doi: 10.1152/ajpheart.00383.2017

Morrison, J. H., Brinton, R. D., Schmidt, P. J., and Gore, A. C. (2006). Estrogen, menopause, and the aging brain: how basic neuroscience can inform hormone therapy in women. *J. Neurosci.* 26, 10332–10348. doi: 10.1523/JNEUROSCI.3369-06.2006

Mosconi, L., Berti, V., Guyara-Quinn, C., McHugh, P., Petrongolo, G., Osorio, R. S., et al. (2017). Perimenopause and emergence of an Alzheimer's bioenergetic phenotype in brain and periphery. *PLoS One* 12:e0185926. doi: 10.1371/journal.pone.0185926

Narisawa-Saito, M., and Nawa, H. (1996). Differential regulation of hippocampal neurotrophins during aging in rats. *J. Neurochem.* 67, 1124–1131. doi: 10.1046/j.1471-4159.1996.67031124.x

Narisawa-Saito, M., Wakabayashi, K., Tsuji, S., Takahashi, H., and Nawa, H. (1996). Regional specificity of alterations in NGF, BDNF and NT-3 levels in Alzheimer's disease. *Neuroreport* 7, 2925–2928. doi: 10.1097/00001756-199611250-00024

Onyango, P., Celic, I., Mccaffery, J. M., Boeke, J. D., and Feinberg, A. P. (2002). SIRT3, a human SIR2 homologue, is an NAD-dependent deacetylase localized to mitochondria. *Proc. Natl. Acad. Sci. U S A* 99, 13653–13658. doi: 10.1073/pnas.222538099

Pamplona, R. (2008). Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity. *Biochim. Biophys. Acta* 1777, 1249–1262. doi: 10.1016/j.bbabio.2008.07.003

Pantazi, E., Zaouali, M. A., Bejaoui, M., Folch-Puy, E., Ben Abdennebi, H., and Rosello-Catafau, J. (2013). Role of sirtuins in ischemia-reperfusion injury. *World J. Gastroenterol.* 19, 7594–7602. doi: 10.3748/wjg.v19.i43.7594

Panza, S., Santoro, M., De Amicis, F., Morelli, C., Passarelli, V., D'Aquila, P., et al. (2017). Estradiol via estrogen receptor β influences ROS levels through the transcriptional regulation of SIRT3 in human seminoma TCam-2 cells. *Tumour Biol.* 39:1010428317701642. doi: 10.1177/1010428317701642

Papadopoulos, V., and Miller, W. L. (2012). Role of mitochondria in steroidogenesis. *Best Pract. Res. Clin. Endocrinol. Metab.* 26, 771–790. doi: 10.1016/j.beem.2012.05.002

Petrosillo, G., Matera, M., Moro, N., Ruggiero, F. M., and Paradies, G. (2009). Mitochondrial complex I dysfunction in rat heart with aging: critical role of reactive oxygen species and cardiolipin. *Free Radic. Biol. Med.* 46, 88–94. doi: 10.1016/j.freeradbiomed.2008.09.031

Phillips, H. S., Hains, J. M., Armanini, M., Laramee, G. R., Johnson, S. A., and Winslow, J. W. (1991). BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron* 7, 695–702. doi: 10.1016/0896-6273(91)90273-3

Pollard, A. K., Craig, E. L., and Chakrabarti, L. (2016). Mitochondrial complex 1 activity measured by spectrophotometry is reduced across all brain regions in ageing and more specifically in neurodegeneration. *PLoS One* 11:e0157405. doi: 10.1371/journal.pone.0157405

Quinlan, C. L., Perevoshchikova, I. V., Hey-Mogensen, M., Orr, A. L., and Brand, M. D. (2013). Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. *Redox Biol.* 1, 304–312. doi: 10.1016/j.redox.2013.04.005

Rajendran, R., Garva, R., Krstic-Demonacos, M., and Demonacos, C. (2011). Sirtuins: molecular traffic lights in the crossroad of oxidative stress, chromatin remodeling, and transcription. *J. Biomed. Biotechnol.* 2011:368276. doi: 10.1155/2011/368276

Razmara, A., Sunday, L., Stirone, C., Wang, X. B., Krause, D. N., Duckles, S. P., et al. (2008). Mitochondrial effects of estrogen are mediated by estrogen receptor α in brain endothelial cells. *J. Pharmacol. Exp. Ther.* 325, 782–790. doi: 10.1124/jpet.107.134072

Rekkas, P. V., Wilson, A. A., Lee, V. W., Yogalingam, P., Sacher, J., Rusjan, P., et al. (2014). Greater monoamine oxidase a binding in perimenopausal age as measured with carbon 11-labeled harmine positron emission tomography. *JAMA Psychiatry* 71, 873–879. doi: 10.1001/jamapsychiatry.2014.250

Rettberg, J. R., Yao, J., and Brinton, R. D. (2014). Estrogen: a master regulator of bioenergetic systems in the brain and body. *Front. Neuroendocrinol.* 35, 8–30. doi: 10.1016/j.yfrne.2013.08.001

Rizzuto, R., De Stefani, D., Raffaello, A., and Mammucari, C. (2012). Mitochondria as sensors and regulators of calcium signalling. *Nat. Rev. Mol. Cell Biol.* 13, 566–578. doi: 10.1038/nrm3412

Rosenzweig, E. S., and Barnes, C. A. (2003). Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. *Prog. Neurobiol.* 69, 143–179. doi: 10.1016/s0301-0082(02)00126-0

Rossouw, J. E., Anderson, G. L., Prentice, R. L., LaCroix, A. Z., Kooperberg, C., Stefanick, M. L., et al. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 288, 321–333. doi: 10.1016/s1062-1458(02)00919-4

Sandhu, S. K., and Kaur, G. (2003). Mitochondrial electron transport chain complexes in aging rat brain and lymphocytes. *Biogerontology* 4, 19–29. doi: 10.1023/A:1022473219044

Sanz, A., Hiona, A., Kujoth, G. C., Seo, A. Y., Hofer, T., Kouwenhoven, E., et al. (2007). Evaluation of sex differences on mitochondrial bioenergetics and apoptosis in mice. *Exp. Gerontol.* 42, 173–182. doi: 10.1016/j.exger.2006.10.003

Sastre-Serra, J., Nadal-Serrano, M., Pons, D. G., Roca, P., and Oliver, J. (2012). Mitochondrial dynamics is affected by 17β-estradiol in the MCF-7 breast cancer cell line. Effects on fusion and fission related genes. *Int. J. Biochem. Cell Biol.* 44, 1901–1905. doi: 10.1016/j.biocel.2012.07.012

Scharfman, H. E., and MacLusky, N. J. (2006). Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS. *Front. Neuroendocrinol.* 27, 415–435. doi: 10.1016/j.yfrne.2006.09.004

Scheller, K., and Sekeris, C. E. (2003). The effects of steroid hormones on the transcription of genes encoding enzymes of oxidative phosphorylation. *Exp. Physiol.* 88, 129–140. doi: 10.1113/eph8802507

Schmitt, K., Grimm, A., Dallmann, R., Oettinghaus, B., Restelli, L. M., Witzig, M., et al. (2018). Circadian control of DRP1 activity regulates mitochondrial dynamics and bioenergetics. *Cell Metab.* 27, 657.e5–666.e5. doi: 10.1016/j.cmet.2018.01.011

Schwer, B., North, B. J., Frye, R. A., Ott, M., and Verdin, E. (2002). The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. *J. Cell Biol.* 158, 647–657. doi: 10.1083/jcb.200205057

Scott, S. A., Liang, S., Weingartner, J. A., and Crutcher, K. A. (1994). Increased NGF-like activity in young but not aged rat hippocampus after septal lesions. *Neurobiol. Aging* 15, 337–346. doi: 10.1016/0197-4580(94)90029-9

Scott, E., Zhang, Q. G., Wang, R., Vadlamudi, R., and Brann, D. (2012). Estrogen neuroprotection and the critical period hypothesis. *Front. Neuroendocrinol.* 33, 85–104. doi: 10.1016/j.yfrne.2011.10.001

Sidorova-Darmos, E., Wither, R. G., Shulyakova, N., Fisher, C., Ratnam, M., Aarts, M., et al. (2014). Differential expression of sirtuin family members in the developing, adult, and aged rat brain. *Front. Aging Neurosci.* 6:333. doi: 10.3389/fnagi.2014.00333

Silhol, M., Bonnichon, V., Rage, F., and Tapia-Arancibia, L. (2005). Age-related changes in brain-derived neurotrophic factor and tyrosine kinase receptor isoforms in the hippocampus and hypothalamus in male rats. *Neuroscience* 132, 613–624. doi: 10.1016/j.neuroscience.2005.01.008

Slebos, R. J., de Graeff, A., Van Zandwijk, N., Mooi, W. J., Bos, J. L., and Rodenhuis, S. (1988). Recurrent breast cancer and an adenocarcinoma of the lung occurring in one patient: c-myc oncogene amplification and K-ras codon 12 point mutation as tumour markers. *Eur. J. Cancer Clin. Oncol.* 24, 1529–1530. doi: 10.1016/0277-5379(88)90347-1

Stauch, K. L., Purnell, P. R., and Fox, H. S. (2014). Aging synaptic mitochondria exhibit dynamic proteomic changes while maintaining bioenergetic function. *Aging* 6, 320–334. doi: 10.18632/aging.100657

Sun, N., Youle, R. J., and Finkel, T. (2016). The mitochondrial basis of aging. *Mol. Cell* 61, 654–666. doi: 10.1016/j.molcel.2016.01.028

Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. *J. Physiol.* 552, 335–344. doi: 10.1113/jphysiol.2003.049478

Vasconsuelo, A., Milanesi, L., and Boland, R. (2013). Actions of 17β-estradiol and testosterone in the mitochondria and their implications in aging. *Ageing Res. Rev.* 12, 907–917. doi: 10.1016/j.arr.2013.09.001

Velarde, M. C. (2013). Reply to turner and kerber. *Physiol. Genomics* 45:448. doi: 10.1152/physiolgenomics.00053.2013

Velarde, M. C. (2014). Mitochondrial and sex steroid hormone crosstalk during aging. *Longev. Healthspan* 3:2. doi: 10.1186/2046-2395-3-2

Vermulst, M., Bielas, J. H., Kujoth, G. C., Ladiges, W. C., Rabinovitch, P. S., Prolla, T. A., et al. (2007). Mitochondrial point mutations do not limit the natural lifespan of mice. *Nat. Genet.* 39, 540–543. doi: 10.1038/ng1988

Vest, R. S., and Pike, C. J. (2013). Gender, sex steroid hormones, and Alzheimer's disease. *Horm. Behav.* 63, 301–307. doi: 10.1016/j.yhbeh.2012.04.006

Viña, J., and Borrás, C. (2010). Women live longer than men: understanding molecular mechanisms offers opportunities to intervene by using estrogenic compounds. *Antioxid. Redox Signal.* 13, 269–278. doi: 10.1089/ars.2009.2952

Viña, J., Gambini, J., García-García, F. J., Rodriguez-Mañas, L., and Borrás, C. (2013). Role of oestrogens on oxidative stress and inflammation in ageing. *Horm. Mol. Biol. Clin. Investig.* 16, 65–72. doi: 10.1515/hmbci-2013-0039

Wanagat, J., Dai, D. F., and Rabinovitch, P. (2010). Mitochondrial oxidative stress and mammalian healthspan. *Mech. Ageing Dev.* 131, 527–535. doi: 10.1016/j.mad.2010.06.002

Webster, M. J., Herman, M. M., Kleinman, J. E., and Shannon Weickert, C. (2006). BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan. *Gene. Expr. Patterns* 6, 941–951. doi: 10.1016/j.modgep.2006.03.009

Wei, S. M., Baller, E. B., Kohn, P. D., Kippenhan, J. S., Kolachana, B., Soldin, S. J., et al. (2018). Brain-derived neurotrophic factor $Val^{66}Met$ genotype and ovarian steroids interactively modulate working memory-related hippocampal function in women: a multimodal neuroimaging study. *Mol. Psychiatry* 23, 1066–1075. doi: 10.1038/mp.2017.72

Wu, M. V., Manoli, D. S., Fraser, E. J., Coats, J. K., Tollkuhn, J., Honda, S., et al. (2009). Estrogen masculinizes neural pathways and sex-specific behaviors. *Cell* 139, 61–72. doi: 10.1016/j.cell.2009.07.036

Yang, S. H., Liu, R., Perez, E. J., Wen, Y., Stevens, S. M. Jr., Valencia, T., et al. (2004). Mitochondrial localization of estrogen receptor β. *Proc. Natl. Acad. Sci. U S A* 101, 4130–4135. doi: 10.1073/pnas.0306948101

Yao, J., Hamilton, R. T., Cadenas, E., and Brinton, R. D. (2010). Decline in mitochondrial bioenergetics and shift to ketogenic profile in brain during reproductive senescence. *Biochim. Biophys. Acta* 1800, 1121–1126. doi: 10.1016/j.bbagen.2010.06.002

Yin, F., Boveris, A., and Cadenas, E. (2014). Mitochondrial energy metabolism and redox signaling in brain aging and neurodegeneration. *Antioxid. Redox Signal.* 20, 353–371. doi: 10.1089/ars.2012.4774

Yin, F., Yao, J., Sancheti, H., Feng, T., Melcangi, R. C., Morgan, T. E., et al. (2015). The perimenopausal aging transition in the female rat brain: decline in bioenergetic systems and synaptic plasticity. *Neurobiol. Aging* 36, 2282–2295. doi: 10.1016/j.neurobiolaging.2015.03.013

Yu, Z., Sunchu, B., Fok, W. C., Alshaikh, N., and Pérez, V. I. (2015). Gene expression in the liver of female, but not male mice treated with rapamycin resembles changes observed under dietary restriction. *Springerplus* 4:174. doi: 10.1186/s40064-015-0909-7

Yurek, D. M., and Fletcher-Turner, A. (2001). Differential expression of GDNF, BDNF, and NT-3 in the aging nigrostriatal system following a neurotoxic lesion. *Brain Res.* 891, 228–235. doi: 10.1016/s0006-8993(00)03217-0

Zárate, S., Stevnsner, T., and Gredilla, R. (2017). Role of estrogen and other sex hormones in brain aging. Neuroprotection and DNA repair. *Front. Aging Neurosci.* 9:430. doi: 10.3389/fnagi.2017.00430

Ziegenhorn, A. A., Schulte-Herbrüggen, O., Danker-Hopfe, H., Malbranc, M., Hartung, H. D., Anders, D., et al. (2007). Serum neurotrophins—a study on the time course and influencing factors in a large old age sample. *Neurobiol. Aging* 28, 1436–1445. doi: 10.1016/j.neurobiolaging.2006.06.011

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

REVIEW
published: 07 December 2017
doi: 10.3389/fnagi.2017.00406

Role of Sex Hormones on Brain Mitochondrial Function, with Special Reference to Aging and Neurodegenerative Diseases

*Pauline Gaignard[1,2], Philippe Liere[1], Patrice Thérond[2], Michael Schumacher[1], Abdelhamid Slama[2] and Rachida Guennoun[1]**

[1]U1195 Inserm and University Paris-Sud and University Paris-Saclay, Le Kremlin-Bicêtre, France, [2]Biochemistry Laboratory, Bicêtre Hospital, Assistance Publique-Hôpitaux de Paris, Le Kremlin-Bicêtre, France

The mitochondria have a fundamental role in both cellular energy supply and oxidative stress regulation and are target of the effects of sex steroids, particularly the neuroprotective ones. Aging is associated with a decline in the levels of different steroid hormones, and this decrease may underline some neural dysfunctions. Besides, modifications in mitochondrial functions associated with aging processes are also well documented. In this review, we will discuss studies that describe the modifications of brain mitochondrial function and of steroid levels associated with physiological aging and with neurodegenerative diseases. A special emphasis will be placed on describing and discussing our recent findings concerning the concomitant study of mitochondrial function (oxidative phosphorylation, oxidative stress) and brain steroid levels in both young (3-month-old) and aged (20-month-old) male and female mice.

Keywords: aging, progesterone, estrogens, oxidative phosphorylation, oxidative stress, sex differences, neurodegenerative diseases

OPEN ACCESS

Edited by:
Luis Miguel Garcia-Segura, Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
Marco Fidel Avila-Rodriguez, Universidad del Tolima, Colombia
Valerio Magnaghi, Università degli Studi di Milano, Italy

****Correspondence:***
Rachida Guennoun
rachida.guennoun@inserm.fr

Received: *09 October 2017*
Accepted: *24 November 2017*
Published: *07 December 2017*

Citation:
Gaignard P, Liere P, Thérond P, Schumacher M, Slama A and Guennoun R (2017) Role of Sex Hormones on Brain Mitochondrial Function, with Special Reference to Aging and Neurodegenerative Diseases. Front. Aging Neurosci. 9:406. doi: 10.3389/fnagi.2017.00406

INTRODUCTION

The cross-talk between mitochondria and sex steroids plays a major role in the brain. Indeed, sex steroids influence numerous functions of mitochondria: energy production, oxidative stress regulation, calcium homeostasis, cell proliferation or apoptosis (Nilsen and Diaz Brinton, 2003; Chen et al., 2009a,b; Sayeed et al., 2009; Gaignard et al., 2017). In addition, because mitochondria are also the site of the first step of steroidogenesis, dysfunctions of mitochondria may impact on steroidogenesis. Since sex steroids decrease and mitochondrial alterations are known to be implicated in aging, understanding the relationship between both is a key to explore normal and pathological brain aging. This review will focus on two intricate mitochondrial functions: the energy production by oxidative phosphorylation and the regulation of oxidative stress. After a brief summary of age-associated mitochondrial dysfunction in brain and the decrease of brain steroids, we discuss the regulation of mitochondrial metabolism by sex steroids in the context of aging. We then expose the current knowledge about sexual dimorphism in mitochondrial function during normal brain aging and in neurodegenerative diseases, and we emphasize the role of sex steroids on it.

THE DECLINE OF MITOCHONDRIAL FUNCTION DURING AGING

Energy production and oxidative stress regulation by mitochondria are critical for cell life and particularly in the brain that has both a high metabolic rate and an increased sensitivity to oxidative damages (Kann et al., 2007). The mitochondrial ATP production from pyruvate coming from glycolysis requires three principal enzymatic systems (**Figure 1**): the pyruvate dehydrogenase complex (PDHc), composed of three subunits E1, E2 and E3 that convert pyruvate to acetyl-coA; the tricarboxylic acid (TCA) cycle that produces reduced co-enzymes (reduced nicotinamide adenine nucleotide NADH and reduced flavin adenine dinucleotide $FADH_2$) and the respiratory chain (RC) that finally produces ATP. The RC is composed of five complexes, the first four complexes (complexes I, II, III and IV) form the electron transfer chain (ETC) that creates an electrochemical gradient and reduces O_2 into H_2O ("respiration"). The complex V then catalyzes the phosphorylation of ADP into ATP using the proton motive force generated by the ETC. The coupling between the electron transport and the ADP phosphorylation is called "oxidative phosphorylation".

In addition to energy production, mitochondria are a major cellular regulators of oxidative stress by producing reactive oxygen species (ROS) and harboring powerful antioxidant systems (Wüllner et al., 1999; Murphy, 2009; Fukui and Zhu, 2010). ROS generation and mitochondrial respiration are intrinsically linked. The close proximity between high concentrations of O_2 and of electrons promotes the production of superoxide anions ($O_2^{\cdot -}$), especially when the ETC is accelerated or, on the contrary, is slowed down (Murphy, 2009). The regulation of oxidative stress is principally mediated by the mitochondrial superoxide dismutase SOD2 (or MnSOD) that catalyzes the dismutation of superoxide anions to H_2O_2 and by the reduced glutathione (GSH) pool that allows the detoxification of H_2O_2 into H_2O (**Figure 1**). The maintenance of the GSH pool and of the ratio between reduced and oxidized glutathione forms (GSH/GSSG) is especially crucial for the brain, as the activity of catalase, the other enzyme that detoxifies H_2O_2, is low in this tissue (Kudin et al., 2012).

In 1956, Harman (1956) exposed the "free radical theory of aging" suggesting that oxidative damages accumulate with age and are responsible for aging. Based on the central role of mitochondria in ROS production and detoxification, this theory has evolved into the "mitochondrial aging theory": oxidative damages induced by free radicals on mitochondrial DNA (mtDNA) particularly cause mitochondrial impairments that enhance ROS production in a vicious circle, finally leading to cellular failure (Harman, 1972). Since several decades, the age-induced mitochondrial alterations have been largely studied and numerous experimental evidence support Harman's theory. However, the direct link between free radicals, mtDNA mutations and cellular alterations during aging has become controversial, and it has been proposed that mitochondrial ROS are involved in aging process by their roles in cellular signaling rather than by their altering effects (Stuart et al., 2014).

With regard to brain aging, investigating the "mitochondrial aging theory" is particularly relevant. The brain is highly vulnerable to oxidative damages because of the scarcity of antioxidant defense systems in this tissue (Poon et al., 2006; Kann et al., 2007). The accumulation of oxidative damages in brain mitochondrial proteins, lipids and nucleotides has been demonstrated in numerous studies (reviewed in Chakrabarti et al., 2011). Thus, it has been reported that the activity of the TCA cycle enzyme α-keto-glutarate dehydrogenase (αKGDH) was diminished under pro-oxidative conditions (Tretter and Adam-Vizi, 2005); the cardiolipin, a specific mitochondrial membrane lipid, was subjected to peroxidation during aging and the peroxidized cardiolipin form inhibited complex IV activity in brain, which could potentially disrupt RC complexes interactions (Petrosillo et al., 2008, 2013; Lenaz and Genova, 2010). In pigmented neurons of the human substantia nigra, the accumulation of mtDNA deletions and the decrease in complex IV activity were correlated (Kraytsberg et al., 2006). Nevertheless, the aging consequences on RC efficacy are much less established: the studies reported either a decline or no change on RC function (reviewed in Gilmer et al., 2010; Chakrabarti et al., 2011). Methodological and experimental points could explain some of discrepancies between the age-effect studies. The age selected for the "aged" rodent group is not always similar (either 12- or 24-month-old generally) and has to be in concordance with the lifespan specificities of the species and strains studied (Turturro et al., 1999); the methods exploring RC function do not reach the same conclusions according to the energetic substrate used to measure oxygen consumption or the exhaustiveness in the analysis of enzymatic activities. Importantly, the aging-effect observed in mitochondria isolated from whole brain could differ from those observed in mitochondria isolated from brain sub-regions, since different patterns of age-related changes on RC function were described between brain areas. For example, mitochondria from hippocampus of male rats presented higher age-induced changes in mitochondrial bioenergetics than those isolated from various other areas (Pandya et al., 2016). In accordance, the rate of mtDNA mutations and activation of base excision repair pathways were not affected by aging in the same way according to brain sub-regions (McInerny et al., 2009; Gredilla et al., 2010). At the cellular level, differences between synaptic and non-synaptic mitochondria were reported; with a decrease of complex I activity with age in non-synaptic mitochondria but not in synaptic ones (Ferrándiz et al., 1994).

In humans, the effect of aging on mitochondrial metabolism has been mainly analyzed in skeletal muscle and less in brain. Two studies reported a decrease in complex IV activity or protein quantity in different brain sub-regions (Ojaimi et al., 1999; Cottrell et al., 2001), and another reported an increase in oxidative damages and a decreasing trend in complex I activity in frontal cortex and in hippocampus (Venkateshappa et al., 2012).

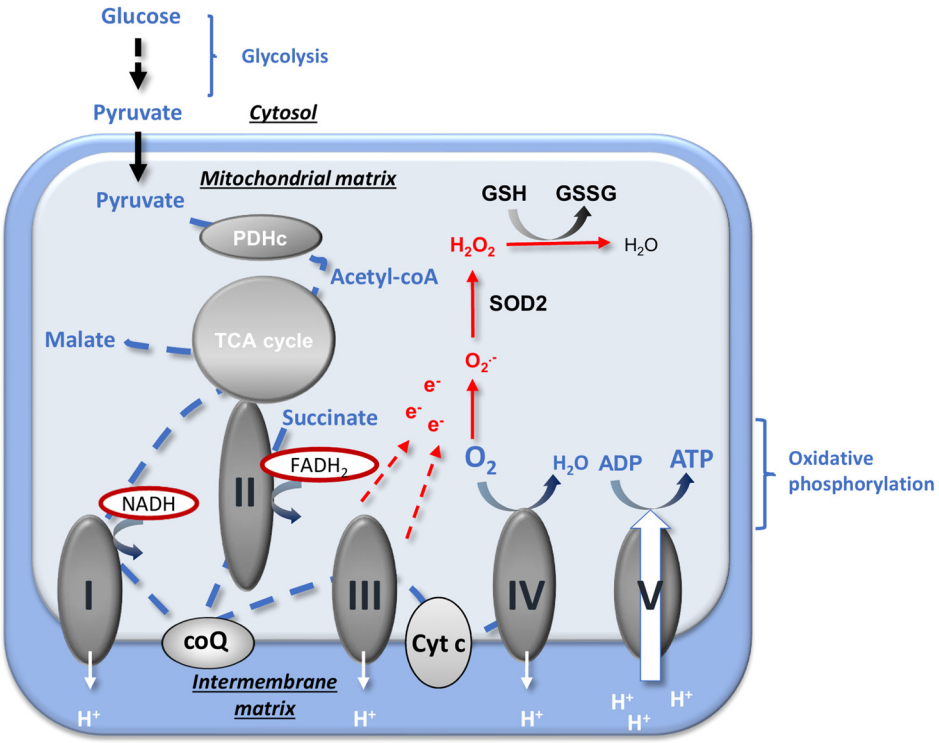


FIGURE 1 | Mitochondrial energy production and reactive oxygen species (ROS) regulation. Pyruvate from an aerobic glycolysis is converted by pyruvate dehydrogenase complex (PDHc) into acetyl-coA that enters into the tricarboxylic acid (TCA) cycle. The reducing equivalents NADH and $FADH_2$ produced are used by the respiratory chain (RC) complexes (complexes I, II, III and IV) to reduce O_2 (« respiration ») and to create the proton gradient necessary to ATP production by complex V. The oxidative phosphorylation is the coupling between ADP phosphorylation and the electron transport. The production of anion superoxide $O_2^{\cdot -}$ is inherent to mitochondrial respiration because of the proximity between free electrons and O_2. ROS detoxification is provided by the SOD2 that catalyzes the dismutation of $O_2^{\cdot -}$ into H_2O_2 and by GSH that allows the reduction of H_2O_2 into H_2O. CoQ, ubiquinone; Cyt c, cytochrome c; e^-, electron; GSH, reduced glutathione; GSSG, oxidized glutathione; $O_2^{\cdot -}$, anion superoxide; PDHc, pyruvate dehydrogenase complex; SOD, superoxide dismutase; TCA, tricarboxylic acid. Adapted from Gaignard et al. (2017).

THE DECLINE OF BRAIN STEROID LEVELS DURING AGING

The decrease in the peripheral synthesis of sex steroids is a major feature of aging, with a more drastic drop in women at the time of menopause when compared to men. Of note, the age-induced changes in sex steroid levels observed in blood may differ from those observed in brain, since the brain pool of sex steroids depends both on endocrine gland production and on the local synthesis of neurosteroids (Schumacher et al., 2003). Nevertheless, the brain also suffers from a decline in sex steroids. We have recently reported the profile of progesterone and its metabolites in the brain of aged male and female mice and showed an overall age-induced decrease (Gaignard et al., 2015). In the limbic region, Caruso et al. (2013) described also a decrease of 17β-estradiol, progesterone, testosterone and some metabolites by comparing 7- and 24-month-old male mice. Due to evident technical limitations, the knowledge of age-dependent changes in human brain levels of sex steroids is still fragmented. A recent RIA study reported that androgen but not estrogen levels declined in mid-frontal gyrus of men from 50 years to 97 years, whereas no correlation between androgen or estrogen brain levels and age were found in aged women (Rosario et al., 2011). Complementary studies are now needed in younger populations but also in various brain regions, since changes in steroid profiles differ between brain areas (Weill-Engerer et al., 2002).

Interestingly, the age-induced decline in mitochondrial function could modify sex steroid levels on its own. Thus, mitochondria are not only the targets of sex steroid actions but also the site of the initial steps of steroidogenesis. Cholesterol, the precursor of all steroid hormones, enters the mitochondria via the transduceosome complex where it is converted into pregnenolone by the mitochondrial cholesterol side-chain cleavage enzyme cytochrome P450 (P450scc). Then, the 3β-hydroxysteroid dehydrogenase (3βHSD) produces progesterone from pregnenolone. The 3βHSD is located both in mitochondria and in the endoplasmic reticulum (Miller, 2013). Some data suggest a potential regulation between mitochondrial metabolism and steroidogenesis (**Figure 2**). The exact composition and the functioning of the transduceosome are not fully elucidated (Papadopoulos et al., 2015; Selvaraj and Stocco, 2015), but it has been suggested that the adenine nucleotide transporter (ANT) may be part of it (Midzak et al., 2011). Yet,

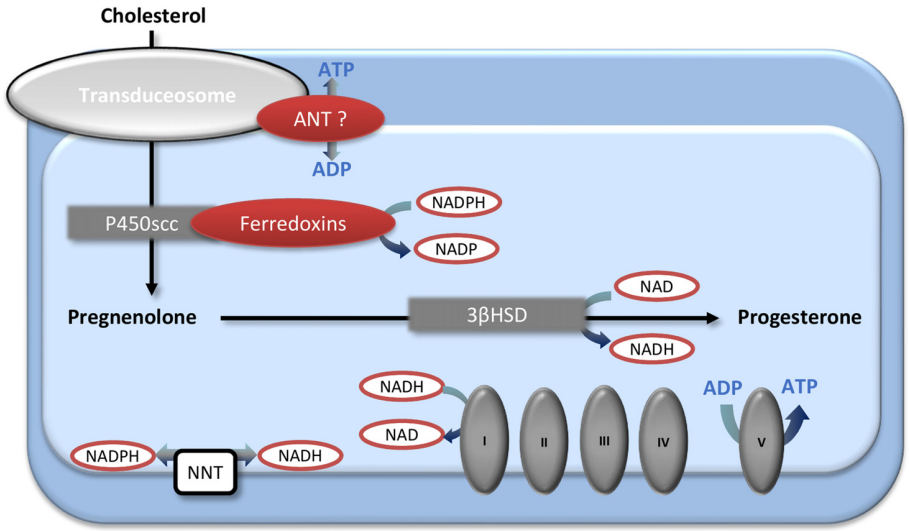


FIGURE 2 | The mitochondrial first steps of steroidogenesis and their interactions with energetic metabolism. Cholesterol enters into the mitochondria by the transduceosome and is converted into pregnenolone by the P450scc. The 3βHSD, located both in mitochondria and endoplasmic reticulum, produces progesterone from pregnenolone. Mitochondrial energetic metabolism and steroidogenesis could interact by several ways. The ADP/ATP transporter adenine nucleotide transporter (ANT) may be a part of the transduceosome; the P450scc-associated ferredoxins are regulated by the NADPH/NADP ratio that is linked with the NADH/NAD ratio by the transhydrogenase NNT; NAD is a co-factor of 3βHSD. 3βHSD, 3β-hydroxysteroid dehydrogenase; ANT, adenine nucleotide transporter; NNT, nicotinamide nucleotide transhydrogenase; P450scc, cholesterol side-chain cleavage enzyme cytochrome P450.

ANT is also responsible for the ADP/ATP transfer through the mitochondrial membrane. Second, the ferredoxins associated with cytochrome P450scc are regulated by the NADP/NADPH ratio (reviewed in Miller, 2005). NADP/NADPH ratio is the phosphorylated counterpart of the NAD/NADH ratio which is mainly regulated by the TCA cycle and RC in mitochondria. The NAD(H) and the NADP(H) pools are compartmentalized in cells, but it has been demonstrated that they are connected by the nicotinamide nucleotide transhydrogenase (NNT; Fisher-Wellman et al., 2015). In addition, the 3βHSD utilizes NAD as a co-factor (Chapman et al., 2005). Finally, as hydroxysteroid dehydrogenases enzymes use the nicotinamide cofactors to interconvert steroid hormones, and as their activities depend mainly on cofactors abundance, levels of steroids may be modulated by the levels of these cofactors and consequently by the redox state of the cells (Agarwal and Auchus, 2005). In light of this information, further specific investigations must determine if alterations in brain mitochondrial energetic function may impact neurosteroidogenesis. This would be another mechanism leading to age-induced brain steroids decrease, in addition to the decline of the peripheral synthesis and to the decrease of expression of the brain enzymes involved in *in situ* production of steroids (Velarde, 2014; Rossetti et al., 2015).

THE REGULATION OF MITOCHONDRIAL METABOLISM BY SEX STEROIDS AND THE PARTICULAR CONTEXT OF AGING

Since few years, there is a growing interest in the effects of sex steroids on brain mitochondrial metabolism. While the mitochondrial effects of testosterone are not well documented, several pharmacological studies have shown that exogenous administration of 17β-estradiol and/or progesterone increases RC function and decreases oxidative stress in brain mitochondria (reviewed in Chen et al., 2009a,b; Rettberg et al., 2014; Gaignard et al., 2017). It has also been demonstrated that these mitochondrial effects contribute to the neuroprotective properties of sex steroids after brain injury (Robertson et al., 2006; Guo et al., 2012; Robertson and Saraswati, 2015; Webster et al., 2015; Gaignard et al., 2016; Yousuf et al., 2016; Andrabi et al., 2017).

It has been shown in young or ovariectomized animals that sex steroids could regulate mitochondrial energy production by transcriptional and post-transcriptional mechanisms (**Figure 3**). The transcriptional regulation of RC by 17β-estradiol is the best demonstrated so far. The RC compounds are encoded by two genomes, the nuclear one and the mitochondrial one, but the last encodes a few parts (only 13 RC complexes subunits out of up to 80 identified). Besides the rest of RC compounds, nuclear DNA (nDNA) encodes all machineries for the replication, the transcription and the translation of mtDNA. The nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) together with the coactivator peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC1-α) and the mitochondrial transcription factor A (TFAM), operate the cross-talk between nuclear and mitochondrial genomes. The activation of the estrogen nuclear receptors ("classic" receptors) ERα or ERβ induces the expression of NRF-1, NRF-2, TFAM and of the nuclear-encoded RC subunits (reviewed in Chen et al., 2009b). Interestingly, some studies showed that the effects of ERα- and ERβ-agonists were slightly different and varied according

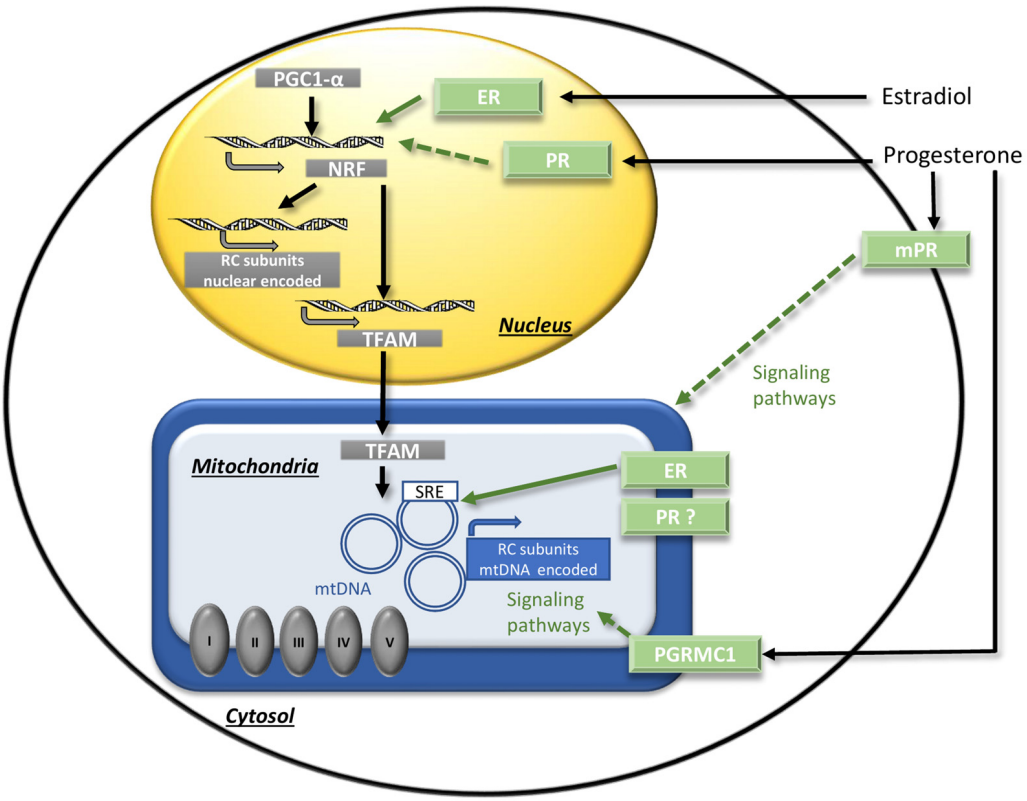


FIGURE 3 | The regulation of mitochondrial function by sex steroids. The binding of estradiol to the nuclear receptors ER activates transcriptional factors (NRF/PGC1-α) that induce the expression of nuclear DNA-encoded RC subunits and of transcription factor A (TFAM). TFAM induces the expression of mitochondrial DNA (mtDNA)-encoded RC subunits. Direct transcriptional effects on mtDNA mediated by mitochondrial ER receptors could also occur. Concerning progesterone, the potential mechanisms have to be investigated. Putative signaling effects *via* NRF/PGC1-α activation by nuclear PR, or *via* membrane receptors mPR or PGRMC1 are represented by dotted arrows. ER, estrogen nuclear receptors; mPRs, progesterone membrane receptors; mtDNA, mitochondrial DNA; NRF, nuclear respiratory factor; PGC1-α, peroxisome proliferator-activated receptor gamma co-activator 1 α; PGRMC1, progesterone receptor membrane component 1; PR, progesterone nuclear receptor; RC, respiratory chain; SRE, steroid response element; TFAM, mitochondrial transcription factor A. Adapted from Gaignard et al. (2017).

to brain regions. Thus, in hippocampus of ovariectomized female rats, the ERβ-agonist diarylpropionitrile (DPN) was more efficient to enhance mitochondrial respiration and to decrease oxidative stress than the ERα-agonist propylpyrazoletriol (PPT; Irwin et al., 2012). By contrast, in primary human brain microvascular endothelial cells, the PPT addition decreased anion superoxide production while the DPN addition did not (Razmara et al., 2008). Considering that ERα and ERβ cerebral expressions are sexually dimorphic (Zhang et al., 2002; Sharma and Thakur, 2006), the estrogen effects on mitochondrial metabolism could be different in males and females. Recently, Riar et al. (2017) demonstrated a significant sex difference in the mitochondrial unfolded protein response (UPR^{mt}), a transcriptional program that restore proteostasis, in the spinal cord of G93A-SOD1 mice and suggested that the sex difference could be due to a difference in ERα activation.

Whether or not progesterone and/or 5-α-dihydroprogesterone exert their effects via the nuclear progesterone receptor PR has to be investigated. Progesterone and derivates could also acts independently of PR. Recently, it has been shown that progesterone promoted a mild decoupling (i.e., the dissociation between the reduction of O_2 by ETC and the ATP synthesis) in yeast cells, even when the yeast ortholog of PR was removed. This effect could be due to a direct effect on mitochondrial membranes or could be mediated by other receptors than PR (Stekovic et al., 2017). It has also been reported that 3α,5α-tetrahydroprogesterone (also known as allopregnanolone), a reduced derivate of progesterone, enhanced ATP levels and mitochondrial respiration, especially in a cellular model of Alzheimer's disease (AD). The authors suggested that these effects were not mediated by $GABA_A$ receptors, like most of the other effects of allopregnanolone, but rather by non-GABAergic receptors such as membrane PR (mPRs; Pang et al., 2013; Lejri et al., 2017).

In addition, number of arguments suggest that sex steroids could also exert their regulation via mitochondrial receptors. Thus, ERα and ERβ were detected in the mitochondria of neurons (Yang et al., 2004; Alvarez-Delgado et al., 2010) and they could potentially exert direct transcriptional effects of

mtDNA because mtDNA harbors steroid response element (SRE) sequences (Demonacos et al., 1996). The existence of a similar mechanism for progesterone action is possible but must be demonstrated, since the reality of the mitochondrial form of PR is still debated. A splicing variant of *PR* gene was described in some tissues, but it encodes a truncated form of the receptor and its functionality is doubtful (Samalecos and Gellersen, 2008). Furthermore, the progesterone receptor membrane component 1 (PGRMC1) was detected in both membrane and mitochondrial fractions and co-localized with markers of the endoplasmic reticulum and the mitochondria (Meffre et al., 2005; Xu et al., 2011; Guennoun et al., 2015). By this way, progesterone could exert post-transcriptional regulation on mitochondria by activating signaling pathways (**Figure 3**).

After effects demonstrated in young animals, a challenging issue now is to determine whether steroids exert mitochondrial effects in aged individuals. In addition to a decline in steroid levels, the question arises of the persistence of the receptors and of their functionalities during aging. The interpretation of the experimental data is however delicate because of methodological pitfalls (mRNA levels do not necessary reflect protein levels, the presence of the receptor does not signify that it is fully functional. . .). Besides, the coexistence of several isoforms of nuclear sex steroid receptors adds even more complexity. In female mice, a study reported that the quantity of nuclear receptor ERα was increased in the anteroventral periventricular nucleus of the hypothalamus in aged females, but that the quantity of ERβ was decreased in the same region (Chakraborty and Gore, 2004). In the cortex, one study showed a decrease in both ERα and ERβ protein levels in aged female mice when compared to young females (Cai et al., 2014), but another one did not detect significant changes in ERα protein levels (Dietrich et al., 2015). Interestingly, it has been reported that the mitochondrial ERα and ERβ content did not vary between young and aged female rats in cortex, hippocampus and hypothalamus (Alvarez-Delgado et al., 2010). In male mice, no significant variations in mRNA levels of ERα and ERβ was observed between 12- and 24-month-old mice, but protein levels were not examined (Munetomo et al., 2015). Recently, the age-associated changes in ER levels and distribution were reviewed by Hara et al. (2015), underpinning that they vary across species and brain regions.

Age-associated decrease in progesterone receptor (PR) expression has been reported in the hypothalamus of male and female rhesus macaques; however their expression still responded to estradiol supplementation (Naugle et al., 2014; Eghlidi and Urbanski, 2015; Eghlidi et al., 2017). A decrease in PR mRNA expression during reproductive aging in the hypothalamus of female rats was also demonstrated (Mills et al., 2002). Analysis of estrogenic regulation of PR mRNA in hypothalamus of aged female rats showed that the induction of PR mRNA by estrogen treatment in middle-aged rats is as strong as in young rats (Funabashi et al., 2000). However, analysis by immuno-histochemistry, showed that the ability of estradiol to stimulate PR expression is attenuated with aging in female rats (Furuta et al., 2010). In the cerebral cortex of aged female mice, one study showed that PR mRNA expression declined with age, but PR protein levels were sustained (Dietrich et al., 2015). The age-related change in membrane progesterone receptors, which play an important role in progesterone and its derivative actions (Guennoun et al., 2015), has still to be determined.

Concerning androgen receptors, mRNA levels of AR have been reported to decrease in cerebral cortex and to increase in the hypothalamus of aged male mice (Munetomo et al., 2015). In contrast, a decrease in AR mRNA expression has been reported in the hypothalamic arcuate nucleus of the male rhesus macaques (Eghlidi et al., 2017).

MITOCHONDRIAL SEXUAL DIMORPHISM IN BRAIN AGING

The question of sex differences in normal aging goes beyond a fundamental scientific interest and is an essential step in medical research. The pharmacological evidences describing the enhancement of brain mitochondrial metabolism following systemic supplementation with sex steroids suggest that the age-induced decrease in sexual steroid production could contribute to brain mitochondrial decay (Chen et al., 2009b; Gaignard et al., 2017). It must be stressed however that almost all data about age-induced effects on mitochondrial RC or oxidative stress were obtained in male rodents. This feature is relatively common in experimental research (Zucker and Beery, 2010), but constitutes a serious lack for our thematic, as sex steroids influence mitochondrial function. The decrease of sex steroid hormones is an important turning point in the aging process in both sexes, but the reduction in estrogen blood levels during menopause is more abrupt than the reduction in testosterone levels during andropause. Thus, the consequences of aging on mitochondrial metabolism likely differ between women (or females) and men (or males). Surprisingly, this issue has been rarely addressed and especially in normal aging.

We designed a specific study to determine whether brain mitochondrial metabolism is sexually dimorphic, in young and aged mice. We have measured the mitochondrial oxygen consumption and the activities of associated enzymes (the NADH-linked respiration, that depends on PDHc, TCA cycle enzymes and complexes I, III and IV activities and the $FADH_2$-linked respiration that depends on complexes II, III and IV activities), the mitochondrial content (estimated by the citrate synthase activity and the mtDNA to nDNA ratio), the mitochondrial anti-oxidant protection (mitochondrial and total GSH pools) and the mitochondrial oxidative damages (oxidative inactivation of mitochondrial aconitase) in intact male and female mice at two ages: 3-month-old and 20-month-old. To standardize, the young adult females used were all in the diestrus stage and the old females were aged of 20 months to ensure that they were reproductively senescent. We showed that the NADH-linked respiration rate was higher in young females when compared to young males, and that it was related to a higher PDHc activity; the oxidative stress was lower in young females than in young males. By comparison, no significant difference was detected between 20-month-old male and female mice, neither in the respiration rate, the mitochondrial content nor in

mitochondrial oxidative stress (**Figure 4**; Gaignard et al., 2015). Thus, the elevated mitochondrial metabolism observed in young females compared to young males disappears with aging.

By contrast, using Wistar rats, Guevara et al. (2009, 2011) reported that mitochondrial respiration (expressed per gram of tissue) was not different between 6-month-old males and females and between 24-month-old males and females. Oxidative damages were lower in young females when compared to males and this difference persisted in aged animals. The comparison between Guevara's studies in Wistar rats and ours in C57BL/6 mice illustrates some essential methodological points that must be taken into account. First, discrepancy in the protocols used to measure oxygen consumption could explain why conclusions differ: the use of a high concentration of malate (associated with pyruvate) to start NADH-linked respiration may lead to a bypass of PDHc activity and may mask differences on it. Second, the choice of the unit expression is crucial: the complex IV activity, expressed per gram of tissue, was higher in aged female rats when compared to aged male rats but was not statistically different when expressed per milligram of mitochondrial proteins in Guevara's study. These data suggest that mitochondrial content per cell or per gram of tissue varies according to sex and age. Guevara et al. (2009, 2011) reported that mitochondrial proteins to mtDNA ratio was higher in females than in males, young or aged. In our study, we evaluated mitochondrial content by two well-known markers (mtDNA to nDNA ratio and citrate synthase activity; Chretien et al., 1994; Medeiros, 2008), but we did not detect any difference between male and female mice either young or aged (**Figure 4**). It has been recently demonstrated that the brain expression of PGC1-α mRNA, the co-activator of NRF-1 and NRF-2, was lower in 22-month-old female mice when compared to male mice of the same age, but the impact on mitochondrial content was not evaluated (Zawada et al., 2015). Further studies, specially designed to analyze mitochondrial biogenesis by complementary methods, are necessary to determine whether mitochondrial content varies between males and females and the effect of aging. Third, it must be noted that rat and mouse mitochondrial metabolisms are not comparable. Studies revealed that, in males, ROS generation in the brain differed between Sprague-Dawley rats and C57BL/6 mice (Panov et al., 2007). Besides, in Wistar rats, young females produced less H_2O_2 than their

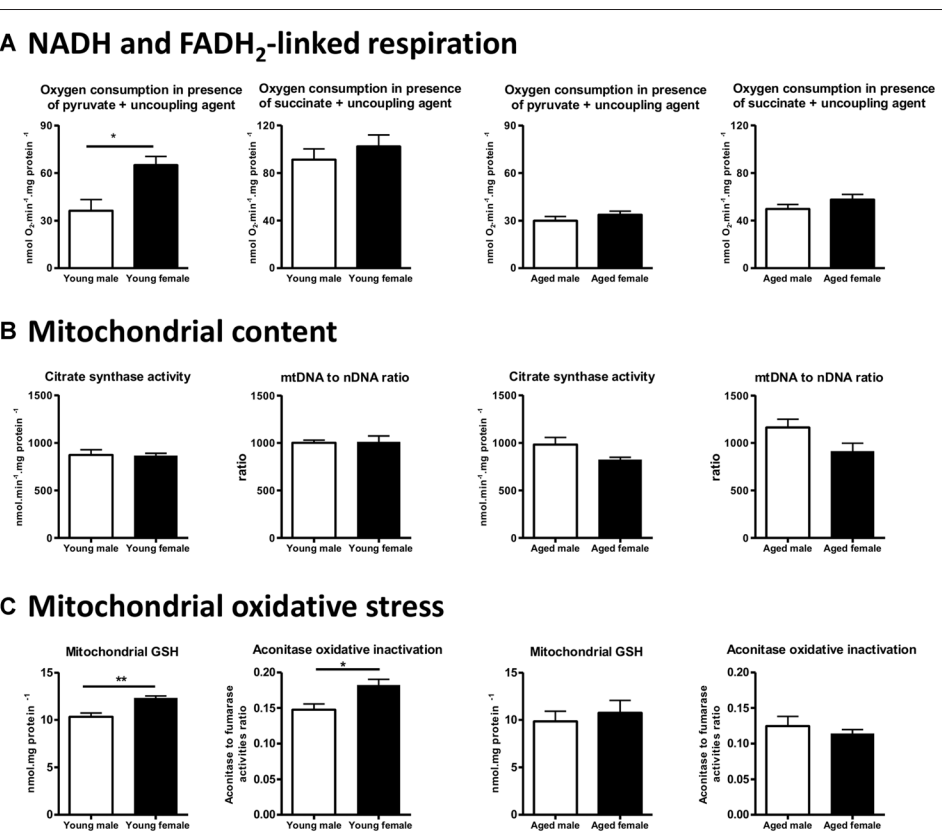


FIGURE 4 | Sex differences in brain mitochondrial metabolism in young (3-month-old) and aged (20-month-old) male and female mice. **(A)** NADH-linked respiration rate (measured by the oxygen consumption in presence of pyruvate) was higher in young females (diestrus stage) than in young males and this sex difference disappeared in aged mice. **(B)** Mitochondrial content estimated by the citrate synthase activity and the mtDNA to nDNA ratio was not different between males and females in both young and aged mice. **(C)** Mitochondrial GSH pool was higher and oxidative inactivation of aconitase was lower in young females than in young males. These differences did not persist in aged mice. Data represent mean ± SEM of 5–6 mice. Statistical analysis: *t*-test. Significance: $^{*}p < 0.05$; $^{**}p < 0.01$. mtDNA, mitochondrial DNA; nDNA, nuclear DNA; GSH, reduced glutathione. Data from Gaignard et al. (2015).

male counterparts (Borrás et al., 2003), whereas the rate of anion superoxide generation was equivalent between males and females young C57BL/6 mice (Ali et al., 2006). Moreover, both brain steroid levels and their effects present substantial differences between mice and rat (Kellogg and Frye, 1999; Ebner et al., 2006; Liu et al., 2012; Porcu and Morrow, 2014).

The latter point is essential since we demonstrated that sex differences observed in brain mitochondrial metabolism are dependent on steroid levels. Thus, in our study, we performed gonadectomy in young adult male and female mice and we reported that the higher respiration rate and anti-oxidant protection in young female comparatively to young male mice were suppressed 3 weeks after ovariectomy. This finding strongly suggests a role of sex steroids in the male/female differences observed in young adults. By contrast, we have shown that three-week orchidectomy was without effect on male mitochondrial metabolism. Taken together, these results oriented us towards a major role played by the "ovarian steroids", *i.e.*, progesterone and 17β-estradiol, in the observed sex-difference. To test this hypothesis, we then have concomitantly analyzed mitochondrial function in one hemisphere and brain steroid levels in the contralateral hemisphere from the same animals using both male and female, young and aged mice. We showed that pregnenolone and progesterone levels were higher in young female mice (diestrus stage) when compared to young males. The 5α-reduced progesterone derivates levels (5α-dihydroprogesterone, 3α,5α-tetrahydroprogesterone and 3α,5β-tetrahydroprogesterone) were not statistically different between both sexes. In aged mice, pregnenolone and progesterone levels were lower than in young mice and the sex differences were no longer observed (**Figure 5**). Therefore, we postulated that the strong decrease of sex steroid levels and especially progesterone levels in aged female mice could diminish the "metabolic advantage" observed in young females (Gaignard et al., 2015).

Regarding the role of 17β-estradiol, its brain levels were too low to be measured by the accurate GC/MS method in our study. Nevertheless, Brinton (2008) has well described the role of a drop in 17β-estradiol in mitochondrial metabolism regulation. Based on pharmacological studies using exogenous 17β-estradiol administration to ovariectomized female rats, they showed that 17β-estradiol increased PDHc activity (that links glycolytic and oxidative glucose metabolisms), mitochondrial oxygen consumption and complex IV activity in brain (Nilsen et al., 2007; Irwin et al., 2008). They next postulated that the estrogenic impregnation during the reproductive period could enhance brain metabolism in young females by promoting the aerobic glycolysis. In women at the time of menopause, the reduction of ovarian hormones could lead to an hypo-metabolism that could predispose to neurodegenerative diseases (Brinton, 2008). Experimental studies by the same team then confirmed that PDHc activity, mitochondrial oxygen consumption and complex IV activity declined during reproductive senescence in female mice and, using the model of ovariectomized females, that these decreases were reversed by 17β-estradiol substitution (Yao et al., 2010, 2012; Irwin et al., 2011). Interestingly, in our work comparing males and females, we also showed that young females had higher PDHc activity than young males (Gaignard et al., 2015). Recently, a proteomic and functional study concerning the evolution of brain mitochondrial metabolism during aging performed in males, has completed the picture. Stauch et al. (2015) reported that the expression of glycolytic enzymes was higher in mitochondria isolated from brains of 12-month-old and 24-month-old C57BL/6 male mice when compared to those isolated from 5-month-old male mice. By contrast, the TCA cycle enzymes and RC complexes expressions declined from 12 months to 24 months. Functional analysis revealed that oxygen consumption appeared unchanged with aging in males. In light of these data, the role played by the cytoplasmic glycolytic enzymes and PDHc in the sexually dimorphic effects of age on mitochondrial metabolism has to be more considered.

It seems that the mitochondrial effects of sex steroids in endogenous conditions could be more important in the brain than in the other tissues. In fact, no male/female differences were observed in energetic function of mitochondria isolated from heart, skeletal muscle and liver of C57BL/6 mice (Sanz et al., 2007; Khalifa et al., 2017). Besides, the sex difference on PGC1-α mRNA expression seen in brain of aged mice was not retrieved neither in the liver nor in the kidney (Zawada et al., 2015). Detailed studies in several species are now necessary to better determine the extent and the mechanisms of mitochondrial sexual dimorphism during aging.

The two strains of the senescence-accelerated mice (SAM), one prone to accelerated senescence (SAMP) and one resistant (SAMR), are attractive models for aging research. Data about brain mitochondrial metabolism in either SAMR1 or SAMP8 10-month-old male and female mice were reported by two studies. These studies were designed to analyze melatonin effects on mitochondria but the comparison between male and female control groups may provide information. In SAMR1 mice (the "young" model), no sex-related difference was detected in brain mitochondria lipid peroxidation, GSH/GSSG ratio and RC complexes activities. In contrast, in the SAMP8 mice (the "aged" model), females presented a higher lipid peroxidation, a lower GSH/GSSG ratio and lower complexes I and III activities when compared to their male counterparts (Carretero et al., 2009; Escames et al., 2013). In brief, mitochondrial sexual dimorphism was present in the aged model but not in the young model. The discrepancy between these results and ours is probably due to the particular endocrine profile of the SAM mice, making them unsuitable for studying endogenous steroid influences (Yuan et al., 2005). Nevertheless, the classic rodent models are also not entirely satisfactory, since the decrease in circulating sex steroids during reproductive senescence (the "menopause" in rodents) is primarily caused by the decrease in hypothalamic pituitary axis signal and not by the decline of follicles supply, like in women (Yin and Gore, 2006).

Dedicated studies exploring sexual dimorphism in aging-induced variations in brain metabolism are still rare in humans and particularly in cognitively intact individuals. The studies previously cited about the decrease of RC complexes activities or quantities, included men and women but did not specifically address the question of sex difference (Ojaimi et al., 1999; Cottrell

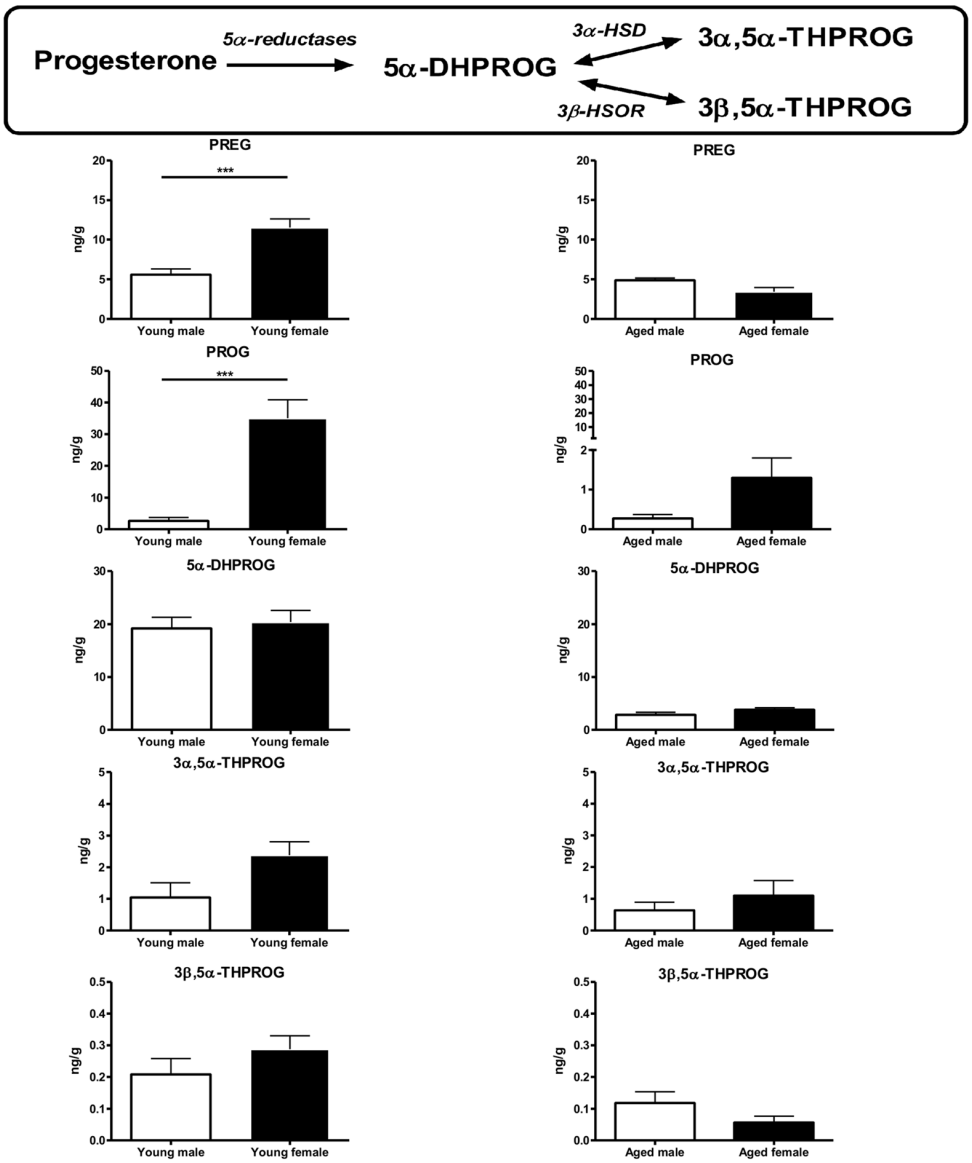


FIGURE 5 | Brain levels of progesterone and its metabolites in young (3-month-old) and aged (20-month-old) male and female mice. Young females (diestrus stage) had higher brain levels of pregnenolone and progesterone when compared to young males. These differences disappeared in aged mice. The levels of 5α-reduced metabolites were not statistically different between sexes in young or aged mice. Data represent mean ± SEM of 4–6 mice. Statistical analysis: *t*-test. Significance: $^{***}p < 0.001$. 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3β-HSOR, 3β-hydroxysteroid oxidoreductase; 5α-DHPROG, 5α-dihydroprogesterone; 3α,5α-THPROG, 3α,5α-tetrahydroprogesterone or allopregnanolone; 3β,5α-THPROG, 3β,5α-tetrahydroprogesterone or iso-allopregnanolone; PREG, pregnenolone; PROG, progesterone. Data from Gaignard et al. (2015).

et al., 2001; Venkateshappa et al., 2012). At the transcriptome level, two gene expression studies revealed that responses in aging are sexually dimorphic in human brain, and particularly concerning metabolic regulation (Berchtold et al., 2008; Guebel and Torres, 2016). In an analysis of age and sex effects on hippocampus transcriptome in men and women, 500 probes related to mitochondrial function were identified, and the age-induced effects were different according to the sex for one third of them. Among the major features, the expression of genes involved in mitochondrial fission (i.e., the fragmentation of one mitochondrion to generate other mitochondria) was higher in older women than in older men, suggesting possible sex differences in brain mitochondrial network (Guebel and Torres, 2016). The biological significance of the strong age and sex interactions observed in the expression of *HMGCS2*, a gene encoding the 3-hydroxy-3-methylglutaryl-CoA synthase involved in hepatic ketogenesis but with splice variants expressed in brain (Puisac et al., 2012) and in the expression of *UCP3*, a gene encoding uncoupling protein and expressed at low level in brain (Alán et al., 2009), has to be determined. In

another study Berchtold et al. (2008) showed that age-related changes in gene expression were more accentuated in men than in women. Especially, the expression of genes involved in energy production (electron transfer, ATP production) was more decreased during aging in men than in women in several regions of the brain. These data run rather counter the Brinton's and Stauch's teams works in rats showing a hypo-metabolism during female aging but not during male aging (see above).

MITOCHONDRIAL SEXUAL DIMORPHISM IN AGE-RELATED NEURODEGENERATIVE DISEASES

The influence of sex on various pathologies, and specially on neurodegenerative diseases, has become an extensive field of investigations, and mitochondrial metabolism seems to play a key role (Ventura-Clapier et al., 2017). We will focus on mitochondrial bioenergetics and oxidative stress regulations and the influences of sex steroids on it in the two major age-related neuronal diseases: AD and Parkinson's disease (PD).

Alzheimer's Disease

AD is the most common cause of dementia in aged people and is characterized by a gradual cognitive decline. The histopathological features of AD are: extracellular plaques constituted by accumulation of β-amyloid peptides (Aβ); and intraneuronal inclusions of neurofibrillary tangles composed of hyperphosphorylated forms of tau, a microtubule-associated protein. The lesions predominate in cortex and hippocampus (Grundke-Iqbal et al., 1987; Hardy and Higgins, 1992).

Among the multiple and intricate physiopathological mechanisms of AD, mitochondria play a pivotal role by controlling calcium homeostasis, intrinsic apoptosis but also energy production and oxidative stress. Deficits in the oxidative phosphorylation system have been described in AD patients by Sims et al. (1987) 30 years ago and confirmed by numerous studies so far (for review, Johri and Beal, 2012; Wang and Brinton, 2016). Beyond the RC failure, a decline in PDHc and in some TCA cycle enzymes were also described in AD human brains as well as a reduction in cerebral glucose utilization (Ishii et al., 1997; Blass et al., 2000). The decrease in energy production is associated with an increase in oxidative stress (for review, Wang and Brinton, 2016). More recently, dysregulation in mitochondrial dynamics has also been described in AD (Zorzano and Claret, 2015).

Mitochondrial dysfunction has been proposed to be either as a consequence or a cause of Aβ and hyperphosphorylated tau accumulations. In the "amyloid cascade hypothesis", the Aβ accumulation causes mitochondrial toxicity, which leads to an impairment of mitochondrial energy (Hardy and Higgins, 1992). By contrast, in the "mitochondrial cascade hypothesis" elaborated by Swerdlow and Khan, the mitochondrial dysfunction initiates Aβ accumulation (Swerdlow and Khan, 2004). According to this theory, the combination of inherited mutations on mtDNA (called mtDNA haplogroup) determines the baseline of mitochondrial function for each individual. The age-associated mitochondrial decline rate is then influenced by genetic and environmental factors. If the mitochondrial decline surpasses a threshold, it results in less energy synthesis, increased ROS production and disrupted calcium homeostasis. This mitochondrial failure would next perturb the control of Aβ production and tau phosphorylation. For example, it has been proposed that excess of mitochondrial oxidative stress and impaired mitophagy (the selective degradation of mitochondria) could disturb amyloidogenic processing and trigger hyperphosphorylation of tau (Melov et al., 2007; Kerr et al., 2017). The accumulation of Aβ and hyperphosphorylated tau then exacerbates mitochondrial failure by a vicious circle process (Swerdlow and Khan, 2004; Swerdlow et al., 2014).

Various epidemiological studies showed that AD affects more aged women than aged men; almost two thirds of the individuals diagnosed are women. The higher incidence of AD cases in women compared to men could be attributed to several mechanisms like the longer lifespan of women and the frequency of associated co-morbidities promoting AD development. However, it was demonstrated that gender is an independent risk factor for AD, which could suggest an influence of chromosomal sex (XX or XY) and/or of sex steroid hormones (Mielke et al., 2014). Moreover, the evolution of the pathogenic hallmarks in AD animal models is also sexually dimorphic (for review, Grimm et al., 2016b). Probably because AD is highly multifactorial, epidemiological data failed to clearly support the hypothesis of sex steroid hormones influence. Several studies reported an increased risk of AD with early menopause, however no significant correlation between the age of menopause and AD incidence was established (Henderson and Brinton, 2010; Yao and Brinton, 2012). Nevertheless, pharmacological evidence in preclinical studies clearly indicates a beneficial effect of estrogen supplementation (Barron and Pike, 2012). Moreover, in women, the protective effects of hormone replacement therapy (HRT) were reported by several observational studies. However, these effects were not confirmed by interventional clinical trials, in particular the Women's Health Initiative Memory Study (Hogervorst et al., 2009). The age of HRT initiation seems crucial to explain this discrepancy and introducing HRT early in the disease progress could be more beneficial (Chen et al., 2006; Henderson and Brinton, 2010). Associated risk factors such as mtDNA haplogroup or apolipoprotein E (ApoE) genotype should also be taken into account to better evaluate the role of endogenous steroids on AD incidence and protection and on the eventual benefit of HRT. Thus, some mtDNA haplogroups were found to be associated with increased or decreased risk of AD, but only in women or in contrary only in men (Wang and Brinton, 2016). For example, carrying the allele E4 of the gene encoding the ApoE is an important risk factor for AD because it could promote plaque aggregation and alter neuronal membrane regeneration and this genotype is also associated with a reduced efficacy of HRT (Thornton et al., 2011; Depypere et al., 2016; Wang and Brinton, 2016).

Based on the central position of mitochondria in AD pathogenesis and on the influence of sex on brain mitochondria

function, it could be hypothesized that mitochondrial energetic metabolism and oxidative stress regulation are involved in AD sex bias. This hypothesis was explored in the model of the triple transgenic-AD mice (3xTg-AD mice) that develop both Aβ plaques and tangles. Coskun et al. (2012) showed that the evolution of brain mitochondrial respiration, complexes I and IV activities in 3xTg-AD mice during aging was different according to the sex: male 3xTg-AD mice presented lower mitochondrial respiration and complexes I and IV activities than age-matched control male mice as early as 1 month of age but these activities did not decline throughout the life (between 1 and 24 months of age). By contrast, young female 3xTg-AD mice had the same rate of mitochondrial respiration and same activities of complexes I and IV than age-matched control female mice but these functions significantly declined with aging. Therefore, old female 3xTg-AD mice exhibited lower mitochondrial functions when compared to age- and sex-matched controls (Coskun et al., 2012). The team of Brinton also showed that brain mitochondrial function (mitochondrial respiration and complex IV activity) decreased since the age of 9 months in female 3xTg-AD mice. In parallel with RC decline, the mitochondrial lipid peroxidation and the free radical leak strongly increased in aged 3xTg-AD female mice (Yao et al., 2009, 2010). The link between low ovarian steroids levels and exacerbated mitochondrial dysfunction was also described in ApoE4 mice, another model of AD mice. The detrimental effect of ApoE4 genotype vs. ApoE3 genotype on synaptic mitochondrial proteome was greater in females than in males. Yet, ApoE4 females presented low cortical 17β-estradiol levels than the ApoE3 control female mice (Shi et al., 2014). In *ex vivo* experimentations using mitochondria isolated from brain of wild-type (WT) Wistar rats incubated with Aβ, it has also been shown that the oxidative stress response was different between young and aged females. Peroxide production and cytochrome c release were strongly enhanced after Aβ addition to mitochondria isolated from young males or from aged females. By contrast, mitochondria isolated from young females were protected against the toxicity of Aβ (Viña et al., 2007).

In addition to RC dysfunction and subsequent ROS production, a decrease of PDHc activity was described in 3xTg-AD female mice since the age of 9 months. Interestingly, PDH subunit E1α expression was decreased as early as 3 months of age, but the PDHc activity was not modified, probably thanks to compensatory post-translational modifications (Yao et al., 2009, 2010). In addition, the activities of the hydroxyacyl-co enzyme A deshydrogenase (HADHA, an enzyme involved in the fatty acid oxidation) and of the 3-oxoacid-CoA transferase 1 (SCOT, an enzyme involved in the ketolysis) were increased in 3-month-old 3xTg-AD female mice when compared to WT mice. The authors suggest that the activation of the fatty acid oxidation (that produces acetyl-coA which is then converted into soluble ketone bodies) and the ketolysis pathway (that reconverts ketone bodies into acetyl-coA) is a way to compensate the reduction in the pool of acetyl-coA due to the decrease of PDHc activity. During reproductive senescence, the expressions of SCOT and HADHA increased in WT aged females in parallel with the PDHc decrease. By contrast, in aged 3xTg-AD female mice, the SCOT expression did not increase; as a consequence, the utilization of ketone bodies as alternative fuel could be limited leading to aggravation of the energetic deficit (Yao et al., 2009, 2010). Recently, the same team has proposed a mechanism for the switch of energetic fuel during normal reproductive senescence: the mitochondrial decline could enhance peroxide production that activates the cytosolic phospholipase A2-sphingomyelinase pathway. The lipids liberated from myelin breakdown could be a source for the fatty acid oxidation by the astrocytes. The astrocytes then provide ketone bodies to the neurons in order to furnish acetyl-coA for the TCA cycle. In the context of AD, the glucose metabolism is decreased right from the prodromal phase and the activation of this adaptive pathway could be a cause of early white matter degeneration (Yao and Brinton, 2012; Klosinski et al., 2015).

All these findings strongly orient toward an important influence of ovarian steroids on the evolution of mitochondrial AD-induced disorders: the impregnation by estrogens and progestagens could protect young females during the reproductive period but the strong drop at the time of menopause could precipitate the mitochondrial decline and disturb the brain homeostasis of Aβ production and tau phosphorylation. Experimental pharmacological studies principally focused on the role of estrogens and showed that 17β-estradiol is protective against the oxidative stress increase and the oxidative phosphorylation decrease observed in AD models (Viña et al., 2007; Yao and Brinton, 2012). The properties of the two other groups of sex steroids, progestagens and androgens, have been less tested (see review, Grimm et al., 2016b). Recently, Grimm et al. (2016a) performed an exhaustive study on the effects of sex steroids on mitochondrial function in two cellular models of AD, one mimicked Aβ accumulation (neuroblastoma cells transfected with the human amyloid precursor protein APP) and the other mimicked tau hyperphosphorylation (neuroblastoma cells transfected with mutant tau P301L). In this work, the effects of progesterone, 17β-estradiol, estrone, testosterone or 3α-androstanediol on mitochondrial ATP level, membrane potential and respiration rate were investigated. This study revealed very interesting findings: progesterone and 17β-estradiol were the most effective steroids to alleviate mitochondrial energetic failure in P301L cells, whereas testosterone was the most effective in APP cells. Therefore, young males could be better protected against Aβ-induced mitochondrial alterations; whereas the ovarian steroids could prevent abnormal tau-induced mitochondrial alterations in young females (Grimm et al., 2016a). One can note that these assays were performed in neuroblastoma cells and should be confirmed in other brain cells. Indeed, in embryonic rat hippocampi from WT rats, it has been reported that progesterone failed to protect neurons against mitochondrial toxins, whereas 17β-estradiol was protective (Yao et al., 2011). However, the Grimm's study conclusions are consistent with an *in vivo* study in 3xTg-AD male mice showing that orchidectomy increased Aβ accumulation more than tau hyperphosphorylation in several brain regions including hippocampus (Rosario et al., 2010). Moreover, dihydrotestosterone (DHT) administration prevented Aβ accumulation but not tau hyperphosphorylation. In contrast to testosterone, DHT is not metabolized to 17β-estradiol by

aromatase. The regulation of Aβ accumulation is therefore well mediated by a specific androgenic effect (Rosario et al., 2010). Thus, it has been suggested that the gradual decrease in testosterone during andropause may participate in AD pathogenesis in aged men (Grimm et al., 2016b).

Besides oxidative phosphorylation and oxidative stress, Aβ and hyperphosphorylated tau also alter mitochondrial dynamics (the balance between fusion and fission; Wang et al., 2008; DuBoff et al., 2012). Interestingly, the regulation of mitochondrial dynamics by sex steroids has been described as sexually dimorphic. Thus, 17β-estradiol or progesterone addition increased the expression of both mitochondrial fusion and fission genes in cortical astrocytes from WT female mice. In contrast, in WT male mice cortical astrocytes, 17β-estradiol enhanced the expression of fission genes and of one fusion gene (*MFN2*) and decreased the expression of the second fusion gene *(MFN1)*; while progesterone decreased both fission and fusion gene expressions (Arnold et al., 2008). In AD models, the effects of sex steroids on mitochondrial network have not been described yet.

Like for normal aging, exploring human brain mitochondrial metabolism in AD patients in order to objectivize putative sexual dimorphism is obviously challenging. Thanks to the progress of proteomics, a recent study described sex-specific changes in proteome of mitochondria from temporal lobes of patients suffering from AD with cerebrovascular disease. Some subunits of complexes I, III and IV were down-regulated and subunits of complex V were dysregulated in women when compared to men (Gallart-Palau et al., 2016). These findings are in agreement with the data obtained in rodents (Yao et al., 2009, 2010; Coskun et al., 2012). Surprisingly, in contrast of what was reported in rodents, the E1β subunit of PDHc was down-regulated in humans, but only in men (Gallart-Palau et al., 2016). Additional studies in other brain regions and in other forms of AD are now necessary to extend the knowledge about sex-specific mitochondrial alterations in AD and their consequences for potential new sex-based therapies.

Together, the recent findings highlight that in addition to sex differences in mitochondrial function, the effects of sex steroids on mitochondria could differ between men and women. Consequently, the development of new sex-specific therapies seems to be a promising way in AD treatment.

Parkinson's Disease

PD is the second most common age-related neurodegenerative disorder and is defined by a triad of motor symptoms associating bradykinesia, rigidity and resting tremor. Non-motor symptoms such as inaugural depression or delayed impaired cognition are also frequent. PD pathological hallmarks are the degeneration of dopaminergic neurons in the substantia nigra and subsequent striatal dopamine loss and the presence of intra-neuronal protein inclusions of aggregated α-synuclein proteins called Lewy bodies in the brainstem and the neocortex (Lees et al., 2009).

The involvement of mitochondrial dysfunction in PD pathogenesis is supported by evidence accumulated over the last several decades. The most described mitochondrial dysfunction is the decrease of complex I activity. Deficiencies in complex I were reported in substantia nigra, platelets and skeletal muscle of patients with PD. Moreover, when complex I is selectively inhibited by rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or its metabolized form N-methyl-4-phenylpyridinium ion (MPP^+), the symptoms and the neuropathological features in corresponding animal models are similar to those observed in humans (for review, Johri and Beal, 2012; Chaturvedi and Flint Beal, 2013). Like in AD, an association between PD cases and some mtDNA haplogroups has been reported, suggesting that some patterns of inherited mtDNA mutations could promote mitochondrial dysfunction. Moreover, complex I deficiency was observed in "cybrids", a technic that consists in introducing mitochondria from PD patients in a healthy nuclear background (Chaturvedi and Flint Beal, 2013). However, it seems that down-regulation of RC subunits is not only due to a reduction of mtDNA-encoded subunits expression. Recently, a meta-analysis of several genome-wide expression studies revealed that the expression of nuclear genes encoding complex I subunits but also complexes II, III, IV and V subunits were down-regulated in dopaminergic neurons of patients with PD. The authors underline that most of these genes are under the control of PGC1-α, a major nuclear regulator of mitochondrial biogenesis (Zheng et al., 2010). Upstream the RC, a decrease of αKGDH activity, a TCA cycle enzyme, was also described in PD patients (Gibson et al., 2003). The rare familial forms of PD, due to mutations in nuclear genes, also strongly suggest that mitochondrial dysfunction is pivotal in PD pathogenesis. For example, mutations that affect the protein Parkin (the phosphatase and tensin homolog induced kinase 1, PINK1) lead to impairments in mitophagy or in mitochondrial dynamics (Chaturvedi and Flint Beal, 2013). Finally, the deficiency of complex I seems to be the most ostentatious but not the only mitochondrial alteration in PD. The precise process remains elusive but the energy production failure and the subsequent oxidative stress are certainly involved in dopaminergic neuron degeneration.

Sex ratio in PD is opposed to the one observed in AD: the men are twice more affected than women. The clinical profile and the response to treatment are also marked by sex differences. This trend is reproduced in animal models: the administration of 6-hydroxydopamine (6-OHDA, a toxic hydroxylated analog of dopamine) induces more loss of dopaminergic neurons in males than in females (for review, Gillies et al., 2014). The ability of the sex-determining region Y (SRY) protein to regulate dopamine synthesis suggests that chromosomal sex is involved in PD sexual dimorphism (Czech et al., 2012). Nevertheless, ovarian hormones are most probably involved too, as proved by epidemiological data like the increased risk of PD in women after bilateral oophorectomy (Rocca et al., 2008) or by some data suggesting a protective effect of early HRT in women (Bourque et al., 2009; Gillies et al., 2014). Importantly, estrogens seem to be protective only in females. In animal experiments with 6-OHDA administration, it has been shown that ovariectomy enhanced the loss of striatal dopamine, whereas orchidectomy limited it and that 17β-estradiol administration reduced striatal lesions in females but increased it in males. It has also been hypothesized that androgens could modulate toxin-induced PD alterations, since men are more affected than women (Gillies et al., 2014).

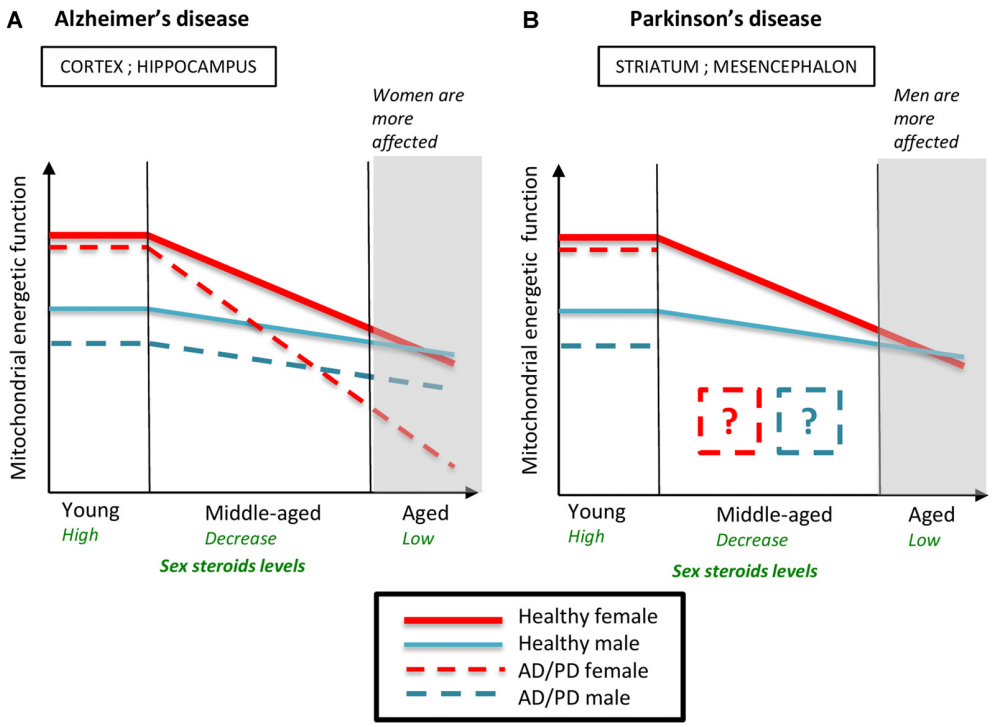


FIGURE 6 | Schematic representation of the evolution of mitochondrial energetic function in males and females during normal and pathological aging. Young healthy females have higher mitochondrial energetic function than young healthy males. The decline of ovarian steroids during reproductive senescence accentuates the age-induced mitochondrial decline. Mitochondrial energetic function is similar between aged males and females. **(A)** In Alzheimer's disease (AD) models, young females are more protected against AD-induced mitochondrial failure than young males but the drop of ovarian steroids levels during reproductive senescence strongly precipitates mitochondrial impairment. Therefore, mitochondrial function is more altered in AD aged females than in AD aged males, which is consistent with the sex ratio observed in AD. **(B)** In Parkinson's disease (PD) models, young females are also more protected than young males. Data on mitochondrial function in middle-aged and aged PD animals are lacking. AD, Alzheimer's disease; PD, Parkinson's disease. Data from Kenchappa et al. (2004), Yao et al. (2009, 2010), Misiak et al. (2010), Sundar Boyalla et al. (2011), Coskun et al. (2012) and Gaignard et al. (2015).

Yet, no study reported a specific androgenic effect (Ekue et al., 2002; Bourque et al., 2009; Gillies et al., 2014).

The mechanisms underlying beneficial effects of estrogens and the resulting PD sex bias are increasingly better understood. It has been demonstrated that estrogens influence the activation of apoptotic pathways in dopaminergic neurons, the dopamine transporter, the brain renin-angiotensin system and the glial inflammation (Morale et al., 2006; Bourque et al., 2009; Gillies et al., 2014; Labandeira-Garcia et al., 2016). Considering the sex influence on mitochondrial metabolism during normal aging and the key role of mitochondria in PD disease, it is essential to investigate male/female differences in mitochondrial functions in PD models. Few studies addressed this issue so far. In 3-month-old Swiss albino mice, neither acute nor chronic administration of MPTP triggered a decrease of complex I activity in striatum or mesencephalon from females; whereas males exhibited a strong deficit. Pretreatment of females with an estrogen receptor antagonist (ICI 182, 170) abolished the sex difference (Kenchappa et al., 2004). This study also reported that intact young females presented a higher glutaredoxin 1 (Grx1) activity than males and that this up-regulation was also mediated by estrogens. Grx1 allows the reactivation of proteins oxidatively inactivated by S-glutathionylation, like some complex I subunits. The high Grx1 activity in females could protect complex I against oxidative stress consequences (Kenchappa et al., 2004). It would be interesting to test if the same phenomenon occurs with αKGDH activity. Indeed, αKGDH is prone to inactivation by S-glutathionylation and its activity is rate-limiting for TCA cycle functioning in brain (Tretter and Adam-Vizi, 2005; Bénit et al., 2014; Ribas et al., 2014). The regulation of RC genes expression is also sexually dimorphic (Misiak et al., 2010). Thus, in primary mesencephalic neuron cultures from male or female fetus mice, it has been shown that the addition of 6-OHDPA induced a decrease of the expressions of mtDNA- and nDNA-encoded RC subunits and that this decrease was more pronounced in male neurons than in female neurons for some mtDNA-encoded subunits. The 6-OHDA addition also triggered a decrease of ATP levels and an increase of ROS levels and these effects were more important in males. Furthermore, the administration of 17β-estradiol counteracted the increase in ROS levels but not the decrease in ATP levels in both sexes (Misiak et al., 2010). Sex-specific toxin-induced mitochondrial alterations were reported in glial cells as well. The addition of MPP^+ to mesencephalic astrocytes increased the expression of the subunits 1 and 2 of the complex IV, decreased ATP levels and increased peroxide

production, but only in cells from male mice pups; whereas no or attenuated effects were observed in cells from female mice pups (Sundar Boyalla et al., 2011). Another study reported that the striatal astrocytes from male mice were more sensitive to MPP^+-induced toxicity than striatal astrocytes from female mice. The higher levels of paraoxonase 2 (PON2), a 17β-estradiol inducible antioxidant lactonase, in females could participate to the protection (Giordano et al., 2013). Together, these results suggest that ovarian steroid impregnation of young females is protective against PD-induced mitochondrial alterations.

By comparing the mitochondrial mechanisms implied in AD and PD pathogenesis, some similarities appear: the energy production failure and the increase of oxidative stress are central in the two diseases; ovarian steroids impregnation in young females is protective (**Figure 6**). In AD, previously cited studies thoroughly suggest that the drop of ovarian steroids leads to a hypo-metabolic state that could precipitate the mitochondrial failure and disturb Aβ and tau homeostasis (Yao et al., 2009, 2010; Coskun et al., 2012). Whether a similar mechanism occurs in PD is not yet determined and is not so obvious because some molecular mechanisms described in brain regions involved in AD or PD pathogenesis could be missing or different in other brain regions. For example, the protective effect of high levels of PON2 in females that was described in striatal astrocytes could not be effective in the cortex and the hippocampus, since PON2 is expressed at low level in these brain regions (Giordano et al., 2011, 2013). After MPP^+ addition, the ATP levels were more decreased in mesencephalic astrocytes from males than from females, as evoked. Conversely, in cortical astrocytes, the MPP^+ addition decreased more ATP levels in cells from females than in cells from males (Sundar Boyalla et al., 2011). Therefore, further explorations should now investigate whether the protection observed in PD young females is abolished or even reversed in PD aged females after the drop of ovarian steroids. In addition, data about sex-specific mitochondrial metabolism differences in human patients are still lacking and should be undertaken to complete the puzzle of PD sex bias and to design the most appropriate therapy according to the sex and the hormonal status.

CONCLUSION

Mitochondria are in charge of energy production, regulate oxidative stress and are the site of the first steps of steroidogenesis. In addition, they are target of sex steroids. As the nervous system has a high metabolic rate and a low capacity of energy storage, dysfunction of brain mitochondria has devastating consequences. Several indications show that brain mitochondrial functions decline with age and that sex steroid loss is involved in the observed dysregulation of mitochondrial functions. Furthermore, sex differences in brain mitochondrial functions may explain, at least partially, the influence of sex steroids on neurodegenerative diseases such as AD and PD. These data describing the complex relationships between age, sex steroids and mitochondrial function should be taken into account when designing therapies for successful aging of both men and women. Investigating the mechanisms and optimizing the strategies of steroid supplementation, will help in better developing sex-specific cerebroprotective approaches.

AUTHOR CONTRIBUTIONS

PG and RG conceived, designed and drafted the manuscript. All authors revised critically and approved the final version.

FUNDING

The publication fees of this review were covered by Inserm funds.

REFERENCES

Agarwal, A. K., and Auchus, R. J. (2005). Minireview: cellular redox state regulates hydroxysteroid dehydrogenase activity and intracellular hormone potency. *Endocrinology* 146, 2531–2538. doi: 10.1210/en.2005-0061

Alán, L., Smolková, K., Kronusová, E., Santorová, J., and Jezek, P. (2009). Absolute levels of transcripts for mitochondrial uncoupling proteins UCP2, UCP3, UCP4, and UCP5 show different patterns in rat and mice tissues. *J. Bioenerg. Biomembr.* 41, 71–78. doi: 10.1007/s10863-009-9201-2

Ali, S. S., Xiong, C., Lucero, J., Behrens, M. M., Dugan, L. L., and Quick, K. L. (2006). Gender differences in free radical homeostasis during aging: shorter-lived female C57BL6 mice have increased oxidative stress. *Aging Cell* 5, 565–574. doi: 10.1111/j.1474-9726.2006.00252.x

Alvarez-Delgado, C., Mendoza-rodríguez, C. A., Picazo, O., Cerbón, M., and Álvarez-delgado, C. (2010). Different expression of α and β mitochondrial estrogen receptors in the aging rat brain: interaction with respiratory complex V. *Exp. Gerontol.* 45, 580–585. doi: 10.1016/j.exger.2010.01.015

Andrabi, S. S., Parvez, S., and Tabassum, H. (2017). Progesterone induces neuroprotection following reperfusion-promoted mitochondrial dysfunction after focal cerebral ischemia in rats. *Dis. Model. Mech.* 10, 787–796. doi: 10.1242/dmm.025692

Arnold, S., de Araújo, G. W., and Beyer, C. (2008). Gender-specific regulation of mitochondrial fusion and fission gene transcription and viability of cortical astrocytes by steroid hormones. *J. Mol. Endocrinol.* 41, 289–300. doi: 10.1677/JME-08-0085

Barron, A. M., and Pike, C. J. (2012). Sex hormones, aging, and Alzheimer's disease. *Front. Biosci. (Elite Ed)* 4, 976–997. doi: 10.2741/e434

Bénit, P., Letouzé, E., Rak, M., Aubry, L., Burnichon, N., Favier, J., et al. (2014). Unsuspected task for an old team: succinate, fumarate and other Krebs cycle acids in metabolic remodeling. *Biochim. Biophys. Acta* 1837, 1330–1337. doi: 10.1016/j.bbabio.2014.03.013

Berchtold, N. C., Cribbs, D. H., Coleman, P. D., Rogers, J., Head, E., Kim, R., et al. (2008). Gene expression changes in the course of normal brain aging are sexually dimorphic. *Proc. Natl. Acad. Sci. U S A* 105, 15605–15610. doi: 10.1073/pnas.0806883105

Blass, J. P., Sheu, R. K., and Gibson, G. E. (2000). Inherent abnormalities in energy metabolism in Alzheimer disease. Interaction with cerebrovascular compromise. *Ann. N Y Acad. Sci.* 903, 204–221. doi: 10.1111/j.1749-6632.2000.tb06370.x

Borrás, C., Sastre, J., García-Sala, D., Lloret, A., Pallardó, F. V., and Viña, J. (2003). Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. *Free Radic. Biol. Med.* 34, 546–552. doi: 10.1016/s0891-5849(02)01356-4

Bourque, M., Dluzen, D. E., and Di Paolo, T. (2009). Neuroprotective actions of sex steroids in Parkinson's disease. *Front. Neuroendocrinol.* 30, 142–157. doi: 10.1016/j.yfrne.2009.04.014

Brinton, R. D. (2008). The healthy cell bias of estrogen action: mitochondrial bioenergetics and neurological implications. *Trends Neurosci.* 31, 529–537. doi: 10.1016/j.tins.2008.07.003

Cai, M., Ma, Y.-L. L., Qin, P., Li, Y., Zhang, L.-X. X., Nie, H., et al. (2014). The loss of estrogen efficacy against cerebral ischemia in aged postmenopausal female mice. *Neurosci. Lett.* 558, 115–119. doi: 10.1016/j.neulet.2013.11.007

Carretero, M., Escames, G., López, L. C., Venegas, C., Dayoub, J. C., García, L., et al. (2009). Long-term melatonin administration protects brain mitochondria from aging. *J. Pineal Res.* 47, 192–200. doi: 10.1111/j.1600-079x.2009.00700.x

Caruso, D., Barron, A. M., Brown, M. A., Abbiati, F., Carrero, P., Pike, C. J., et al. (2013). Age-related changes in neuroactive steroid levels in 3xTg-AD mice. *Neurobiol. Aging* 34, 1080–1089. doi: 10.1016/j.neurobiolaging.2012.10.007

Chakrabarti, S., Munshi, S., Banerjee, K., Thakurta, I. G., Sinha, M., and Bagh, M. B. (2011). Mitochondrial dysfunction during brain aging: role of oxidative stress and modulation by antioxidant supplementation. *Aging Dis.* 2, 242–256.

Chakraborty, T. R., and Gore, A. C. (2004). Aging-related changes in ovarian hormones, their receptors and neuroendocrine function. *Exp. Biol. Med. (Maywood)* 229, 977–987. doi: 10.1177/153537020422901001

Chapman, J. C., Polanco, J. R., Min, S., and Michael, S. D. (2005). Mitochondrial 3 beta-hydroxysteroid dehydrogenase (HSD) is essential for the synthesis of progesterone by corpora lutea: an hypothesis. *Reprod. Biol. Endocrinol.* 3:11. doi: 10.1186/1477-7827-3-11

Chaturvedi, R. K., and Flint Beal, M. (2013). Mitochondrial diseases of the brain. *Free Radic. Biol. Med.* 63, 1–29. doi: 10.1016/j.freeradbiomed.2013.03.018

Chen, J. Q., Brown, T. R., and Russo, J. (2009a). Regulation of energy metabolism pathways by estrogens and estrogenic chemicals and potential implications in obesity associated with increased exposure to endocrine disruptors. *Biochim. Biophys. Acta* 1793, 1128–1143. doi: 10.1016/j.bbamcr.2009.03.009

Chen, J. Q., Cammarata, P. R., Baines, C. P., and Yager, J. D. (2009b). Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and pharmacological implications. *Biochim. Biophys. Acta* 1793, 1540–1570. doi: 10.1016/j.bbamcr.2009.06.001

Chen, S., Nilsen, J., and Brinton, R. D. (2006). Dose and temporal pattern of estrogen exposure determines neuroprotective outcome in hippocampal neurons: therapeutic implications. *Endocrinology* 147, 5303–5313. doi: 10.1210/en.2006-0495

Chretien, D., Rustin, P., Bourgeron, T., Rötig, A., Saudubray, J. M., and Munnich, A. (1994). Reference charts for respiratory chain activities in human tissues. *Clin. Chim. Acta* 228, 53–70. doi: 10.1016/0009-8981(94)90056-6

Coskun, P., Wyrembak, J., Schriner, S. E., Chen, H. W., Marciniack, C., Laferla, F., et al. (2012). A mitochondrial etiology of Alzheimer and Parkinson disease. *Biochim. Biophys. Acta* 1820, 553–564. doi: 10.1016/j.bbagen.2011.08.008

Cottrell, D. A., Blakely, E. L., Johnson, M. A., Ince, P. G., Borthwick, G. M., and Turnbull, D. M. (2001). Cytochrome c oxidase deficient cells accumulate in the hippocampus and choroid plexus with age. *Neurobiol. Aging* 22, 265–272. doi: 10.1016/s0197-4580(00)00234-7

Czech, D. P., Lee, J., Sim, H., Parish, C. L., Vilain, E., and Harley, V. R. (2012). The human testis-determining factor SRY localizes in midbrain dopamine neurons and regulates multiple components of catecholamine synthesis and metabolism. *J. Neurochem.* 122, 260–271. doi: 10.1111/j.1471-4159.2012.07782.x

Demonacos, C. V., Karayanni, N., Hatzoglou, E., Tsiriyiotis, C., Spandidos, D. A., and Sekeris, C. E. (1996). Mitochondrial genes as sites of primary action of steroid hormones. *Steroids* 61, 226–232. doi: 10.1016/0039-128x(96)00019-0

Depypere, H., Vierin, A., Weyers, S., and Sieben, A. (2016). Alzheimer's disease, apolipoprotein E and hormone replacement therapy. *Maturitas* 94, 98–105. doi: 10.1016/j.maturitas.2016.09.009

Dietrich, A. K., Humphreys, G. I., and Nardulli, A. M. (2015). Expression of estrogen receptor α in the mouse cerebral cortex. *Mol. Cell. Endocrinol.* 406, 19–26. doi: 10.1016/j.mce.2015.02.013

DuBoff, B., Götz, J., and Feany, M. B. (2012). Tau promotes neurodegeneration via DRP1 mislocalization *in vivo*. *Neuron* 75, 618–632. doi: 10.1016/j.neuron.2012.06.026

Ebner, M. J., Corol, D. I., Havlíková, H., Honour, J. W., and Fry, J. P. (2006). Identification of neuroactive steroids and their precursors and metabolites in adult male rat brain. *Endocrinology* 147, 179–190. doi: 10.1210/en.2005-1065

Eghlidi, D. H., Garyfallou, V. T., Kohama, S. G., and Urbanski, H. F. (2017). Age-associated gene expression changes in the arcuate nucleus of male rhesus macaques. *J. Mol. Endocrinol.* 59, 141–149. doi: 10.1530/JME-17-0094

Eghlidi, D. H., and Urbanski, H. F. (2015). Effects of age and estradiol on gene expression in the rhesus macaque hypothalamus. *Neuroendocrinology* 101, 236–245. doi: 10.1159/000381063

Ekue, A., Boulanger, J.-F., Morissette, M., and Di Paolo, T. (2002). Lack of effect of testosterone and dihydrotestosterone compared to 17β-oestradiol in 1-methyl-4-phenyl-1,2,3,6, tetrahydropyridine-mice. *J. Neuroendocrinol.* 14, 731–736. doi: 10.1046/j.1365-2826.2002.00833.x

Escames, G., Díaz-Casado, M. E., Doerrier, C., Luna-Sánchez, M., López, L. C., and Acuña-Castroviejo, D. (2013). Early gender differences in the redox status of the brain mitochondria with age: effects of melatonin therapy. *Horm. Mol. Biol. Clin. Investig.* 16, 91–100. doi: 10.1515/hmbci-2013-0026

Ferrándiz, M. L., Martínez, M., De Juan, E., Díez, A., Bustos, G., and Miquel, J. (1994). Impairment of mitochondrial oxidative phosphorylation in the brain of aged mice. *Brain Res.* 644, 335–338. doi: 10.1016/0006-8993(94)91699-3

Fisher-Wellman, K. H., Lin, C.-T., Ryan, T. E., Reese, L. R., Gilliam, L. A. A., Cathey, B. L., et al. (2015). Pyruvate dehydrogenase complex and nicotinamide nucleotide transhydrogenase constitute an energy-consuming redox circuit. *Biochem. J.* 467, 271–280. doi: 10.1042/BJ20141447

Fukui, M., and Zhu, B. T. (2010). Mitochondrial superoxide dismutase SOD2, but not cytosolic SOD1, plays a critical role in protection against glutamate-induced oxidative stress and cell death in HT22 neuronal cells. *Free Radic. Biol. Med.* 48, 821–830. doi: 10.1016/j.freeradbiomed.2009.12.024

Funabashi, T., Kleopoulos, S. P., Brooks, P. J., Kimura, F., Pfaff, D. W., Shinohara, K., et al. (2000). Changes in estrogenic regulation of estrogen receptor α mRNA and progesterone receptor mRNA in the female rat hypothalamus during aging: an *in situ* hybridization study. *Neurosci. Res.* 38, 85–92. doi: 10.1016/s0168-0102(00)00150-4

Furuta, M., Fukushima, A., Chiba, S., Sano, A., Akema, T., Kimura, F., et al. (2010). Progesterone receptor immunoreactivity in the brains of ovariectomized aged rats. *Neuroreport* 21, 777–781. doi: 10.1097/WNR.0b013e32833c5b6f

Gaignard, P., Fréchou, M., Liere, P., Thérond, P., Schumacher, M., Slama, A., et al. (2017). Sex differences in brain mitochondrial metabolism: influence of endogenous steroids and stroke. *J. Neuroendocrinol.* doi: 10.1111/jne.12497 [Epub ahead of print].

Gaignard, P., Fréchou, M., Schumacher, M., Thérond, P., Mattern, C., Slama, A., et al. (2016). Progesterone reduces brain mitochondrial dysfunction after transient focal ischemia in male and female mice. *J. Cereb. Blood Flow Metab.* 36, 562–568. doi: 10.1177/0271678X15610338

Gaignard, P., Savouroux, S., Liere, P., Pianos, A., Thérond, P., Schumacher, M., et al. (2015). Effect of sex differences on brain mitochondrial function and its suppression by ovariectomy and in aged mice. *Endocrinology* 156, 2893–2904. doi: 10.1210/en.2014-1913

Gallart-Palau, X., Lee, B. S. T., Adav, S. S., Qian, J., Serra, A., Park, J. E., et al. (2016). Gender differences in white matter pathology and mitochondrial dysfunction in Alzheimer's disease with cerebrovascular disease. *Mol. Brain* 9:27. doi: 10.1186/s13041-016-0205-7-3

Gibson, G. E., Kingsbury, A. E., Xu, H., Lindsay, J. G., Daniel, S., Foster, O. J. F., et al. (2003). Deficits in a tricarboxylic acid cycle enzyme in brains from patients with Parkinson's disease. *Neurochem. Int.* 43, 129–135. doi: 10.1016/s0197-0186(02)00225-5

Gillies, G. E., Pienaar, I. S., Vohra, S., and Qamhawi, Z. (2014). Sex differences in Parkinson's disease. *Front. Neuroendocrinol.* 35, 370–384. doi: 10.1016/j.yfrne.2014.02.002

Gilmer, L. K., Ansari, M. A., Roberts, K. N., and Scheff, S. W. (2010). Age-related changes in mitochondrial respiration and oxidative damage in the cerebral cortex of the Fischer 344 rat. *Mech. Ageing Dev.* 131, 133–143. doi: 10.1016/j.mad.2009.12.011

Giordano, G., Cole, T. B., Furlong, C. E., and Costa, L. G. (2011). Paraoxonase 2 (PON2) in the mouse central nervous system: a neuroprotective role? *Toxicol. Appl. Pharmacol.* 256, 369–378. doi: 10.1016/j.taap.2011.02.014

Giordano, G., Tait, L., Furlong, C. E., Cole, T. B., Kavanagh, T. J., and Costa, L. G. (2013). Gender differences in brain susceptibility to oxidative stress are

mediated by levels of paraoxonase-2 expression. *Free Radic. Biol. Med.* 58, 98–108. doi: 10.1016/j.freeradbiomed.2013.01.019

Gredilla, R., Garm, C., Holm, R., Bohr, V. A., and Stevnsner, T. (2010). Differential age-related changes in mitochondrial DNA repair activities in mouse brain regions. *Neurobiol. Aging* 31, 993–1002. doi: 10.1016/j.neurobiolaging.2008.07.004

Grimm, A., Biliouris, E. E., Lang, U. E., Götz, J., Mensah-Nyagan, A. G., and Eckert, A. (2016a). Sex hormone-related neurosteroids differentially rescue bioenergetic deficits induced by amyloid-β or hyperphosphorylated tau protein. *Int. J. Mol. Sci.* 73, 201–215. doi: 10.1007/s00018-015-1988-x

Grimm, A., Mensah-Nyagan, A. G., and Eckert, A. (2016b). Alzheimer, mitochondria and gender. *Neurosci. Biobehav. Rev.* 67, 89–101. doi: 10.1016/j.neubiorev.2016.04.012

Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., Quinlan, M., Wisniewski, H., and Binder, L. (1987). Abnormal phosphorylation of the microtubule-associated protein? (tau) in Alzheimer cytoskeletal pathology. *Alzheimer Dis. Assoc. Disord.* 1:202. doi: 10.1097/00002093-198701030-00020

Guebel, D. V., and Torres, N. V. (2016). Sexual dimorphism and aging in the human hyppocampus: identification, validation, and impact of differentially expressed genes by factorial microarray and network analysis. *Front. Aging Neurosci.* 8:229. doi: 10.3389/fnagi.2016.00229

Guennoun, R., Labombarda, F., Gonzalez Deniselle, M. C., Liere, P., De Nicola, A. F., and Schumacher, M. (2015). Progesterone and allopregnanolone in the central nervous system: response to injury and implication for neuroprotection. *J. Steroid Biochem. Mol. Biol.* 146, 48–61. doi: 10.1016/j.jsbmb.2014.09.001

Guevara, R., Gianotti, M., Roca, P., and Oliver, J. (2011). Age and sex-related changes in rat brain mitochondrial function. *Cell. Physiol. Biochem.* 27, 201–206. doi: 10.1159/000327945

Guevara, R., Santandreu, F. M., Valle, A., Gianotti, M., Oliver, J., and Roca, P. (2009). Sex-dependent differences in aged rat brain mitochondrial function and oxidative stress. *Free Radic. Biol. Med.* 46, 169–175. doi: 10.1016/j.freeradbiomed.2008.09.035

Guo, J., Duckles, S. P., Weiss, J. H., Li, X., and Krause, D. N. (2012). 17β-estradiol prevents cell death and mitochondrial dysfunction by an estrogen receptor-dependent mechanism in astrocytes after oxygen-glucose deprivation/reperfusion. *Free Radic. Biol. Med.* 52, 2151–2160. doi: 10.1016/j.freeradbiomed.2012.03.005

Hara, Y., Waters, E. M., McEwen, B. S., and Morrison, J. H. (2015). Estrogen effects on cognitive and synaptic health over the lifecourse. *Physiol. Rev.* 95, 785–807. doi: 10.1152/physrev.00036.2014

Hardy, J. A., and Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184–185. doi: 10.1126/science.1566067

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11, 298–300. doi: 10.1093/geronj/11.3.298

Harman, D. (1972). The biologic clock: the mitochondria? *J. Am. Geriatr. Soc.* 20, 145–147. doi: 10.1111/j.1532-5415.1972.tb00787.x

Henderson, V. W., and Brinton, R. D. (2010). Menopause and mitochondria: windows into estrogen effects on Alzheimer's disease risk and therapy. *Prog. Brain Res.* 182, 77–96. doi: 10.1016/S0079-6123(10)82003-5

Hogervorst, E., Yaffe, K., Richards, M., and Huppert, F. A. H. (2009). Hormone replacement therapy to maintain cognitive function in women with dementia. *Cochrane Database Syst. Rev.* 1:CD003799. doi: 10.1002/14651858.cd003799

Irwin, R. W., Yao, J., Ahmed, S. S., Hamilton, R. T., Cadenas, E., and Brinton, R. D. (2011). Medroxyprogesterone acetate antagonizes estrogen up-regulation of brain mitochondrial function. *Endocrinology* 152, 556–667. doi: 10.1210/en.2010-1061

Irwin, R. W., Yao, J., Hamilton, R. T., Cadenas, E., Brinton, R. D., and Nilsen, J. (2008). Progesterone and estrogen regulate oxidative metabolism in brain mitochondria. *Endocrinology* 149, 3167–3175. doi: 10.1210/en.2007-1227

Irwin, R. W., Yao, J., To, J., Hamilton, R. T., Cadenas, E., and Brinton, R. D. (2012). Selective oestrogen receptor modulators differentially potentiate brain mitochondrial function. *J. Neuroendocrinol.* 24, 236–248. doi: 10.1111/j.1365-2826.2011.02251.x

Ishii, K., Sasaki, M., Kitagaki, H., Yamaji, S., Sakamoto, S., Matsuda, K., et al. (1997). Reduction of cerebellar glucose metabolism in advanced Alzheimer's disease. *J. Nucl. Med.* 38, 925–928.

Johri, A., and Beal, M. F. (2012). Mitochondrial dysfunction in neurodegenerative diseases. *J. Pharmacol. Exp. Ther.* 342, 619–630. doi: 10.1124/jpet.112.192138

Kann, O., Kova, R., and Kovács, R. (2007). Mitochondria and neuronal activity. *Am. J. Physiol. Cell Physiol.* 292, C641–C657. doi: 10.1152/ajpcell.00222.2006

Kellogg, C. K., and Frye, C. A. (1999). Endogenous levels of 5 alpha-reduced progestins androgens in fetal vs. adult rat brains. *Brain Res. Dev. Brain Res.* 115, 17–24. doi: 10.1016/s0165-3806(99)00041-3

Kenchappa, R. S., Diwakar, L., Annepu, J., and Ravindranath, V. (2004). Estrogen and neuroprotection: higher constitutive expression of glutaredoxin in female mice offers protection against MPTP-mediated neurodegeneration. *FASEB J.* 18, 1102–1104. doi: 10.1096/fj.03-1075fje

Kerr, J. S., Adriaanse, B. A., Greig, N. H., Mattson, M. P., Cader, M. Z., Bohr, V. A., et al. (2017). Mitophagy and Alzheimer's disease: cellular and molecular mechanisms. *Trends Neurosci.* 40, 151–166. doi: 10.1016/j.tins.2017.01.002

Khalifa, A. R. M., Abdel-Rahman, E. A., Mahmoud, A. M., Ali, M. H., Noureldin, M., Saber, S. H., et al. (2017). Sex-specific differences in mitochondria biogenesis, morphology, respiratory function, and ROS homeostasis in young mouse heart and brain. *Physiol. Rep.* 5:e13125. doi: 10.14814/phy2.13125

Klosinski, L. P., Yao, J., Yin, F., Fonteh, A. N., Harrington, M. G., Christensen, T. A., et al. (2015). White matter lipids as a ketogenic fuel supply in aging female brain: implications for Alzheimer's disease. *EBioMedicine* 2, 1888–1904. doi: 10.1016/j.ebiom.2015.11.002

Kraytsberg, Y., Kudryavtseva, E., McKee, A. C., Geula, C., Kowall, N. W., and Khrapko, K. (2006). Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. *Nat. Genet.* 38, 518–520. doi: 10.1038/ng1778

Kudin, A. P., Augustynek, B., Lehmann, A. K., Kovács, R., and Kunz, W. S. (2012). The contribution of thioredoxin-2 reductase and glutathione peroxidase to H2O2 detoxification of rat brain mitochondria. *Biochim. Biophys. Acta* 1817, 1901–1906. doi: 10.1016/j.bbabio.2012.02.023

Labandeira-Garcia, J. L., Rodriguez-Perez, A. I., Valenzuela, R., Costa-Besada, M. A., and Guerra, M. J. (2016). Menopause and Parkinson's disease. Interaction between estrogens and brain renin-angiotensin system in dopaminergic degeneration. *Front. Neuroendocrinol.* 43, 44–59. doi: 10.1016/j.yfrne.2016.09.003

Lees, A. J., Hardy, J., and Revesz, T. (2009). Parkinson's disease. *Lancet* 373, 2055–2066. doi: 10.1016/S0140-6736(09)60492-X

Lejri, I., Grimm, A., Miesch, M., Geoffroy, P., Eckert, A., and Mensah-Nyagan, A. G. (2017). Allopregnanolone and its analog BR 297 rescue neuronal cells from oxidative stress-induced death through bioenergetic improvement. *Biochim. Biophys. Acta* 1863, 631–642. doi: 10.1016/j.bbadis.2016.12.007

Lenaz, G., and Genova, M. L. (2010). Structure and organization of mitochondrial respiratory complexes: a new understanding of an old subject. *Antioxid. Redox Signal.* 12, 961–1008. doi: 10.1089/ars.2009.2704

Liu, A., Margaill, I., Zhang, S., Labombarda, F., Coqueran, B., Delespierre, B., et al. (2012). Progesterone receptors: a key for neuroprotection in experimental stroke. *Endocrinology* 153, 3747–3757. doi: 10.1210/en.2012-1138

McInerny, S. C., Brown, A. L., and Smith, D. W. (2009). Region-specific changes in mitochondrial D-loop in aged rat CNS. *Mech. Ageing Dev.* 130, 343–349. doi: 10.1016/j.mad.2009.01.008

Medeiros, D. M. (2008). Assessing mitochondria biogenesis. *Methods* 46, 288–294. doi: 10.1016/j.ymeth.2008.09.026

Meffre, D., Delespierre, B., Gouézou, M., Leclerc, P., Vinson, G. P., Schumacher, M., et al. (2005). The membrane-associated progesterone-binding protein 25-Dx is expressed in brain regions involved in water homeostasis and is up-regulated after traumatic brain injury. *J. Neurochem.* 93, 1314–1326. doi: 10.1111/j.1471-4159.2005.03127.x

Melov, S., Adlard, P. A., Morten, K., Johnson, F., Golden, T. R., Hinerfeld, D., et al. (2007). Mitochondrial oxidative stress causes hyperphosphorylation of tau. *PLoS One* 2:e536. doi: 10.1371/journal.pone.0000536

Midzak, A., Rone, M., Aghazadeh, Y., Culty, M., and Papadopoulos, V. (2011). Mitochondrial protein import and the genesis of steroidogenic mitochondria. *Mol. Cell. Endocrinol.* 336, 70–79. doi: 10.1016/j.mce.2010.12.007

Mielke, M. M., Vemuri, P., and Rocca, W. A. (2014). Clinical epidemiology of Alzheimer's disease: assessing sex and gender differences. *Clin. Epidemiol.* 6, 37–48. doi: 10.2147/CLEP.s37929

Miller, W. L. (2005). Minireview: regulation of steroidogenesis by electron transfer. *Endocrinology* 146, 2544–2550. doi: 10.1210/en.2005-0096

Miller, W. L. (2013). Steroid hormone synthesis in mitochondria. *Mol. Cell. Endocrinol.* 379, 62–73. doi: 10.1016/j.mce.2013.04.014

Mills, R. H., Romeo, H. E., Lu, J. K. H., and Micevych, P. E. (2002). Site-specific decrease of progesterone receptor mRNA expression in the hypothalamus of middle-aged persistently estrus rats. *Brain Res.* 955, 200–206. doi: 10.1016/s0006-8993(02)03440-6

Misiak, M., Beyer, C., and Arnold, S. (2010). Gender-specific role of mitochondria in the vulnerability of 6-hydroxydopamine-treated mesencephalic neurons. *Biochim. Biophys. Acta* 1797, 1178–1188. doi: 10.1016/j.bbabio.2010.04.009

Morale, M. C., Serra, P. A., L'episcopo, F., Tirolo, C., Caniglia, S., Testa, N., et al. (2006). Estrogen, neuroinflammation and neuroprotection in Parkinson's disease: glia dictates resistance versus vulnerability to neurodegeneration. *Neuroscience* 138, 869–878. doi: 10.1016/j.neuroscience.2005.07.060

Munetomo, A., Hojo, Y., Higo, S., Kato, A., Yoshida, K., Shirasawa, T., et al. (2015). Aging-induced changes in sex-steroidogenic enzymes and sex-steroid receptors in the cortex, hypothalamus and cerebellum. *J. Physiol. Sci.* 65, 253–263. doi: 10.1007/s12576-015-0363-x

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochem. J.* 417, 1–13. doi: 10.1042/BJ20081386

Naugle, M. M., Nguyen, L. T., Merceron, T. K., Filardo, E., Janssen, W. G. M., Morrison, J. H., et al. (2014). G-protein coupled estrogen receptor, estrogen receptor α and progesterone receptor immunohistochemistry in the hypothalamus of aging female rhesus macaques given long-term estradiol treatment. *J. Exp. Zool. A Ecol. Genet. Physiol.* 321, 399–414. doi: 10.1002/jez.1871

Nilsen, J., and Diaz Brinton, R. (2003). Mechanism of estrogen-mediated neuroprotection: regulation of mitochondrial calcium and Bcl-2 expression. *Proc. Natl. Acad. Sci. U S A* 100, 2842–2847. doi: 10.1073/pnas.0438041100

Nilsen, J., Irwin, R. W., Gallaher, T. K., and Brinton, R. D. (2007). Estradiol *in vivo* regulation of brain mitochondrial proteome. *J. Neurosci.* 27, 14069–14077. doi: 10.1523/JNEUROSCI.4391-07.2007

Ojaimi, J., Masters, C. L., Opeskin, K., McKelvie, P., and Byrne, E. (1999). Mitochondrial respiratory chain activity in the human brain as a function of age. *Mech. Ageing Dev.* 111, 39–47. doi: 10.1016/s0047-6374(99)00071-8

Pandya, J. D., Royland, J. E., MacPhail, R. C., Sullivan, P. G., and Kodavanti, P. R. S. (2016). Age- and brain region-specific differences in mitochondrial bioenergetics in Brown Norway rats. *Neurobiol. Aging* 42, 25–34. doi: 10.1016/j.neurobiolaging.2016.02.027

Pang, Y., Dong, J., and Thomas, P. (2013). Characterization, neurosteroid binding and brain distribution of human membrane progesterone receptors δ and ϵ (mPRδ and mPRϵ) and mPRδ involvement in neurosteroid inhibition of apoptosis. *Endocrinology* 154, 283–295. doi: 10.1210/en.2012-1772

Panov, A., Dikalov, S., Shalbuyeva, N., Hemendinger, R., Greenamyre, J. T., and Rosenfeld, J. (2007). Species- and tissue-specific relationships between mitochondrial permeability transition and generation of ROS in brain and liver mitochondria of rats and mice. *Am. J. Physiol. Cell Physiol.* 292, C708–C718. doi: 10.1152/ajpcell.00202.2006

Papadopoulos, V., Aghazadeh, Y., Fan, J., Campioli, E., Zirkin, B., and Midzak, A. (2015). Translocator protein-mediated pharmacology of cholesterol transport and steroidogenesis. *Mol. Cell. Endocrinol.* 408, 90–98. doi: 10.1016/j.mce.2015.03.014

Petrosillo, G., De Benedictis, V., Ruggiero, F. M., and Paradies, G. (2013). Decline in cytochrome c oxidase activity in rat-brain mitochondria with aging. Role of peroxidized cardiolipin and beneficial effect of melatonin. *J. Bioenerg. Biomembr.* 45, 431–440. doi: 10.1007/s10863-013-9505-0

Petrosillo, G., Matera, M., Casanova, G., Ruggiero, F. M., and Paradies, G. (2008). Mitochondrial dysfunction in rat brain with aging: involvement of complex I, reactive oxygen species and cardiolipin. *Neurochem. Int.* 53, 126–131. doi: 10.1016/j.neuint.2008.07.001

Poon, H. F., Vaishnav, R. A., Getchell, T. V., Getchell, M. L., and Butterfield, D. A. (2006). Quantitative proteomics analysis of differential protein expression and oxidative modification of specific proteins in the brains of old mice. *Neurobiol. Aging* 27, 1010–1019. doi: 10.1016/j.neurobiolaging.2005.05.006

Porcu, P., and Morrow, A. L. (2014). Divergent neuroactive steroid responses to stress and ethanol in rat and mouse strains: relevance for human studies. *Psychopharmacology (Berl)* 231, 3257–3272. doi: 10.1007/s00213-014-3564-8

Puisac, B., Ramos, M., Arnedo, M., Menao, S., Gil-Rodríguez, M. C., Teresa-Rodrigo, M. E., et al. (2012). Characterization of splice variants of the genes encoding human mitochondrial HMG-CoA lyase and HMG-CoA synthase, the main enzymes of the ketogenesis pathway. *Mol. Biol. Rep.* 39, 4777–4785. doi: 10.1007/s11033-011-1270-8

Razmara, A., Sunday, L., Stirone, C., Wang, X. B., Krause, D. N., Duckles, S. P., et al. (2008). Mitochondrial effects of estrogen are mediated by estrogen receptor α in brain endothelial cells. *J. Pharmacol. Exp. Ther.* 325, 782–790. doi: 10.1124/jpet.107.134072

Rettberg, J. R., Yao, J., and Brinton, R. D. (2014). Estrogen: a master regulator of bioenergetic systems in the brain and body. *Front. Neuroendocrinol.* 35, 8–30. doi: 10.1016/j.yfrne.2013.08.001

Riar, A. K., Burstein, S. R., Palomo, G. M., Arreguin, A., Manfredi, G., and Germain, D. (2017). Sex specific activation of the ERα axis of the mitochondrial UPR (UPRmt) in the G93A-SOD1 mouse model of familial ALS. *Hum. Mol. Genet.* 26, 1318–1327. doi: 10.1093/hmg/ddx049

Ribas, V., García-Ruiz, C., and Fernández-Checa, J. C. (2014). Glutathione and mitochondria. *Front. Pharmacol.* 5:151. doi: 10.3389/fphar.2014.00151

Robertson, C. L., Puskar, A., Hoffman, G. E., Murphy, A. Z., Saraswati, M., and Fiskum, G. (2006). Physiologic progesterone reduces mitochondrial dysfunction and hippocampal cell loss after traumatic brain injury in female rats. *Exp. Neurol.* 197, 235–243. doi: 10.1016/j.expneurol.2005.09.014

Robertson, C. L., and Saraswati, M. (2015). Progesterone protects mitochondrial function in a rat model of pediatric traumatic brain injury. *J. Bioenerg. Biomembr.* 47, 43–51. doi: 10.1007/s10863-014-9585-5

Rocca, W. A., Bower, J. H., Maraganore, D. M., Ahlskog, J. E., Grossardt, B. R., de Andrade, M., et al. (2008). Increased risk of parkinsonism in women who underwent oophorectomy before menopause. *Neurology* 70, 200–209. doi: 10.1212/01.wnl.0000280573.30975.6a

Rosario, E. R., Carroll, J., and Pike, C. J. (2010). Testosterone regulation of Alzheimer-like neuropathology in male 3xTg-AD mice involves both estrogen androgen pathways. *Brain Res.* 1359, 281–290. doi: 10.1016/j.brainres.2010.08.068

Rosario, E., Chang, L., Head, E., Stanczyk, F., and Pike, C. (2011). Brain levels of sex steriod hormones in men and women during normal aging and Alzheimer's disease. *Neurobiol. Aging* 32, 604–613. doi: 10.1016/j.neurobiolaging.2009.04.008

Rossetti, M. F., Varayoud, J., Moreno-Piovano, G. S., Luque, E. H., and Ramos, J. G. (2015). Environmental enrichment attenuates the age-related decline in the mRNA expression of steroidogenic enzymes and reduces the methylation state of the steroid 5α-reductase type 1 gene in the rat hippocampus. *Mol. Cell. Endocrinol.* 412, 330–338. doi: 10.1016/j.mce.2015.05.024

Samalecos, A., and Gellersen, B. (2008). Systematic expression analysis and antibody screening do not support the existence of naturally occurring progesterone receptor (PR)-C, PR-M, or other truncated PR isoforms. *Endocrinology* 149, 5872–5887. doi: 10.1210/en.2008-0602

Sanz, A., Hiona, A., Kujoth, G. C., Seo, A. Y., Hofer, T., Kouwenhoven, E., et al. (2007). Evaluation of sex differences on mitochondrial bioenergetics and apoptosis in mice. *Exp. Gerontol.* 42, 173–182. doi: 10.1016/j.exger.2006.10.003

Sayeed, I., Parvez, S., Wali, B., Siemen, D., and Stein, D. G. (2009). Direct inhibition of the mitochondrial permeability transition pore: a possible mechanism for better neuroprotective effects of allopregnanolone over progesterone. *Brain Res.* 1263, 165–173. doi: 10.1016/j.brainres.2009.01.045

Schumacher, M., Weill-Engerer, S., Liere, P., Robert, F., Franklin, R. J. M., Garcia-Segura, L. M., et al. (2003). Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. *Prog. Neurobiol.* 71, 3–29. doi: 10.1016/j.pneurobio.2003.09.004

Selvaraj, V., and Stocco, D. M. (2015). The changing landscape in translocator protein (TSPO) function. *Trends Endocrinol. Metab.* 26, 341–348. doi: 10.1016/j.tem.2015.02.007

Sharma, P. K., and Thakur, M. K. (2006). Expression of estrogen receptor (ER) α and β in mouse cerebral cortex: effect of age, sex and gonadal steroids. *Neurobiol. Aging* 27, 880–887. doi: 10.1016/j.neurobiolaging.2005.04.003

Shi, L., Du, X., Zhou, H., Tao, C., Liu, Y., Meng, F., et al. (2014). Cumulative effects of the ApoE genotype and gender on the synaptic proteome and oxidative stress in the mouse brain. *Int. J. Neuropsychopharmacol.* 17, 1863–1879. doi: 10.1017/s1461145714000601

Sims, N. R., Finegan, J. M., Blass, J. P., Bowen, D. M., and Neary, D. (1987). Mitochondrial function in brain tissue in primary degenerative dementia. *Brain Res.* 436, 30–38. doi: 10.1016/0006-8993(87)91553-8

Stauch, K. L., Purnell, P. R., Villeneuve, L. M., and Fox, H. S. (2015). Proteomic analysis and functional characterization of mouse brain mitochondria during aging reveal alterations in energy metabolism. *Proteomics* 15, 1574–1586. doi: 10.1002/pmic.201400277

Stekovic, S., Ruckenstuhl, C., Royer, P. P., Winkler-Hermaden, C., Carmona-Gutierrez, D., Fröhlich, K.-U., et al. (2017). The neuroprotective steroid progesterone promotes mitochondrial uncoupling, reduces cytosolic calcium and augments stress resistance in yeast cells. *Microb. Cell* 4, 191–199. doi: 10.15698/mic2017.06.577

Stuart, J. A., Maddalena, L. A., Merilovich, M., and Robb, E. L. (2014). A midlife crisis for the mitochondrial free radical theory of aging. *Longev. Healthspan* 3:4. doi: 10.1186/2046-2395-3-4

Sundar Boyalla, S., Barbara Victor, M., Roemgens, A., Beyer, C., and Arnold, S. (2011). Sex- and brain region-specific role of cytochrome c oxidase in 1-methyl-4-phenylpyridinium-mediated astrocyte vulnerability. *J. Neurosci. Res.* 89, 2068–2082. doi: 10.1002/jnr.22669

Swerdlow, R. H., Burns, J. M., and Khan, S. M. (2014). The Alzheimer's disease mitochondrial cascade hypothesis: progress and perspectives. *Biochim. Biophys. Acta* 1842, 1219–1231. doi: 10.1016/j.bbadis.2013.09.010

Swerdlow, R. H., and Khan, S. M. (2004). A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease. *Med. Hypotheses* 63, 8–20. doi: 10.1016/j.mehy.2003.12.045

Thornton, V., Warden, D., Talbot, C., Mastana, S. S., Bandelow, S., and Hogervorst, E. (2011). Modification of estrogen's association with Alzheimer's disease risk by genetic polymorphisms. *Brain Res.* 1379, 213–223. doi: 10.1016/j.brainres.2010.12.074

Tretter, L., and Adam-Vizi, V. (2005). Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 360, 2335–2345. doi: 10.1098/rstb.2005.1764

Turturro, A., Witt, W. W., Lewis, S., Hass, B. S., Lipman, R. D., and Hart, R. W. (1999). Growth curves and survival characteristics of the animals used in the biomarkers of aging program. *J. Gerontol. A Biol. Sci. Med. Sci.* 54, B492–B501. doi: 10.1093/gerona/54.11.b492

Velarde, M. C. (2014). Mitochondrial and sex steroid hormone crosstalk during aging. *Longev. Healthspan* 3:2. doi: 10.1186/2046-2395-3-2

Venkateshappa, C., Harish, G., Mahadevan, A., Srinivas Bharath, M. M., and Shankar, S. K. (2012). Elevated oxidative stress and decreased antioxidant function in the human hippocampus and frontal cortex with increasing age: implications for neurodegeneration in Alzheimer's disease. *Neurochem. Res.* 37, 1601–1614. doi: 10.1007/s11064-012-0755-8

Ventura-Clapier, R., Moulin, M., Piquereau, J., Lemaire, C., Mericskay, M., Veksler, V., et al. (2017). Mitochondria: a central target for sex differences in pathologies. *Clin. Sci. (Lond)* 131, 803–822. doi: 10.1042/cs20160485

Viña, J., Lloret, A., Vallés, S. L., Borrás, C., Badía, M.-C., Pallardó, F. V., et al. (2007). Effect of gender on mitochondrial toxicity of Alzheimer's Aβ peptide. *Antioxid. Redox Signal.* 9, 1677–1690. doi: 10.1089/ars.2007.1773

Wang, Y., and Brinton, R. D. (2016). Triad of risk for late onset alzheimer's: mitochondrial haplotype, apoe genotype and chromosomal sex. *Front. Aging Neurosci.* 8:232. doi: 10.3389/fnagi.2016.00232

Wang, X., Su, B., Siedlak, S. L., Moreira, P. I., Fujioka, H., Wang, Y., et al. (2008). Amyloid-β overproduction causes abnormal mitochondrial dynamics via differential modulation of mitochondrial fission/fusion proteins. *Proc. Natl. Acad. Sci. U S A* 105, 19318–19323. doi: 10.1073/pnas.0804871105

Webster, K. M., Wright, D. K., Sun, M., Semple, B. D., Ozturk, E., Stein, D. G., et al. (2015). Progesterone treatment reduces neuroinflammation, oxidative stress and brain damage and improves long-term outcomes in a rat model of repeated mild traumatic brain injury. *J. Neuroinflammation* 12:238. doi: 10.1186/s12974-015-0457-7

Weill-Engerer, S., David, J.-P., Sazdovitch, V., Liere, P., Eychenne, B., Pianos, A., et al. (2002). Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. *J. Clin. Endocrinol. Metab.* 87, 5138–5143. doi: 10.1210/jc.2002-020878

Wüllner, U., Seyfried, J., Groscurth, P., Beinroth, S., Winter, S., Gleichmann, M., et al. (1999). Glutathione depletion and neuronal cell death: the role of reactive oxygen intermediates and mitochondrial function. *Brain Res.* 826, 53–62. doi: 10.1016/s0006-8993(99)01228-7

Xu, J., Zeng, C., Chu, W., Pan, F., Rothfuss, J. M., Zhang, F., et al. (2011). Identification of the PGRMC1 protein complex as the putative sigma-2 receptor binding site. *Nat. Commun.* 2:380. doi: 10.1038/ncomms1386

Yang, S.-H., Liu, R., Perez, E. J., Wen, Y., Stevens, S. M., Valencia, T., et al. (2004). Mitochondrial localization of estrogen receptor β. *Proc. Natl. Acad. Sci. U S A* 101, 4130–4135. doi: 10.1073/pnas.0306948101

Yao, J., and Brinton, R. D. (2012). Estrogen regulation of mitochondrial bioenergetics: implications for prevention of Alzheimer's disease. *Adv. Pharmacol.* 64, 327–371. doi: 10.1016/b978-0-12-394816-8.00010-6

Yao, J., Chen, S., Cadenas, E., and Brinton, R. D. (2011). Estrogen protection against mitochondrial toxin-induced cell death in hippocampal neurons: antagonism by progesterone. *Brain Res.* 1379, 2–10. doi: 10.1016/j.brainres.2010.11.090

Yao, J., Hamilton, R. T., Cadenas, E., and Brinton, R. D. (2010). Decline in mitochondrial bioenergetics and shift to ketogenic profile in brain during reproductive senescence. *Biochim. Biophys. Acta* 1800, 1121–1126. doi: 10.1016/j.bbagen.2010.06.002

Yao, J., Irwin, R., Chen, S., Hamilton, R., Cadenas, E., and Brinton, R. D. (2012). Ovarian hormone loss induces bioenergetic deficits and mitochondrial β-amyloid. *Neurobiol. Aging* 33, 1507–1521. doi: 10.1016/j.neurobiolaging.2011.03.001

Yao, J., Irwin, R. W., Zhao, L., Nilsen, J., Hamilton, R. T., and Brinton, R. D. (2009). Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U S A* 106, 14670–14675. doi: 10.1073/pnas.0903563106

Yin, W., and Gore, A. C. (2006). Neuroendocrine control of reproductive aging: roles of GnRH neurons. *Reproduction* 131, 403–414. doi: 10.1530/rep.1.00617

Yousuf, S., Atif, F., Sayeed, I., Wang, J., and Stein, D. G. (2016). Neuroprotection by progesterone after transient cerebral ischemia in stroke-prone spontaneously hypertensive rats. *Horm. Behav.* 84, 29–40. doi: 10.1016/j.yhbeh.2016.06.002

Yuan, M., Wen-Xia, Z., Jun-Ping, C., and Yong-Xiang, Z. (2005). Age-related changes in the oestrous cycle and reproductive hormones in senescence-accelerated mouse. *Reprod. Fertil. Dev.* 17, 507–512. doi: 10.1071/RD04099

Zawada, I., Masternak, M. M., List, E. O., Stout, M. B., Berryman, D. E., Lewinski, A., et al. (2015). Gene expression of key regulators of mitochondrial biogenesis is sex dependent in mice with growth hormone receptor deletion in liver. *Aging (Albany NY)* 7, 195–204. doi: 10.18632/aging.100733

Zhang, J.-Q., Cai, W.-Q., Zhou, D. S., and Su, B.-Y. (2002). Distribution and differences of estrogen receptor beta immunoreactivity in the brain of adult male and female rats. *Brain Res.* 935, 73–80. doi: 10.1016/s0006-8993(02)02460-5

Zheng, B., Liao, Z., Locascio, J. J., Lesniak, K. A., Roderick, S. S., Watt, M. L., et al. (2010). PGC-1α, a potential therapeutic target for early intervention in Parkinson's disease. *Sci. Transl. Med.* 2:52ra73. doi: 10.1126/scitranslmed.3001059

Zorzano, A., and Claret, M. (2015). Implications of mitochondrial dynamics on neurodegeneration and on hypothalamic dysfunction. *Front. Aging Neurosci.* 7:101. doi: 10.3389/fnagi.2015.00101

Zucker, I., and Beery, A. K. (2010). Males still dominate animal studies. *Nature* 465:690. doi: 10.1038/465690a

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

REVIEW
published: 22 December 2017
doi: 10.3389/fnagi.2017.00430

Role of Estrogen and Other Sex Hormones in Brain Aging. Neuroprotection and DNA Repair

Sandra Zárate[1,2], *Tinna Stevnsner*[3] *and Ricardo Gredilla*[4]*

[1]*Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina,* [2]*Departamento de Histología, Embriología, Biología Celular y Genética, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina,* [3]*Danish Center for Molecular Gerontology and Danish Aging Research Center, Department of Molecular Biology and Genetics, University of Aarhus, Aarhus, Denmark,* [4]*Department of Physiology, Faculty of Medicine, Complutense University, Madrid, Spain*

Aging is an inevitable biological process characterized by a progressive decline in physiological function and increased susceptibility to disease. The detrimental effects of aging are observed in all tissues, the brain being the most important one due to its main role in the homeostasis of the organism. As our knowledge about the underlying mechanisms of brain aging increases, potential approaches to preserve brain function rise significantly. Accumulating evidence suggests that loss of genomic maintenance may contribute to aging, especially in the central nervous system (CNS) owing to its low DNA repair capacity. Sex hormones, particularly estrogens, possess potent antioxidant properties and play important roles in maintaining normal reproductive and non-reproductive functions. They exert neuroprotective actions and their loss during aging and natural or surgical menopause is associated with mitochondrial dysfunction, neuroinflammation, synaptic decline, cognitive impairment and increased risk of age-related disorders. Moreover, loss of sex hormones has been suggested to promote an accelerated aging phenotype eventually leading to the development of brain hypometabolism, a feature often observed in menopausal women and prodromal Alzheimer's disease (AD). Although data on the relation between sex hormones and DNA repair mechanisms in the brain is still limited, various investigations have linked sex hormone levels with different DNA repair enzymes. Here, we review estrogen anti-aging and neuroprotective mechanisms, which are currently an area of intense study, together with the effect they may have on the DNA repair capacity in the brain.

Keywords: brain aging, neuroprotection, sex hormones, estrogen, DNA repair, mitochondria

OPEN ACCESS

Edited by:
Isabel Varela-Nieto, Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
Christian J. Pike, University of Southern California, United States
Jose L. Labandeira-Garcia, Universidade de Santiago de Compostela, Spain

***Correspondence:**
Ricardo Gredilla
gredilla@ucm.es

Received: *19 October 2017*
Accepted: *14 December 2017*
Published: *22 December 2017*

Citation:
Zárate S, Stevnsner T and Gredilla R (2017) Role of Estrogen and Other Sex Hormones in Brain Aging. Neuroprotection and DNA Repair. Front. Aging Neurosci. 9:430. doi: 10.3389/fnagi.2017.00430

INTRODUCTION

The world's population is aging. Life expectancy at birth has increased by 6 years worldwide in the last 3 decades, and in 2050 the proportion of old adults over 60 years is estimated to reach 22% worldwide. Such an increase has a direct consequence: a rise in the incidence of age-related diseases, in particular neurodegeneration. According to different organizations like the World Health Organization (WHO), neurodegenerative disorders are, together with cardiovascular diseases, the main causes of death in western countries. More than 20% of adults aged 60 and over develop neurological disorders, dementia being the most common one. WHO estimates that around 47 million people suffer from this disorder, with nearly 10 million new cases every year.

Thus, it is estimated that the total number of people with dementia will increase to near 75 million in 2030 and 132 million by 2050 worldwide. Alzheimer's disease (AD) is the most common cause of dementia, contributing to 60%–70% of cases. Although a relationship between the development of cognitive impairment and life-style-related risk factors, such as obesity, tobacco and alcohol use has been reported, age is still the strongest risk factor for dementia and other neurodegenerative disorders (World Health Statistics, 2017).

Despite aging globally affects both male and female animals from all species, including humans, various studies have stressed that sex differences exist. Thus, females have longer life expectancies than males in mammals, and that is also the case for women vs. men in most developed countries (Promislow, 1991; Schroots et al., 1999). Since such higher life expectancy is not exclusive for humans, it cannot be attributed only to socioeconomical factors but rather reflects specific biological characteristics of both sexes. Similarly, the incidence of various age-related neurodegenerative diseases shows a sex-dependency. Thus, Parkinson's disease (PD) has a higher prevalence and earlier onset in males compared to females (Gillies et al., 2014). Likewise, men over the age of 40 have smaller volumes in different brain areas, including the hippocampus (Coffey et al., 1998; Jack et al., 2015) and worse memory performance than age-matched women (Jack et al., 2015). On the other hand, post-menopausal women have a higher prevalence of AD than men, as well as a faster cognition decline after disease onset (Li and Singh, 2014; Zagni et al., 2016). The factors promoting the longer lifespan in women vs. men as well as sex dimorphisms in brain aging and neurodegenerative diseases are not fully understood. The positive effects of estrogen on different cellular processes, such as reactive oxygen species (ROS) production and antioxidant defense, cardiovascular protection, immune competence and telomere maintenance have been well recognized to account, at least in part, for women's longer lifespan (Regan and Partridge, 2013; Austad and Fischer, 2016). However, it may seem paradoxical that women still live longer than men long after hormonal loss due to menopause and consequently long after having benefited from the protective effects of estrogens. Interestingly, a positive correlation between later age at menopause or longer reproductive lifespan with longevity has been recently reported (Shadyab et al., 2017). That suggests that lifetime cumulative exposure to estrogenic stimulation throughout fertile life could exert long-lasting effects and account for such sex dimorphism in longevity. This exposure would also affect brain aging and neurodegenerative processes. Altogether, the above mentioned sex differences suggest that taking sex into account as a biological variable might be critical when approaching therapies to treat neurodenegerative diseases or to delay brain aging (Young and Pfaff, 2014; May, 2016).

Neuroprotective Effects of Sex Hormones

As mentioned above, one factor that is believed to play an important role in the sex differences observed in brain aging and neurodegeneration is sex hormone levels, in particular estrogen. It is well known that estrogen receptors (ERs) are widely distributed in the brain (Hara et al., 2015), having important regulatory function on different processes such as cognition, anxiety, body temperature, feeding and sexual behavior (Do Rego et al., 2009). The neuroprotective effect of estrogen has been stressed by several investigations. Epidemiological studies suggest that late symptom onset of PD in women may be related to such neuroprotective effect (Saunders-Pullman et al., 1999; Rocca et al., 2008; Kowal et al., 2013). It has also been suggested that the reduced concentration of sex steroid hormones after menopause may be responsible for the higher prevalence and greater severity of AD in women than men (Tang et al., 1996; Brann et al., 2007; Li and Singh, 2014). Moreover, in support of the neuroprotective effect of sex steroids, hormone replacement therapy (HRT) has been shown to have beneficial effects on different animal models (Borrás et al., 2003; Ding et al., 2013; Lu et al., 2018). Clinical data also suggest that HRT may have an effect in humans on neurodegenerative diseases such as AD (Li and Singh, 2014), PD and other age-related brain diseases like stroke (Brann et al., 2007), although concerns about its use have arisen as further discussed below.

Different animal models have been used in order to analyze how sex hormones affect neurodegenerative processes. Inhibition of aromatase, the enzyme that catalyzes the nonreversible conversion of aromatizable androgens into estrogens, results in lower estrogen levels. This inhibition is associated with altered beta amyloid deposition and more severe strokes in AD mouse models (McCullough et al., 2003; Overk et al., 2012). Similar results have been observed with aromatase knock out (KO) mice (Yue et al., 2005). Different animal models have been used for investigating the role of sex steroids in neurodegeneration and aging, being gonadectomy one of the most used. This is a standard practice in rodent hormone replacement studies, allowing investigators more control over sex hormone levels in circulation in order to study testosterone, estradiol and/or progesterone specific actions and their interaction at different levels (Frick, 2009). Moreover, ovariectomy (OVX) in female animals, especially in rodents, is a broadly used method to model women's menopause. Like primates, rodents have reproductive cycles, which start to become irregular in middle-aged animals, i.e., 9–12 months of age in rats. However, and unlike primates, rats do not experience complete ovarian follicular loss at middle age, having chronically high circulating estradiol levels. For this reason, many laboratories often turn to the OVX rat model. Thus, using age-appropriate rats at young, middle, and old ages, surgical, peri- and natural menopause can be modeled (Morrison et al., 2006).

Several studies have shown that decreasing estrogen levels by OVX enhances neurodegenerative processes by increasing brain damage (Borrás et al., 2003; Overk et al., 2012; Yao et al., 2012; Ding et al., 2013; Kireev et al., 2014). Furthermore, restoration of estradiol levels in OVX animals by hormonal therapy reverses brain damage (Yao et al., 2012; Ding et al., 2013; Kireev et al., 2014; Lu et al., 2018). Numerous preclinical and epidemiological studies as well as some clinical trials have supported beneficial effects of HRT on memory and cognition

and reduced risk for AD. Yet, an initial evaluation of results from the Women's Health Initiative (WHI), the largest clinical trial involving postmenopausal women to date, suggested the contrary (Rettberg et al., 2014). However, the study population in the WHI considerably differed from women in previous observational studies regarding age or years after menopause onset at the time treatment was initiated. Later sub-analysis of data from the WHI trial as well as other epidemiological studies have provided evidence supporting the concept that there is a limited period of time around menopause during which HRT can exert positive effects on brain function. The "window of opportunity" theory suggests that beneficial effects of estrogens can only be achieved on a healthy brain; i.e., if HRT starts before or at the time of menopause. On the contrary, if HRT is initiated some years after menopause onset, it has detrimental effects on brain function and can even increase the risk of developing AD (Morrison et al., 2006; Brinton, 2008, 2009; Scott et al., 2012; Rettberg et al., 2014; Miller and Harman, 2017). Moreover, it has been suggested that effectiveness of HRT is likely to be dependent on additional factors, such as formulations of treatments regarding type (conjugated equine estrogen vs. 17β- estradiol, medroxyprogesterone acetate vs. progesterone), mode of delivery (oral vs. transdermal) and regimen (continuous vs. cyclic) (Miller and Harman, 2017).

Apart from being a target for sex hormones, the brain is now recognized as a steroidogenic organ, and the degree to what neurosteroidogenesis, i.e., the synthesis on neuroactive steroids in the brain, affects its function has recently started to be unraveled. Neuroactive steroids are detectable in the brain after the removal of peripheral steroidogenic organs and their levels are affected by modifications in the levels of peripheral neuroactive steroids in a region-, time after gonadectomy- and sex-manner, suggesting a compensatory mechanism in the brain to counteract the effects of peripheral hormone loss (Caruso et al., 2010; Sorwell et al., 2012; Arevalo et al., 2015). Similarly to peripheral sex steroids, neurosteroids have also been described to exert neuroprotective effects. Moreover, it has been suggested that endogenously synthesized neurosteroids may reinforce the protective effects of exogenously administered steroids (Chamniansawat and Chongthammakun, 2012; Li et al., 2013). In fact, the level of brain estrogen has been shown to determine the effect of estrogen therapy-induced protection against AD pathology in mice (Li et al., 2013). Neurosteroids are not only synthesized de novo from cholesterol. They can also be produced through transformation of blood peripheral steroid hormones into derivatives with potent neuromodulatory actions. Consequently, although neurosteroidogenesis is regulated independently of peripheral steroidogenesis, it is expected that fluctuations in plasma steroid levels affect the rate of brain steroid synthesis (Veiga et al., 2004).

Although they have received less attention than estrogens on brain function, androgens and progesterone exert neuroprotective actions as well. Testosterone and its metabolites have been described to be neuroprotective under conditions of glucose deprivation both in hippocampal neurons (Ishihara et al., 2016) and astroglial cells (Toro-Urrego et al., 2016). Similarly, testosterone has been shown to prevent dendritic atrophy in motoneurons after induced death of the surrounding neurons (Cai et al., 2017). Moreover, under chronic stress conditions, depletion of testosterone has been shown to increase susceptibility to oxidative damage in different brain areas (Son et al., 2016). Androgens have been suggested to have a positive impact on cognition (Colciago et al., 2015). This effect is likely to be mediated by testosterone and its metabolites in the hippocampus, where a high concentration of androgen receptors has been shown in hippocampal CA1 pyramidal cells (Colciago et al., 2015). Orchidectomy studies have described that testosterone replacement increases spine density in the hippocampus and at the same time improves spatial memory in rats (Jacome et al., 2016). Likewise, dihydrotestosterone (DHT) treatment also restores hippocampal spine density (Maclusky et al., 2006). Moreover, the proportion of immature dendritic spines increase after orchidectomy, suggesting that testosterone not only affects the number of synaptic spines, but also their maturation state (Li M. et al., 2012). The effects of testosterone on spine density and maturation are likely related to the brain-derived neurotrophic factor (BDNF) expression (Gao et al., 2009; Li M. et al., 2012).

Progesterone is another major sex hormone, whose best-characterized function is reproduction regulation. Progesterone receptors are broadly expressed in the brain, and they have been described to be present in all neural cell types (Brinton et al., 2008). Together with its metabolites, progesterone exerts several physiological functions in the brain. It regulates neuronal development of Purkinje cells in cerebellum (Tsutsui et al., 2011), differentiation and proliferation of oligodendrocytes (Ghoumari et al., 2005), and synaptogenesis and neuronal plasticity (Rossetti et al., 2016) among others. On the other hand, progesterone and its metabolites have been described to exert beneficial effects in various animal models of neurodegeneration and brain damage, including PD (Bourque et al., 2009) stroke (Singh and Su, 2013) traumatic brain injury and demyelination (De Nicola et al., 2009; El-Etr et al., 2015). These neuroprotective effects involve different mechanisms of action. Among them, progesterone and its metabolites activate the MAPK/ERK and PI3K/Akt pathways (Kaur et al., 2007; Baudry et al., 2013), which are well-established survival signaling pathways. In addition, progesterone also exerts neurotrophic actions regulating the expression of neurotrophins such as BDNF (Melcangi et al., 2014). Besides, progesterone modulates neuroinflammation. The anti-inflammatory effect of progesterone has been extensively investigated in an experimental multiple sclerosis murine model: autoimmune experimental encephalomyelitis (EAE). Since progesterone and its derivates promote myelin formation in the peripheral nervous system, the role that they might play in demyelinating diseases has received attention for many years. In EAE mice, progesterone itself and one of its reduced metabolites, allopregnanolone, have been shown to reduce inflammatory markers, to inhibit microglia activation and to avoid the penetration of circulating lymphocytes and macrophages in the central nervous system (CNS) (De Nicola et al., 2013, 2017; Noorbakhsh et al., 2014). This reduction in neuroinflammatory processes would mediate, at least in part,

the beneficial effects of progesterone in EAE mice, which show reduced clinical severity, lower demyelination and improved neuronal function after progesterone treatment (Garay et al., 2007, 2009).

Remarkably, the effect of progesterone when delivered in combination with estrogen is not always positive. It is well known that progesterone regulates estrogen actions, particularly at the reproductive level (Graham and Clarke, 1997). At the CNS level, various studies have suggested that when both hormones are administered together, progesterone often antagonizes rather than synergizes estrogen effects (Bimonte-Nelson et al., 2004, 2006; Rosario et al., 2006; Carroll et al., 2008; Yao et al., 2011). Although the precise mechanism behind the antagonistic effect of progesterone on estrogen actions is poorly understood, some studies have suggested that it might be mediated, at least in part, by the regulation of ER expression (Pike et al., 2009).

Sex Hormones: Mechanisms of Action in the Brain

Similar to other steroid hormones, sex steroids exert their multiple actions through binding to nuclear receptors that act as ligand-dependent transcription factors to regulate the expression of target genes (McDevitt et al., 2008). As for estrogens, two classical receptors have been described, ERα and ERβ. However, in recent years, the existence of membrane associated ER and other proteins unrelated to ERs that also trigger estrogen responses, like membrane G protein-coupled ER 1 (GPER1), has become evident (Nilsson et al., 2001; Zárate and Seilicovich, 2010; Rettberg et al., 2014). This provides an additional layer of complexity to the understanding of how sex hormones affect higher cognitive functions and other neural processes including mood, cardiovascular regulation, fine motor skills and neuroprotection (Dumitriu et al., 2010; Hara et al., 2015). Androgen and progestin receptors have also revealed both nuclear and non-nuclear forms and locations of classical and non-classical receptors (McLachlan et al., 1991; Brinton et al., 2008; Sarkey et al., 2008; Petersen et al., 2013; Li et al., 2015).

Apart from being highly expressed in regions related to reproductive behavior and neuroendocrine function like the hypothalamus, sex hormone receptors are widely distributed in the brain and are present both in nuclear and non-nuclear compartments, including mitochondria (Kelly and Levin, 2001; Boulware et al., 2007; Psarra and Sekeris, 2008; Simpkins et al., 2008; Rettberg et al., 2014; McEwen and Milner, 2017). In accordance with the complexity of the brain, sex hormones modulate not only neuronal function; they exert their actions on different cellular targets, modulating an important number of physiological processes. Thus, estrogen and progesterone have been described to regulate proliferation and maturation of oligodendrocytes (Marin-Husstege et al., 2004; Ghoumari et al., 2005) as well as local inflammation processes mediated by astrocytes and microglia (Arevalo et al., 2010). Studies in different animal models of brain injury have described that sex hormones also have an important effect in the cerebral vasculature. ERs have been localized in endothelial as well as in smooth muscle cells (Stirone et al., 2003). During stroke, estrogens exert a protective action due to theirs effect on the cerebral vasculature, increasing endothelial nitric oxide synthase activity, suppressing inflammatory markers like COX-2, and reducing leukocyte adhesion (Suzuki et al., 2009). Similarly, in a rat model of traumatic brain injury, progesterone has been described to promote angiogenic activity of endothelial progenitor cells (Yu et al., 2017).

Estrogens have an important effect on mitochondrial function as well (Klinge, 2017). Although the mechanisms by which estrogen regulates mitochondrial function are not totally understood, both direct and indirect actions have been described to contribute to such regulation. Estrogen's beneficial effects in mitochondria are especially important in those tissues that have high demand of energy like the CNS. Along with metabolism regulation (Brinton, 2008; Klinge, 2017), estrogen exerts different actions involving mitochondrial function in neuronal tissues, including biogenesis (Kemper et al., 2014), apoptosis processes (Garcia-Segura et al., 1998; Nilsen and Diaz Brinton, 2003; Mo et al., 2013) and even morphology (Arnold et al., 2008; Hara et al., 2014). Many estrogen actions in mitochondria are mediated by the presence of ERs in these organelles, which seems to be cell-type specific. In relation to the brain, mitochondrial ERs have been suggested to be present in primary cultured rat neurons, murine hippocampal cell lines (Yang et al., 2004), neurons and glia of rat hippocampus (Milner et al., 2005; Herrick et al., 2006) and also in pre- and post-synaptic mitochondria of hippocampal neurons (Milner et al., 2008). The presence of ERs within mitochondria suggests that estrogen might modulate mitochondrial function by directly affecting transcription of mitochondrial DNA (mtDNA). In fact, mitochondrial ERβ has been described to bind to estrogen response element-like sequences in mtDNA (Demonacos et al., 1996; Chen et al., 2004). However, estrogen actions on mitochondria are not exclusively related to such mechanism. Estrogen also regulates mitochondrial functions through their classical nuclear mechanism, i.e., transcriptional regulation of nuclear-encoded mitochondrial proteins. It is known that estrogen regulates the nuclear transcription of different proteins affecting mitochondrial function such as nuclear respiratory factor-1 (NRF-1) and peroxisome proliferator-activated receptor-gamma coactivator 1 (PCG-1) (Kemper et al., 2013; Klinge, 2017). Hence, this regulation is critical for the activation of nuclear genes encoding proteins involved in mitochondrial biogenesis as well as in the mitochondrial electron transport chain complexes (Scarpulla, 2008; Klinge, 2017). It also regulates the transcription of mitochondrial transcription factor A (TFAM), which translocates into mitochondria and initiates transcription and replication of mtDNA (Virbasius and Scarpulla, 1994; Kang et al., 2007).

Effects of estrogen in mitochondria might be especially relevant in the brain since the accumulation of mtDNA mutations and the related mitochondrial dysfunction have been suggested to play a critical role in the process of brain aging and in the onset of neurological disorders (Barja, 2004; Cantuti-Castelvetri et al., 2005; Kujoth et al., 2007). Accordingly, increased levels of oxidative modifications and mutations in

mtDNA occur in the brain during normal aging (Melov, 2004; Beal, 2005; Vermulst et al., 2007), with enhancement of these levels in neurodegenerative diseases such as AD and PD (Gabbita et al., 1998; Sanders et al., 2014). One of the main factors contributing to mtDNA instability, both during brain aging and in neurodegenerative diseases, is the decline in mtDNA repair capacity (Imam et al., 2006; Weissman et al., 2007a; Gredilla, 2010; Gredilla et al., 2012). Different DNA repair pathways have been described both in the nucleus and mitochondria (Gredilla et al., 2010a; Jeppesen et al., 2011). The major ones are base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER) and double-strand break repair. The main pathway taking place in mitochondria is BER, which repairs mtDNA modifications caused by alkylation, deamination and oxidation. A brief description of these repair pathways and the effect of sex-hormones on them will be described later in this review.

SEX HORMONES AND BRAIN AGING

Aging is an inevitable physiological process orchestrated by a plethora of molecular mechanisms that interact to alter body homeostasis, eventually leading to organismal functional decline and disease. While for many years the brain was not considered to be a sex-hormone-responsive organ, except the hypothalamus for reproductive function regulation, it is now well accepted that the entire brain is both a target and a source of sex hormones (Acaz-Fonseca et al., 2016; McEwen and Milner, 2017). Sex hormones exert numerous protective and antioxidant actions in the adult brain increasing neural function and resilience and promoting neuronal survival. As the organism age, a relatively rapid loss of ovarian hormones in the female after menopause, and a gradual but indeed significant decline of testosterone in men occur. Thus, it is not surprising that reproductive senescence both in males and females has a negative impact on neural function and represents a significant age-associated risk factor for neurodegenerative diseases, such as AD (Barron and Pike, 2012).

Sex Hormones and Synaptic Plasticity during Aging

The key role of estrogens and ERs in the synaptic basis of cognitive functions mediated by the hippocampus and prefrontal cortex (PFC) is well recognized (Dumitriu et al., 2010; Hara et al., 2015). Both ERα and ERβ are localized in synaptic terminals and dendritic spines, dendritic shafts, axons and glial cell processes in a membrane-associated manner (Milner et al., 2005; McEwen and Milner, 2007), suggesting that estrogen mediates its effects on synapses locally rather than via regulating nuclear transcription. As the brain ages, changes in the pattern of ER expression and/or in differentially activated signaling pathways in these brain areas have been suggested to be the basis for the detrimental effects of aging in memory and learning. Briefly, synapse number and spine density decrease with natural or surgical depletion of ovarian hormones in the CA1 area of the hippocampus from female rats (Gould et al., 1990; Woolley and McEwen, 1992, 1993; Adams et al., 2001). Unlike what is observed in young animals, estrogen treatment cannot restore synapse and spine density levels in aged animals, suggesting that the hippocampus becomes unresponsive to estrogen effects with age (Adams et al., 2001). Furthermore, the finding that the number of ERα-containing synapses in the hippocampus of old female rats decreases to half the number found in young animals could explain loss of estrogen actions in the aged hippocampus, which has been suggested to be the basis for lower brain plasticity with aging (Adams et al., 2002). Unlike the hippocampus, hypothalamic levels of ERα and progestin receptor are maintained with age in female rhesus monkeys, while membrane GPER1 expression is increased, indicating that the aged hypothalamus retains the ability to express steroid hormone receptors at levels comparable to young adults (Naugle et al., 2014).

Despite being extensively homologous, ERα and ERβ diverge in their expression and action in the brain. They are widely expressed throughout the adult brain and their expression is differentially regulated in aging and by estrogen treatment. Like ERα, the levels of synaptic ERβ are reduced with age in the hippocampus of female rats. However, ERβ levels are increased following estrogen treatment both in young and old OVX rats, indicating that ERβ-mediated effects at the synaptic level are maintained during aging in the female rat hippocampus. It suggests that ERβ would be a more sensitive target to estrogen actions in the aged female brain (Waters et al., 2011). Since ERβ signaling has been associated with altered synapse formation and plasticity (Szymczak et al., 2006; Waters et al., 2009), the shift to decreased ERα/ERβ ratio has been proposed to be a major contributor to the age-induced loss of synapse formation by estrogens (Waters et al., 2011). Also, since ERα and ERβ are linked to unique second messenger pathways that can oppose one another, the altered ERα/ERβ ratio can contribute to deficits in specific signaling pathways, affecting memory and plasticity in old animals (Waters et al., 2011). Moreover, clinical studies have shown a positive correlation between Mini Mental StateExam (MMSE) score and nuclear ERα levels in the frontal cortex of AD patients, suggesting that decreased ERα responsiveness is directly associated to severity of cognitive impairment (Kelly et al., 2008). On the contrary, an age-realted increase in the ERα/ERβ ratio has been reported in cortical astrocytes from both male and female rats, which correlated with lower glial trophic support to neuronal function. The increased ratio was suggested to be associated to both long-term potentiation and spatial memory impairment (Paris et al., 2011; Arimoto et al., 2013; Yin et al., 2015).

Like females, the male brain is also responsive to variations in androgen levels at the synaptic level. The density of dendritic spines in the hippocampus has been reported to be modulated *in vivo* by androgen depletion and replacement (Leranth et al., 2003). Gonadectomy in male rats decreased CA1 spine synapse density compared to sham-operated animals (Jia et al., 2013). Since it can be metabolized into the androgen DHT and estradiol, testosterone can mediate its effects through androgen

and/or estrogen pathways. Treatment of gonadectomized rats with DHT or testosterone propionate but not with estradiol restored spine synapse density to similar levels of those found in intact males, suggesting a direct role of androgens through androgen receptors rather than indirectly via local estradiol biosynthesis in hippocampal synaptic plasticity (Leranth et al., 2003). Similar results were obtained in SAMP8 mice, an animal model of accelerated aging (Jia et al., 2016; Pan et al., 2016).

Sex Hormone and Growth Factor Interaction during Aging

A functional interplay between ERs and growth factor receptors, such as insulin-like growth factor-1 (IGF-1) or BDNF, has broadly been shown to take place in the brain. Hence, it is expected that conditions that affect the expression and/or activity of these receptors have a reciprocal negative impact on the multiple processes regulated by these systems, from the control of hormonal homeostasis and reproduction to learning and cognition. For example, estrogen-induced transport of glucose in the brain through the insulin-sensitive glucose transporter GLUT-4, adult hippocampal neurogenesis and protection against stroke are processes that require the coupling between ERα and IGF-1 receptor, providing further evidence for the interplay between these two systems in promoting enhanced neuronal metabolism and neuroprotection (Cardona-Gómez et al., 2002; Garcia-Segura et al., 2010; Arevalo et al., 2012; Sohrabji, 2015; Huffman et al., 2017). Besides, in aged OVX animals, which had undergone estrogen replacement treatment during middle age, estrogen-induced improvement in memory function was abolished by treatment with an IGF-1 receptor inhibitor. This finding indicates that estrogen may exert part of its lasting effects on the hippocampus and memory through the IGF-1 receptor signaling pathway (Witty et al., 2013). In female rats, both reproductive senescence and OVX have been shown to consistently decrease the levels of IGF-1 gene expression, which correlates with increased expression of genes involved in Aβ generation (Rettberg et al., 2014). In addition, clinical studies have shown that patients with AD have decreased expression of insulin receptors and impaired insulin signaling in brain areas susceptible to AD pathology, which could account for the early cognitive impairment seen in these patients (Schiöth et al., 2012). These studies suggest that impaired brain estrogen/ER and IGF-1/IGF-1 receptor systems may account, at least in part, for the women's well known higher vulnerability to develop AD after menopause. Despite estrogens and androgens share metabolic pathways and functional properties, far less research has examined a functional link between IGF-1 and androgens in the brain (Huffman et al., 2017). However, some studies have shown that IGF-1/androgen interactions promote beneficial effects in neuroprotection (García-Fernández et al., 2008; Puche et al., 2008). On the other hand, BDNF is a crucial molecule for synaptic plasticity and hippocampal memory formation (Heldt et al., 2007; Bekinschtein et al., 2014). BDNF and estrogens activate a number of common signaling pathways, which converge in the induction of growth, survival, neural plasticity and learning. Estrogens can also induce BDNF gene expression through direct binding to an estrogen-sensitive response element (ERE) on the BDNF gene or by increasing neural activity that in turn upregulates BDNF (Scharfman and MacLusky, 2006). Serum BDNF levels have been reported to decline with increasing age in both men and women (Shimada et al., 2014). In addition, a significant drop in serum BDNF levels was found in women after menopause, suggesting that ovarian hormone and BDNF circulating levels are tightly associated (Bus et al., 2012). Interestingly, a recent report has shown that working memory-related hippocampal function is differentially modulated by estradiol in women carrying the specific BDNF $Val^{66}Met$ functional single-nucleotide polymorphism (SNP; Wei et al., 2017). A decrease in BDNF expression has been observed both during aging and after OVX in murine hippocampus (Singh et al., 1995; Sohrabji et al., 1995; Chapman et al., 2012; Perovic et al., 2013; Lu et al., 2014) and estrogen replacement treatment to OVX rats has been shown to increase BDNF mRNA or protein levels (Singh et al., 1995; Sohrabji et al., 1995; Gibbs, 1998; Kiss et al., 2012; Lu et al., 2014). Recently, it has also been suggested that estrogen-enhanced consolidation of multiple forms of hippocampal memory in middle-aged rats is associated with the induction of BDNF protein levels through non-classical cell signaling mechanisms involving epigenetic regulation of the BDNF gene (Fortress et al., 2014). Although there has been substantial growth of new data on the functional consequences of the interplay between sex hormones and these growth factors in recent years, many key aspects remain to be addressed. In particular, an area that warrants further study is to ascertain the role this interaction plays during aging and menopause, when the levels of sex hormones and IGF-1/BDNF decline and the cells and tissues that respond to them undergo both metabolic and functional changes (Garcia-Segura et al., 2010; Sohrabji, 2015).

Sex Hormones and Mitochondrial Function during Aging

Despite comprising only 2% of the body's mass, the brain consumes 20% of the body fuel to sustain its high demand of energy in the form of ATP, making it highly dependent on proper mitochondria function (Rettberg et al., 2014). As mentioned before, estrogens have beneficial effects on brain energy metabolism, increasing blood flow and glucose uptake and enhancing aerobic glycolysis coupled to the citric acid cycle, mitochondrial respiration and ATP generation (Brinton, 2008). Not surprisingly, a strong link between the drop in circulating ovarian hormones and reduced brain bioenergetics in women during menopause and in animals undergoing natural or surgical reproductive senescence has been reported (Maki and Resnick, 2000; Rasgon et al., 2005; Yao et al., 2009, 2010, 2012). In line with this, it is now recognized that normal aging and several age-related diseases, such as AD and PD, are related to mitochondrial dysfunction (Chakrabarti et al., 2011; Johri and Beal, 2012). The common features observed in aging and AD regarding mitochondria have recently been reviewed (Grimm et al., 2016a). Briefly, the aging process is characterized by decreased mitochondrial

activity, including impaired oxidative phosphorylation, reduced expression and activity of respiratory chain complexes and decreased antioxidant defenses (Grimm et al., 2016a). Extensive evidence indicates that the decline in brain mitochondrial function observed in reproductive senescent female animals is caused by loss of ovarian hormones (Yao et al., 2009, 2010, 2012). Also, considering that the first steps of steroidogenesis take place in mitochondria, it is reasonable to hypothesize that age-related accumulation of mitochondrial deficits may have a detrimental effect in steroid biosynthesis and comprise a potential pathogenic mechanism leading to neurodegeneration (Velarde, 2014).

OVX in young adult animals has been shown to induce adverse effects in brain mitochondrial bioenergetics similar to those found in aged animals, including reduced respiration and ATP production rates, increased oxidative stress and decreased expression and/or activity of metabolic enzymes within this organelle (Irwin et al., 2011; Shi et al., 2011; Yao et al., 2012; Gaignard et al., 2015). A recent report has shown that OVX induces mitochondrial dysfunction in terms of reduced active respiration and ATP production likely associated to alterations in the mitochondrial membrane lipid profile in the hippocampus (Zárate et al., 2017). In particular, OVX induces changes in the fatty acid profile of mitochondrial membranes rendering them more prone to peroxidation, a feature also observed during aging in an organ-dependent manner (Pamplona, 2008). Interestingly, OVX also induces a specific decrease in cardiolipin content and changes in its fatty acid composition. Importantly, cardiolipin is an essential component of mitochondria membranes that plays a crucial role in several mitochondrial processes such as oxidative phosphorylation, apoptosis, mitochondrial protein import and supercomplex formation (Claypool and Koehler, 2012). Reduced cardiolipin content, alterations in its acyl chain composition and/or increased cardiolipin peroxidation have been linked to mitochondrial dysfunction in multiple tissues during aging and in neuropathological disorders (Monteiro-Cardoso et al., 2015). Overall, these studies suggest that loss of ovarian hormones accelerates the decline in mitochondrial bioenergetics promoting a premature aging phenotype (Yao et al., 2009). In line with this, alteration in mitochondrial membrane lipid composition, especially in cardiolipin content, could be an additional player in the aging effects of ovarian hormone loss contributing to the early bioenergetic decay during menopause (Zárate et al., 2017).

Although activation of both ERα and ERβ favors mitochondrial function, ERβ activation often results in greater mitochondria functional capacity (Irwin et al., 2012; Yao et al., 2013). It has also been suggested that estrogen effects in brain metabolism mainly relies on ERβ signaling through directly promoting mtDNA gene expression, mitochondrial antioxidant defenses and oxidative and calcium buffering capacity (Nilsen and Diaz Brinton, 2003; Yang et al., 2004; Simpkins et al., 2008; Rettberg et al., 2014).

In addition to be the main source of ATP in cells, mitochondria also play important roles in other cellular functions, such as cell growth and differentiation, regulation of intracellular calcium homeostasis, apoptosis, alteration of the cellular redox state and synaptic plasticity (Grimm et al., 2016b). Mitochondria are considered the major source of ROS production in cells under physiological conditions. Electrons leak the electron transport chain during mitochondrial respiration, combining with molecular oxygen to generate $O_2^{-\bullet}$, which subsequently can be converted to H_2O_2 by superoxide dismutase (SOD). When compared to most other tissues, the brain has very low antioxidant capacity and it is subjected to particularly high levels of oxidative DNA damage. Considerable evidence supports the role of oxidative damage in the aging process (Golden et al., 2002; Samarghandian et al., 2016) and an increasing number of studies implicate ROS as an important contributor to cognitive impairment during aging as well as in age-associated neurodegenerative diseases (Grimm et al., 2016b). Estrogens have well-known antioxidant effects. Clinical evidence points to lower brain oxidative stress and better antioxidant defenses in premenopausal women compared to men, parameters which gradually decrease as women age or if they undergo bilateral oophorectomy (Mandal et al., 2012; Bellanti et al., 2013; Rekkas et al., 2014). Similar results were obtained in animals, where brain mitochondria from young adult females showed lower peroxide production and higher levels of MnSOD, glutathione (GSH) and glutathione peroxidase (GPx) compared to males of the same age (Borrás et al., 2003). Estrogens also increase the expression of peroxirodoxin 5 and glutaredoxin in brain mitochondria (Nilsen et al., 2007). Remarkably, OVX blunted the differences in peroxide and GSH levels between females and males while estrogen treatment prevented them, highlighting the protective effects of this steroid against oxidative stress (Borrás et al., 2003). A similar pattern of a positive correlation between circulating testosterone levels and the activity of antioxidant enzymes both in serum and in the hippocampus have been reported in men and in orchidectomized rats, respectively (Meydan et al., 2010; Cunningham et al., 2014). Again, these effects were prevented in animals after testosterone administration (Meydan et al., 2010).

Some brain areas seem to have a particularly high vulnerability to the effects of sex hormone deprivation, aging and oxidative stress. Thus, several studies have reported a link between hippocampal synaptic decline, cognitive impairment and increased risk of neurodegeneration after OVX in animal models and menopause in women (Morrison et al., 2006; Brinton, 2009; Velarde, 2014; Hara et al., 2015). Mitochondrial dysfunction and oxidative damage are also more evident in the hippocampus than in brain cortex or whole brain in aged male rodents (Navarro et al., 2008). Moreover, OVX severely induces a decrease in the activity of SOD together with an increase in the pro-oxidant enzyme monoamine oxidase (MAO) in the hippocampus but not in the cortex of young rats (Huang and Zhang, 2010). Overall, increasing evidence indicates that the hippocampus is an early target of aging, sex hormone loss and oxidative stress (Navarro et al., 2008; Paradies et al., 2011; Hara et al., 2015).

Another brain region highly sensitive to ovarian hormones is the PFC, an area tightly associated to cognitive function in humans. A recent report has shown that aging in non human

primates induces morphological changes in mitochondria from this brain area leading to a mitochondrial phenotype related to enhanced oxidative stress and ROS production. Noteworthy, this correlates with worsening of working memory. Interestingly, OVX promoted similar effects in mitochondrial morphology and cognitive behavior, which was reversed by estradiol treatment. These studies suggest that estrogen effects on PFC-related memory can result from its antioxidant capacity leading to improved mitochondrial health (Hara et al., 2014).

Sex Hormones and Neurosteroidogenesis during Aging

Another process that has been described to be affected by aging is the synthesis of neurosteroids. The transport of cholesterol from the outer to the inner mitochondrial membrane is the first and rate-limiting step in steroidogenesis. For many years, there has been general agreement that this trafficking relies on the activity of at least two proteins, steroidogenic acute regulatory protein (StAR) and translocator protein of 18 kDa (TSPO), previously known as peripheral benzodiazepine receptor (PBR; Veiga et al., 2004; Acaz-Fonseca et al., 2016). However, recent research using genetic depletion of TSPO both in *in vivo* and *in vitro* models has challenged the view of TSPO as a critical enzyme for steroidogenesis (Selvaraj et al., 2015). A recent report suggested that TSPO may be functionally redundant in achieving baseline steroidogenesis although it may play an important role in maintaining androgen levels during aging (Barron et al., 2017). It is important to stress that the expression and activity of both enzymes are increased with aging and after brain injury, suggesting that the production of brain steroids can be modulated as a protective mechanism to cope with decreased peripheral steroids or pathological conditions (Veiga et al., 2004).

Also, other enzymes catalyzing different steps in neurosteroidogenesis are differentially expressed in neurons and glia in a region and pathophysiological-condition manner. Under physiological conditions, neurons are the main sites for brain estrogen production, relying on their high expression of aromatase (Acaz-Fonseca et al., 2016). However, enhanced aromatase expression in astrocyes has been reported following brain injury in rats and also in the human PFC in the late stages of AD, suggesting that neuronal impairment can induce estrogen production as a glial protective mechanism against neuronal death (Veiga et al., 2004; Luchetti et al., 2011; Acaz-Fonseca et al., 2016). A recent report has shown decreased levels of aromatase in the hippocampus of aged female mice when compared to the levels detected in adult mice (Zhao et al., 2017). OVX also downregulates aromatase gene expression in the hippocampus of middle-aged rats (Sárvári et al., 2014). Female brain-derived estradiol levels have been reported to mirror estradiol circulating levels and thus significantly decline in postmenopausal compared to premenopausal women (Rosario et al., 2011). Since the level of the estradiol precursor and aromatase substrate testosterone is also decreased in the female cerebral cortex after OVX (Caruso et al., 2010), it is tempting to speculate that brain-derived estradiol levels would also be decreased in the brain after surgical loss of ovarian hormones. It has been reported that the inhibition or null mutation of brain aromatase results in accelerated neurodegeneration (Azcoitia et al., 2003). Furthermore, genetic variants in human aromatase have been reported to confer an increased risk for AD (Iivonen et al., 2004; Huang and Poduslo, 2006). Depletion of aromatase in an animal model of AD also led to earlier and more severe neuropathology than what was observed in OVX control mice, suggesting that depletion of brain-derived estrogen rather that peripheral blood estrogen is a more direct and significant risk factor for developing AD and points to the importance of preserving neurosteroidogenesis for healthy brain aging (Cui et al., 2013). Indeed, targeting key enzymes involved in brain estrogen production have been proposed as pharmacological targets to ameliorate brain function decline during aging and to prevent neurodegenerative diseases (Veiga et al., 2004).

Sex Hormones and Neuroinflammation during Aging

As reviewed so far, a wide variety of profound physiological changes occur during aging in the brain. The immune system is not an exception, shifting from a resting, surveying state to a chronic mild inflammatory one (Nissen, 2017). Neuroinflammation is choreographed by microglia and astroglia, both of which are affected with aging. Microglia constitute the resident immunocompetent cells of the CNS. It modulates the inflammatory response under pathological conditions but it also maintains homeostasis in the healthy brain through immune surveillance of the brain parenchyma. Changes in microglial cells during aging and in neurodegenerative processes as well as sex-related differences have recently been reviewed (von Bernhardi et al., 2015; Nissen, 2017). Aging promotes the dysregulation of microglia. As a result, an impairment of their physiological neuroprotective functions occurs. At the same time, a mild chronic inflammatory environment characterized by an increased production of inflammatory cytokines and ROS takes place in the CNS. Aged microglia displays morphological changes and a less dynamic response to injury; however, they appear to be activated under mild stimulatory events or minor injuries, responding in an exacerbated way to local and peripheral signals (Nissen, 2017).

Sex differences in gene expression across age in adult human brain have been reported. Thus, women display higher age-related increases in expression of genes associated with immune and inflammatory functions than men (Christensen and Pike, 2015). While both men and women show increased expression of these genes in the hippocampus and entorhinal cortex, only women have significant increases in other brain regions, suggesting a more global pro-inflammatory state in the aged female brain (Berchtold et al., 2008). Also, postmenopausal women display higher expression of macrophage-associated genes in the aging frontal cortex than premenopausal women, suggesting that ovarian hormone loss shifts the microglia phenotype from the resting towards the reactive state (Sárvári et al., 2012).

It is well known that activated microglia can be polarized into a proinflammatory/cytotoxic M1 or an anti-inflammatory/neuroprotective M2 phenotype in response to a myriad of physiological and pathological stimuli (Villa et al., 2016; Labandeira-Garcia et al., 2017). It has been broadly reported that estrogens trigger the polarization of microglia to an M2 phenotype (Habib and Beyer, 2015). This estrogen action becomes especially relevant under chronic inflammation conditions, where perpetuation of microglial proinflammatory status induce neuronal damage. Hence, it could provide an explanation for the neuroprotective effects of estrogens observed in aging and neurodegenerative diseases (Gyenes et al., 2010; Selvamani et al., 2012; Siani et al., 2017). Besides, growing evidence has highlighted the role of the local renin-angiotensin system in aging and several processes mediated by microglial activation and neuroinflammation (Hellner et al., 2005; Kerr et al., 2005; Rey et al., 2007; Rodriguez-Pallares et al., 2008; Torika et al., 2016). Interestingly, estrogen-induced inhibition of this system leads to reduced oxidative stress, neuroinflammation, and neurodegeneration of dopaminergic neurons in murine models of PD, which may explain, at least in part, the lower risk of developing the disease in premenopausal vs. postmenopausal women and men (Rodriguez-Perez et al., 2010; Labandeira-Garcia et al., 2016).

Like humans, female rodents display enhanced inflammation in the brain with aging, which is regulated, at least in part, by estrogen status. When compared to age-matched males, female mice show higher induction of inflammation-related genes, especially of microglia-specific ones, in the hippocampus (Mangold et al., 2017). In addition, gene expression studies in the rat frontal cortex and hippocampus have shown that both aging and ovarian hormone loss increase the expression of several microglial and immune function genes, leading to a shift towards a more inflammatory and reactive microglia phenotype (Sárvári et al., 2011, 2012, 2014). Estrogen replacement treatment attenuates the OVX-induced effects and most of these effects are mimicked by both ERα and ERβ agonists, suggesting that both ERs are targets of estrogens in microglia (Sárvári et al., 2011, 2014). Besides, both aging and OVX increase the gene expression of pro-inflammatory cytokines such as TNFα and IL-1β in the hippocampus of aged mice (Benedusi et al., 2012). It has been shown that aging exacerbates microglial response to OVX, indicating that loss of sex hormones increase the susceptibility of aged microglia to inflammation (Lei et al., 2003). Recently, it has been suggested that a form of estrogen resistance may be involved in the impaired ability of microglia to resolve inflammation during aging (Villa et al., 2016). Astroglial cells also display a proinflammatory phenotype during aging, expressing and secreting increased levels of inflammatory markers such as TNF-α, IL-1β and IL-6 and hence contributing to brain neuroinflammation. They also display age-related increased levels of intermediate glial fibrillary acidic protein (GFAP) and vimentin filaments as well as increased accumulation of proteotoxic aggregates (Salminen et al., 2011). Taken together, both preclinical and clinical data indicate that both aging and menopause lead to increased neuroinflammation, which may contribute to sex differences in age-related neurological diseases such as stroke and AD.

Although the age-induced increase in the expression of GFAP has been the most classical change reported in astroglial cells (Schipper, 1996; Unger, 1998; Cotrina and Nedergaard, 2002; Lynch et al., 2010), experimental data suggest that physiological brain aging and early stages of neurodegenerative disease are characterized by an increased number of dystrophic astrocytes (Oddo et al., 2003; Broe et al., 2004; Mena and García de Yébenes, 2008; Rossi et al., 2008; Bradford et al., 2010; Olabarria et al., 2011; Cerbai et al., 2012; Kulijewicz-Nawrot et al., 2012; Beauquis et al., 2013). These cells are smaller and less complex, with reduced capacity for glutamate uptake and decreased activity of glutamine synthetase, hence displaying reduced neuroprotective and homeostatic potential (Verkhratsky et al., 2014).

Taken together, aging promotes profound changes in morphological and functional parameters within the brain, most of which are recapitulated by sex hormone loss, particularly in the female (**Figure 1**).

DNA REPAIR AND BRAIN AGING

The DNA in brain cells is frequently damaged and if this damage is not removed it can have serious consequences such as compromised genomic stability. Although exogenous sources of DNA damage exist and replication errors may lead to DNA strand breaks, the majority of DNA lesions in non-replicating brain cells are introduced endogenously by ROS. It is important to note, though, that there are other common DNA lesions that have recently been recognized to also substantially impact genome stability. Among these, rNTP incorporation into DNA, genome damage from transcription-associated R-loop formation (hybridization of nascent primary RNA transcripts to the transcribed DNA strand), and aberrant topoisomerase activity are potential threats to the neural genome (McKinnon, 2016; Williams et al., 2016). Also, the generation of DNA damage through topoisomerase I cleavage complexes formed during transcription is likely to play a significant role in neurons due to the high transcription rates in these cells (Katyal et al., 2014). The difference in replicative status influences to some extent the damage and repair processes in cells. In this context it is important to note that the brain is composed of both non-dividing and dividing cells. As mentioned above, neurons are in a post-mitotic state. But glial cells (e.g., astrocytes, oligodendrocytes and microglia) are in either a proliferative or non-proliferative state, depending on their differentiation status. Because both neurons and glial cells are required to carry out the various higher-order brain functions, it is critically important to maintain all cell types in an appropriate number and configuration (Iyama and Wilson, 2013).

When DNA is exposed to ROS, it may result in oxidative base modifications. Among these, 8-oxo-dG is one of the most abundant and well characterized (Dizdaroglu et al., 2002). It has been estimated that approximately 180 guanines are oxidized to 8-oxo-dG per mammalian genome per day (Lindahl,

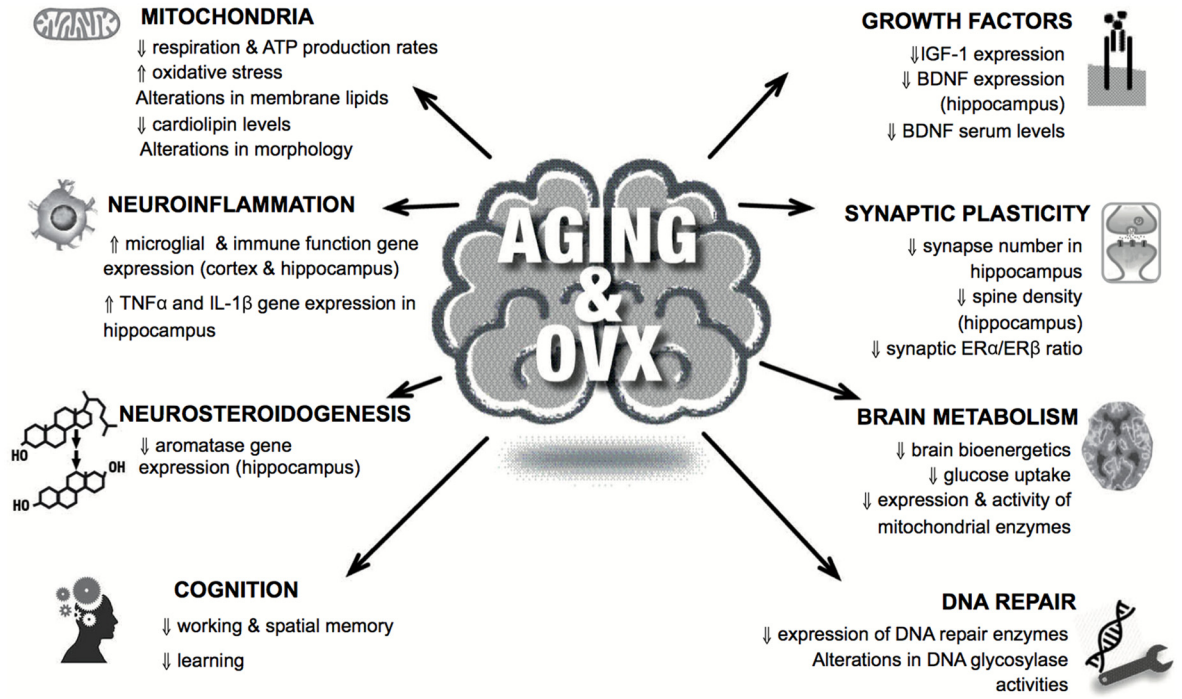


FIGURE 1 | Organismal, cellular and molecular hallmarks of brain aging are frequently observed in ovariectomized animal brains. Examples of common features shared in aging and lack of sex hormones in the brain are shown. For details, see text.

1993). These lesions may cause G:C to T:A transversion mutations because 8-oxo-dG can base pair with adenine as well as cytosine during DNA replication (Shibutani et al., 1991). 8-oxodG has also been implicated in an event called transcriptional mutagenesis (TM), whereby a mis-incorporated adenine in the transcribing mRNA leads to the generation of mutated species of protein (Bregeon et al., 2009). Interestingly, it has recently been suggested that TM may contribute to α-synuclein aggregation and the pathogenesis of PD (Basu et al., 2015). It is important to note that e.g., 8-oxo-dG may also become further oxidized into secondary oxidation products such as guanidinohydantoin 2′-deoxynucleoside (dGh) in doublestranded DNA or spiroiminodihydantoin 2′-deoxynucleoside (dSp) in single stranded DNA (Suzuki et al., 2001; Fleming and Burrows, 2013). ROS may also give rise to DNA single strand breaks (SSBs), which are some of the most common DNA lesions arising at an estimated rate of tens of thousands per cell per day (Lindahl, 1993). Persistent SSBs can lead to the collapse of the replication fork during chromosome duplication, but they may also block transcription. Double strand breaks (DSBs) may also be formed as a result of the actions of ROS. Although these lesions normally are relatively rare, DSBs are some of the most deleterious forms of DNA damage, causing translocations and loss of genomic information. Finally, ROS can also lead to lipid peroxidation, whose byproducts can also react with DNA to produce exocyclic DNA lesions (Yu et al., 2016).

There is growing evidence for the accumulation of unrepaired DNA lesions in the CNS during both normal and accelerated aging and progressive neurodegeneration, but the observed changes depend on brain region, cell types and sub-cellular location of the DNA. Rutten et al. (2007) reported that the number of SSBs increases substantially with aging in the nuclear DNA (nDNA) of hippocampal pyramidal and granule cells as well as in cerebellar granule cells but not in cerebellar Purkinje cells in the mouse brain. For rat brain it has been reported that Ogg1-sensitive sites (i.e., mainly 8-oxo-d-G) accumulate continuously through adulthood and old age in both neurons and astrocytes (Swain and Subba Rao, 2011). Furthermore, it has been shown that susceptibility to oxidative DNA damage is lower and BER capacity is higher in undifferentiated human SH-SY5Y neuro-blastoma cells than in neuronally differentiated SH-SY5Y cells (Sykora et al., 2013). Due to the close proximity of the mtDNA to the site where most of the cellular ROS is formed, mtDNA is particularly vulnerable to oxidative damage. Accordingly, age-associated accumulation of DNA damage is mostly reported for mtDNA. It has been reported that human brain cells experience a progressive increase in the levels of 8-oxo-dG and the magnitude of the age-related damage is approximately ten-fold greater in mtDNA than in nDNA (Wang et al., 2005, 2006; Lovell and Markesbery, 2007).

If left unrepaired, DNA damage can give rise to genomic instability and trigger signaling cascades leading to cellular senescence or cell death, which are phenotypes associated with aging (Rodier et al., 2009). Accordingly, increased levels of mutations in the DNA have been described to occur in the brain and other tissues during normal aging leading to DNA

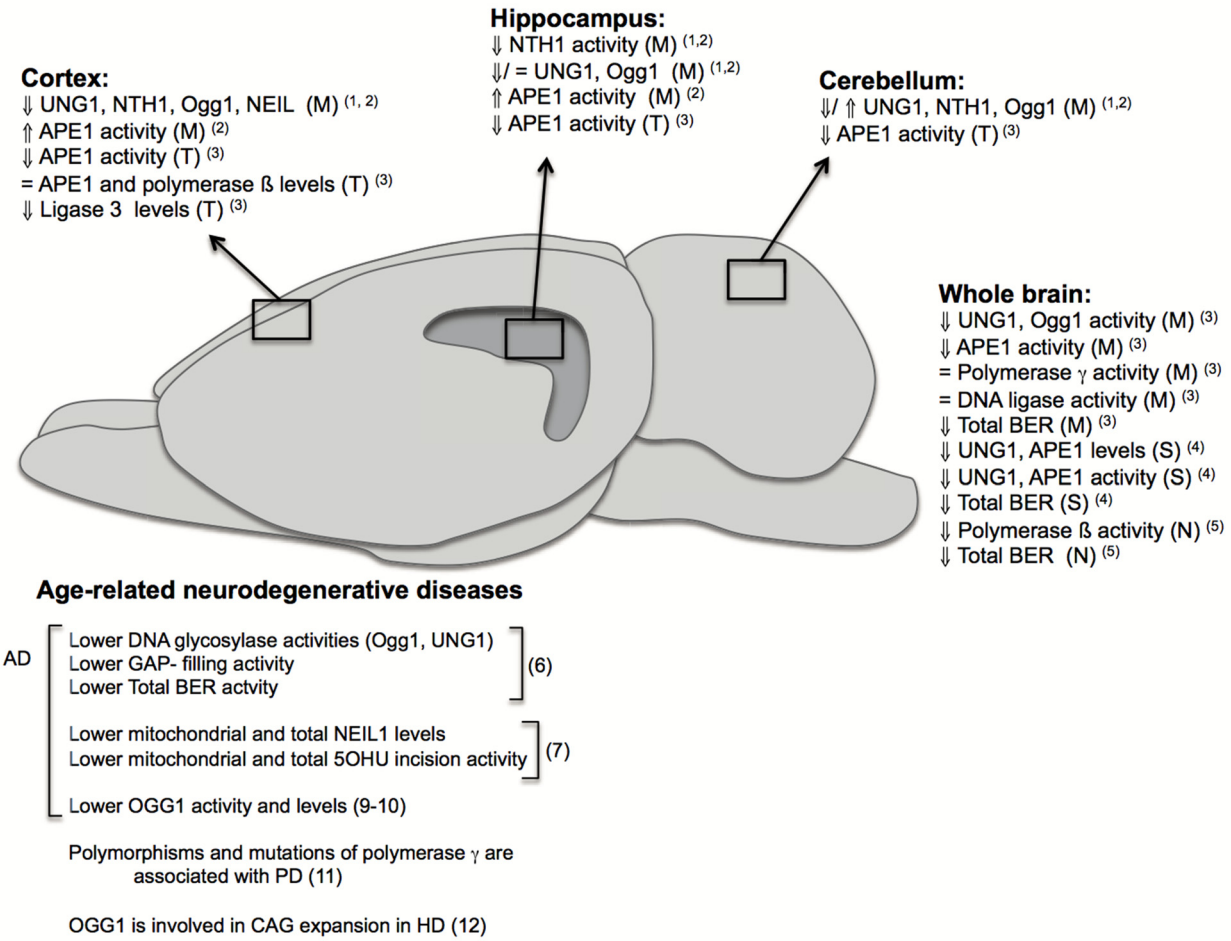


FIGURE 2 | Changes in base excision repair (BER) in brain aging and neurodegeneration. The figure depicts age-related changes in the BER pathway from different investigations performed in specific brain regions: cortex, hippocampus, cerebellum; or using whole brains and in different fractions: (M): mitochondrial, (N): nuclear, (S): synaptosomal, (T): total. It also summarizes some of the major findings in humans and rodents implicating BER in age-related neurodegenerative diseases. Numbers in brackets correspond to references: (1) Imam et al. (2006); (2) Gredilla et al. (2010b); (3) Kisby et al. (2010); (4) Gredilla et al. (2012); (5) Cabelof et al. (2003); (6) Weissman et al. (2007b); (7) Canugovi et al. (2014); (8) Lovell et al. (2000); (9) Iida et al. (2002); (10) Shao et al. (2008); (11) Coppedè and Migliore (2015); (12) Kovtun et al. (2007).

instability (Gredilla, 2010). Although the majority of known DNA repair pathways are present in neurons and glia cells, many investigations suggest that BER impairment and increased DNA instability are the principal contributors to brain aging and age- associated neurodegenerative diseases (**Figure 2**). The BER pathway is basically divided in four distinct steps. First, DNA glycosylases recognize and remove the modified bases. These DNA glycosylases, such as NTH1 and Ogg1, have distinct substrate specificities. They render an abasic site, which is mainly processed by the AP endonuclease (APE1). BER may proceed through two different sub-pathways, both in nuclei and mitochondria: short- or long-patch BER. Both pathways mainly differ in the number of nucleotides that are incorporated into the gap by a DNA polymerase. Different accessory proteins are involved in this step. Finally ligation of the DNA strand takes place by a DNA ligase (Robertson et al., 2009; Liu and Demple, 2010).

Together with BER, it has been suggested that proteins involved in MMR and DSBs repair are present in mitochondria; however, no evidence of mitochondrial NER activity has been reported (Gredilla et al., 2010a). The NER pathway repairs different types of helix distorting and bulky lesions. Moreover, this pathway plays a critical role in DNA crosslinks repair. Similarly to other DNA repair pathways, NER involves various steps, including damage recognition, opening of the DNA helix, incision of the nucleotides surrounding the lesion, gap filling and ligation. Two NER sub-pathways exist: the global genome (GG)-NER and transcription coupled (TC)-NER. The first step of the pathway, recognition of the damage, involves the action of multiple proteins. In GG-NER destabilization of the base pairing is detected by XPC together with the human homolog Rad23 protein, which is suggested by many studies to be the first protein factor to arrive at the lesion. For specific types of lesions, such as UV-induced photoproducts, other proteins are

involved i.e., UV-damaged DNA-binding protein (UV-DDB), thereby recruiting XPC and extending the substrate specificity (Sugasawa, 2011). In TC-NER, damage recognition is caused by the blockage of the transcribing RNA polymerase II on the damaged DNA template. TC-NER is initiated by the CSB protein, followed by CSA. In both GG-NER and TC-NER, the lesion recognition step is followed by recruitment of TFIIH. DNA is unwound around the lesion and the open complex is stabilized by XPG and XPA. Specific endonucleases XPG and ERCC1/XPF cleave the lesion, and after removal of a 24–32 nucleotide fragment, the remaining single-strand gap is filled by the replication machinery and the resulting nick is sealed by ligase I or ligase III (de Boer and Hoeijmakers, 2000; Jeppesen et al., 2011). In MMR, MSH2–MSH6 or MSH2–MSH3 heterodimeric ATPase complexes recognize and bind the mismatch. After recruitment of different proteins e.g., MLH1, PMS2, PCNA, excision is performed by the exonuclease EXO1. Repair synthesis is accurately performed by Polδ, and ligation of the remaining nicks after DNA synthesis is performed by ligase I (Jiricny, 2006). The DSB repair pathway is regulated by several phosphorylation events, starting immediately after DSB formation, where large numbers of the histone protein H2AX are phosphorylated (γH2AX) and accumulate in the chromatin around the break (Bonner et al., 2008; Muslimovic et al., 2008). Moreover, different DSB damage response proteins accumulate in foci around DSBs, activating signaling pathways that affect events related to DNA repair, cell cycle checkpoints, and transcription. The repair of DSBs involves one of two mechanisms: non-homologous end-joining (NHEJ), directly joining the broken ends and involving loss of genetic material, or homologous recombination (HR), which can only take place in replicating cells and uses the intact sister chromatid as a template for repair.

Several studies have shown that oxidative DNA damage do accumulate with age, especially in the mitochondria. The DNA glycosylase Ogg1, is likely to be particularly important for DNA maintenance in the brain due to its specificity for the common 8-oxo-dG lesion. Ogg1 activity decreases significantly with age in neuronal extracts of rat brain, and a similar trend is observed, to a lesser extent though, in rat brain astrocytes (Swain and Subba Rao, 2011). The glycosylases NEIL1 and NEIL2, are also considered to be essential for neuronal DNA repair, due to their ability to remove various oxidatively damaged bases in single stranded regions of DNA, since neuronal DNA is heavily transcribed and during that process the DNA is transiently in a single stranded conformation. In aged rats the activity of APE1, the major enzyme responsible for incision of the DNA backbone in BER, is reduced in the frontal/parietal cortex, cerebellum, brainstem, midbrain and hypothalamus compared to young rats (Kisby et al., 2010) and APE1 activity seems to be reduced both in neurons and astrocytes (Swain and Subba Rao, 2011). While APE1 activity declines with age, there does not seem to be any change in the protein levels of either APE1, Pol β or LIG3 in the rat frontal/parietal cortex, suggesting that the reduced APE activity could be due to altered post-translational modification.

Mitochondrial DNA repair is likely to be particularly important due to the heavy exposure of mtDNA to ROS. Studies by Hollensworth et al. (2000) indicate that mtBER is more efficient in astrocytes than oligodendrocytes or microglia, and that the efficient repair associates with reduced susceptibility to apoptosis. We have previously reported age-associated changes in mtBER activity in the murine brain and showed that these changes are region specific. Thus, in cortical mitochondria, DNA glycosylase activities peak at middle-age followed by a significant drop at old age. However, only minor changes are observed in hippocampal mitochondria during the whole lifespan. Furthermore, DNA glycosylase activities are lower in hippocampal than in cortical mitochondria (Gredilla et al., 2010b). Noteworthy, we have also reported that an age-related decline in mouse brain mtBER occurs specifically at the synapses, which is associated with a decrease in the level of BER proteins (Gredilla et al., 2012). In the human brain, the expression of at least some genes coding for proteins involved in BER has been shown to fluctuate with aging. Interestingly, this seems to some extent to be due to an age-associated accumulation of oxidative lesions in the promotor regions of these genes (Lu et al., 2004). Recently, work from Lillenes et al. (2011, 2017), suggests a potential link between specific SNPs in APE1 and DNA polymerase β in humans and reduced cognitive performance in healthy elderly individuals, which is in support of a role for BER in the maintenance of brain function late in life.

SEX HORMONES AND DNA REPAIR IN THE BRAIN

As mentioned above, DNA instability is one of the major hallmarks of aging. Several investigations have associated brain aging and age-related neurodegenerative disorders with higher accumulation of DNA mutations due, at least in part, to a reduction in DNA repair capacity (Vermulst et al., 2007; Jeppesen et al., 2011; Sanders et al., 2014). Since sex steroids have been described to exert neuroprotective effects, it is likely that such effects might be partly linked to a direct impact on DNA repair mechanisms. In fact, the effect of sex steroids on DNA damage responses have been extensively investigated in cancer, where sex steroids have been described to interact with different DNA repair pathways (Caldon, 2014). Estrogen and androgens positively regulate the repair of DSBs by activation of NHEJ in breast and prostate cancers (Schiewer and Knudsen, 2016). However, conflicting results have been reported regarding the effect of sex steroids on HR depending on the type of cancer, with positive regulation in prostatic cancer and melanoma (Fang et al., 2013; Bowen et al., 2015), but negative in medulloblastoma (Urbanska et al., 2009). Estrogen has also been associated with an enhanced DNA repair via MMR in colorectal cancer (Lu J. Y. et al., 2017). Regarding NER, estrogen up-regulates the repair of UV-induced DNA damage in breast cancer cells (Boulay and Perdiz, 2005) while reducing the repair of thymine dimers in human keratinocytes (Evans et al., 2003). Moreover, in patients with basal cell carcinoma, postmenopausal women show a significant drop in lymphocyte DNA repair capacity compared to postmenopausal women on estrogen supplementation (Grossman and Wei, 1995).

Poly (ADP-ribose) polymerases (PARP) are members of a family of enzymes that are particularly abundant in cell nuclei and can function as sensors of DNA damage. Various investigations have reported sex differences in PARP1 activity. A recent report has shown that female and estrogen-treated male mice are completely protected from alkylation-induced nephrotoxicity in a transgenic model of enhanced alkyladenine DNA glycosylase (Aag) expression together with PARP-1 KO (AagTg/Parp1−/−). This sex dimorphism suggests a direct interaction between Aag and/or PARP1 with estrogen pathways leading to changes in DNA repair activity and/or gene expression (Calvo et al., 2016). Estrogen supplementation also affects PARP-1 activity differently in peripheral blood mononuclear cells from male and female mice (Zaremba et al., 2011). Moreover, PARP-1 is an important regulator of neuronal cell death and cellular responses to DNA damage and it has been reported that PARP-1 mediated cell death is dependent on androgen-receptor signaling after stroke (Vagnerova et al., 2010).

In contrast to cancer research, the relation of sex steroid hormones and DNA repair pathways and whether the former regulates the latter contributing to their neuroprotective effect has not been extensively investigated. However, various studies have reported that sex steroids, particularly estrogen and progesterone, regulate DNA repair mechanisms in the brain. Most of those studies have used OVX animals as a model for analyzing their effect on specific activities or expression of DNA repair enzymes. Studies on how estrogen levels affect DNA repair in the brain have mainly focused on enzymes involved in BER. Estrogen has been described to regulate the transcription as well as the translocation within different cellular compartments of BER enzymes. Neuroprotection of estrogens has been shown in cerebral cortex where they have been described to reduce oxidative DNA damage after hypoxia (Rao et al., 2011), an effect that is associated with an enhancement in the transcription of DNA repair enzymes like APE1 in that brain region (Dietrich et al., 2013). Moreover, estrogen supplementation in OVX old female macaques has been shown to increase the transcription levels of different DNA repair enzymes in the dorsal raphe (Bethea et al., 2016). Bethea et al. (2016) described a significant increase in the transcription of DNA repair enzymes involved in different pathways, including BER (APE1, NTH1 among others), NER (RAD23 and GTF2H5, a subunit of THIIF) and HR (NBS1 and SHFM1). Interestingly, they also reported that when estrogen is combined with progesterone the effect is reduced or even absent (Bethea et al., 2016). That is in agreement with those studies that have previously reported an antagonistic effect of progesterone over estrogen, e.g., in cognitive function of hormone-treated OVX rats (Bimonte-Nelson et al., 2006) and in hippocampal cellular survival after treatment with kainate or mitochondrial toxins (Rosario et al., 2006; Carroll et al., 2008; Yao et al., 2011). Similarly, progesterone has also been described to reduce the estrogen-related enhancement of BDNF in OVX rats (Bimonte-Nelson et al., 2004). This is especially interesting, because BDNF has been described to enhance neuronal survival, at least in part by inducing the transcription of DNA repair enzymes such as APE1 (Yang et al., 2014). Thus, the effect of estrogen on DNA repair might also be related to BDNF levels, since as we previously described, estrogens induce BDNF expression. Various studies have also linked beneficial effects of estrogens with an up-regulation of Nrf-2 via the PI3K/Akt signaling pathway. This is a relevant link since Nrf2 has been associated to the transcription of antioxidant response elements, including different DNA repair enzymes (Jayakumar et al., 2015; Habib et al., 2016). The expression of Nrf2 has been described to be reduced after OVX and restored to normal levels after estrogen supplementation in murine hippocampus (Li et al., 2017). This mechanism has also been described to play an important role in the protective role of estrogens after light-induced degeneration in retina (Zhu et al., 2015). Moreover, it has been described that certain phytestrogens exert their beneficial effects by activating the PI3K/Akt/Nrf2 pathway through ER binding (Hwang and Jeong, 2010).

Estrogen not only regulates the expression/activity of DNA repair enzymes, it has also been described to regulate their subcellular distribution. Leclère et al. (2013) have shown that in whole brain extracts, APE1 activity is increased in mitochondrial fractions after OVX. The same effect is observed in liver extracts, being associated with a translocation of APE1 from the cytosol to the mitochondria. Moreover, this effect of estrogen on the trafficking of DNA repair enzymes has been suggested to be brain region-dependent. Araneda et al. (2005) have shown that after estrogen supplementation in OVX rats, Ogg1 was translocated within the nucleus and to other cellular compartments in the paraventricular nucleus of the hypothalamus but not in the bed nucleus of the stria terminalis. This translocation of DNA repair enzymes dependency on estrogen levels might be associated with the increased oxidative stress that has been described to occur after OVX (Borrás et al., 2003; Baeza et al., 2008). Various studies have reported that DNA repair enzymes can be specifically translocated to nuclei and mitochondria in response to increased oxidative stress and DNA damage (Mitra et al., 2007; Boesch et al., 2011; Li M. X. et al., 2012).

CONCLUDING REMARKS

Brain aging is associated with an important decline in neuronal function. The drop in sex hormone levels during aging is believed to play an important role in the loss of neuronal function, which may further contribute to the onset of age-related neurodegenerative diseases. A broadly used animal model for investigating the effects of sex hormones in brain aging and neurodegeneration is gonadectomy, especially in rodents. Gonadectomized young rodents display several features of intact aged animals, including changes in brain metabolism, mitochondrial function, and neuroinflammation among others. Interestingly, estrogen supplementation in female rodents has been described to revert the negative effects of OVX in brain functionality. Different studies have supported the neuroprotective effect of estrogen at different levels. Such effect may be extremely complex, and it may depend not only on the type of receptor involved, but also on the timing of estrogen therapy. In view of ERβ sustained actions on plasticity during aging in the female brain and its effects

on mitochondrial function, ERβ appears as a target worth exploring to counteract age-associated detrimental effects in the brain. A better understanding of the underlying mechanisms of sex hormone actions may lead to new avenues for treatment of age-associated neurodegeneration. Recently, some studies have reported a significant effect of estrogens on DNA repair enzymes in the brain. However, the investigations on this particular issue and the related mechanisms are still scarce. Since reduction in DNA repair capacity has been suggested to contribute to brain aging and the onset of neurodegenerative diseases, it is critical to fully understand how estrogens affect DNA repair mechanisms. This novel interplay warrants further study in an attempt to find new therapeutic targets to promote healthy brain aging and prevent age-related diseases.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from different institutions. SZ was supported by a short-term EMBO fellowship, an ISN-CAEN grant and a mobility grant from University of Buenos Aires. TS was supported by a grant from the Velux Foundation to the Danish AgingResearch Center. RG was supported by a "Salvador de Madariaga" mobility grant from the Spanish Education Ministry and a grant from Complutense University.

REFERENCES

Acaz-Fonseca, E., Avila-Rodriguez, M., Garcia-Segura, L. M., and Barreto, G. E. (2016). Regulation of astroglia by gonadal steroid hormones under physiological and pathological conditions. *Prog. Neurobiol.* 144, 5–26. doi: 10.1016/j.pneurobio.2016.06.002

Adams, M. M., Fink, S. E., Shah, R. A., Janssen, W. G., Hayashi, S., Milner, T. A., et al. (2002). Estrogen and aging affect the subcellular distribution of estrogen receptor-α in the hippocampus of female rats. *J. Neurosci.* 22, 3608–3614.

Adams, M. M., Shah, R. A., Janssen, W. G., and Morrison, J. H. (2001). Different modes of hippocampal plasticity in response to estrogen in young and aged female rats. *Proc. Natl. Acad. Sci. U S A* 98, 8071–8076. doi: 10.1073/pnas.141215898

Araneda, S., Pelloux, S., Radicella, J. P., Angulo, J., Kitahama, K., Gysling, K., et al. (2005). 8-oxoguanine DNA glycosylase, but not Kin17 protein, is translocated and differentially regulated by estrogens in rat brain cells. *Neuroscience* 136, 135–146. doi: 10.1016/j.neuroscience.2005.06.080

Arevalo, M. A., Azcoitia, I., and Garcia-Segura, L. M. (2015). The neuroprotective actions of oestradiol and oestrogen receptors. *Nat. Rev. Neurosci.* 16, 17–29. doi: 10.1038/nrn3856

Arevalo, M. A., Ruiz-Palmero, I., Scerbo, M. J., Acaz-Fonseca, E., Cambiasso, M. J., and Garcia-Segura, L. M. (2012). Molecular mechanisms involved in the regulation of neuritogenesis by estradiol: recent advances. *J. Steroid Biochem. Mol. Biol.* 131, 52–56. doi: 10.1016/j.jsbmb.2011.09.004

Arevalo, M. A., Santos-Galindo, M., Bellini, M. J., Azcoitia, I., and Garcia-Segura, L. M. (2010). Actions of estrogens on glial cells: implications for neuroprotection. *Biochim. Biophys. Acta* 1800, 1106–1112. doi: 10.1016/j.bbagen.2009.10.002

Arimoto, J. M., Wong, A., Rozovsky, I., Lin, S. W., Morgan, T. E., and Finch, C. E. (2013). Age increase of estrogen receptor-α (ERα) in cortical astrocytes impairs neurotrophic support in male and female rats. *Endocrinology* 154, 2101–2113. doi: 10.1210/en.2012-2046

Arnold, S., De Araújo, G. W., and Beyer, C. (2008). Gender-specific regulation of mitochondrial fusion and fission gene transcription and viability of cortical astrocytes by steroid hormones. *J. Mol. Endocrinol.* 41, 289–300. doi: 10.1677/JME-08-0085

Austad, S. N., and Fischer, K. E. (2016). Sex differences in lifespan. *Cell Metab.* 23, 1022–1033. doi: 10.1016/j.cmet.2016.05.019

Azcoitia, I., Sierra, A., Veiga, S., and Garcia-Segura, L. M. (2003). Aromatase expression by reactive astroglia is neuroprotective. *Ann. N Y Acad. Sci.* 1007, 298–305. doi: 10.1196/annals.1286.028

Baeza, I., Alvarado, C., Ariznavarreta, C., Castillo, C., Tresguerres, J. A., and De la Fuente, M. (2008). Effect of growth hormone treatment on lymphocyte functions in old male rats. *Neuroimmunomodulation* 15, 279–284. doi: 10.1159/000156471

Barja, G. (2004). Free radicals and aging. *Trends Neurosci.* 27, 595–600. doi: 10.1016/j.tins.2004.07.005

Barron, A. M., Ji, B., Kito, S., Suhara, T., and Higuchi, M. (2017). Steroidogenic abnormalities in translocator protein knockout mice and significance in the aging male. *Biochem. J.* doi: 10.1042/BCJ20170645 [Epub ahead of print].

Barron, A. M., and Pike, C. J. (2012). Sex hormones, aging, and Alzheimer's disease. *Front. Biosci.* 4, 976–997. doi: 10.2741/e434

Basu, S., Je, G., and Kim, Y. S. (2015). Transcriptional mutagenesis by 8-oxodG in α-synuclein aggregation and the pathogenesis of Parkinson's disease. *Exp. Mol. Med.* 47:e179. doi: 10.1038/emm.2015.54

Baudry, M., Bi, X., and Aguirre, C. (2013). Progesterone-estrogen interactions in synaptic plasticity and neuroprotection. *Neuroscience* 239, 280–294. doi: 10.1016/j.neuroscience.2012.10.051

Beal, M. F. (2005). Mitochondria take center stage in aging and neurodegeneration. *Ann. Neurol.* 58, 495–505. doi: 10.1002/ana.20624

Beauquis, J., Pavia, P., Pomilio, C., Vinuesa, A., Podlutskaya, N., Galvan, V., et al. (2013). Environmental enrichment prevents astroglial pathological changes in the hippocampus of APP transgenic mice, model of Alzheimer's disease. *Exp. Neurol.* 239, 28–37. doi: 10.1016/j.expneurol.2012.09.009

Bekinschtein, P., Cammarota, M., and Medina, J. H. (2014). BDNF and memory processing. *Neuropharmacology* 76, 677–683. doi: 10.1016/j.neuropharm.2013.04.024

Bellanti, F., Matteo, M., Rollo, T., De Rosario, F., Greco, P., Vendemiale, G., et al. (2013). Sex hormones modulate circulating antioxidant enzymes: impact of estrogen therapy. *Redox Biol.* 1, 340–346. doi: 10.1016/j.redox.2013.05.003

Benedusi, V., Meda, C., Della Torre, S., Monteleone, G., Vegeto, E., and Maggi, A. (2012). A lack of ovarian function increases neuroinflammation in aged mice. *Endocrinology* 153, 2777–2788. doi: 10.1210/en.2011-1925

Berchtold, N. C., Cribbs, D. H., Coleman, P. D., Rogers, J., Head, E., Kim, R., et al. (2008). Gene expression changes in the course of normal brain aging are sexually dimorphic. *Proc. Natl. Acad. Sci. U S A* 105, 15605–15610. doi: 10.1073/pnas.0806883105

Bethea, C. L., Kohama, S. G., Reddy, A. P., and Urbanski, H. F. (2016). Ovarian steroids regulate gene expression in the dorsal raphe of old female macaques. *Neurobiol. Aging* 37, 179–191. doi: 10.1016/j.neurobiolaging.2015.10.004

Bimonte-Nelson, H. A., Francis, K. R., Umphlet, C. D., and Granholm, A. C. (2006). Progesterone reverses the spatial memory enhancements initiated by tonic and cyclic oestrogen therapy in middle-aged ovariectomized female rats. *Eur. J. Neurosci.* 24, 229–242. doi: 10.1111/j.1460-9568.2006.04867.x

Bimonte-Nelson, H. A., Nelson, M. E., and Granholm, A. C. (2004). Progesterone counteracts estrogen-induced increases in neurotrophins in the aged female rat brain. *Neuroreport* 15, 2659–2663. doi: 10.1097/00001756-200412030-00021

Boesch, P., Weber-Lotfi, F., Ibrahim, N., Tarasenko, V., Cosset, A., Paulus, F., et al. (2011). DNA repair in organelles: pathways, organization, regulation, relevance in disease and aging. *Biochim. Biophys. Acta* 1813, 186–200. doi: 10.1016/j.bbamcr.2010.10.002

Bonner, W. M., Redon, C. E., Dickey, J. S., Nakamura, A. J., Sedelnikova, O. A., Solier, S., et al. (2008). γH2AX and cancer. *Nat. Rev. Cancer* 8, 957–967. doi: 10.1038/nrc2523

Borrás, C., Sastre, J., García-Sala, D., Lloret, A., Pallardö, F. V., and Viña, J. (2003). Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. *Free Radic. Biol. Med.* 34, 546–552. doi: 10.1016/s0891-5849(02)01356-4

Boulay, F., and Perdiz, D. (2005). 17β-estradiol modulates UVB-induced cellular responses in estrogen receptors positive human breast cancer cells. *J. Photochem. Photobiol. B Biol.* 81, 143–153. doi: 10.1016/j.jphotobiol.2005.05.008

Boulware, M. I., Kordasiewicz, H., and Mermelstein, P. G. (2007). Caveolin proteins are essential for distinct effects of membrane estrogen receptors in neurons. *J. Neurosci.* 27, 9941–9950. doi: 10.1523/jneurosci.1647-07.2007

Bourque, M., Dluzen, D. E., and Di Paolo, T. (2009). Neuroprotective actions of sex steroids in Parkinson's disease. *Front. Neuroendocrinol.* 30, 142–157. doi: 10.1016/j.yfrne.2009.04.014

Bowen, C., Zheng, T., and Gelmann, E. P. (2015). NKX3.1 suppresses TMPRSS2-ERG gene rearrangement and mediates repair of androgen receptor-induced DNA damage. *Cancer Res.* 75, 2686–2698. doi: 10.1158/0008-5472.CAN-14-3387

Bradford, J., Shin, J. Y., Roberts, M., Wang, C. E., Sheng, G., Li, S., et al. (2010). Mutant huntingtin in glial cells exacerbates neurological symptoms of Huntington disease mice. *J. Biol. Chem.* 285, 10653–10661. doi: 10.1074/jbc.M109.083287

Brann, D. W., Dhandapani, K., Wakade, C., Mahesh, V. B., and Khan, M. M. (2007). Neurotrophic and neuroprotective actions of estrogen: basic mechanisms and clinical implications. *Steroids* 72, 381–405. doi: 10.1016/j.steroids.2007.02.003

Bregeon, D., Peignon, P. A., and Sarasin, A. (2009). Transcriptional mutagenesis induced by 8-oxoguanine in mammalian cells. *PLoS Genet.* 5:e1000577. doi: 10.1371/journal.pgen.1000577

Brinton, R. D. (2008). The healthy cell bias of estrogen action: mitochondrial bioenergetics and neurological implications. *Trends Neurosci.* 31, 529–537. doi: 10.1016/j.tins.2008.07.003

Brinton, R. D. (2009). Estrogen-induced plasticity from cells to circuits: predictions for cognitive function. *Trends Pharmacol. Sci.* 30, 212–222. doi: 10.1016/j.tips.2008.12.006

Brinton, R. D., Thompson, R. F., Foy, M. R., Baudry, M., Wang, J., Finch, C. E., et al. (2008). Progesterone receptors: form and function in brain. *Front. Neuroendocrinol.* 29, 313–339. doi: 10.1016/j.yfrne.2008.02.001

Broe, M., Kril, J., and Halliday, G. M. (2004). Astrocytic degeneration relates to the severity of disease in frontotemporal dementia. *Brain* 127, 2214–2220. doi: 10.1093/brain/awh250

Bus, B. A., Tendolkar, I., Franke, B., de Graaf, J., den Heijer, M., Buitelaar, J. K., et al. (2012). Serum brain-derived neurotrophic factor: determinants and relationship with depressive symptoms in a community population of middle-aged and elderly people. *World J. Biol. Psychiatry* 13, 39–47. doi: 10.3109/15622975.2010.545187

Cabelof, D. C., Yanamadala, S., Raffoul, J. J., Guo, Z., Soofi, A., and Heydari, A. R. (2003). Caloric restriction promotes genomic stability by induction of base excision repair and reversal of its age-related decline. *DNA Repair (Amst)* 2, 295–307. doi: 10.1016/s1568-7864(02)00219-7

Cai, Y., Chew, C., Muñoz, F., and Sengelaub, D. R. (2017). Neuroprotective effects of testosterone metabolites and dependency on receptor action on the morphology of somatic motoneurons following the death of neighboring motoneurons. *Dev. Neurobiol.* 77, 691–707. doi: 10.1002/dneu.22445

Caldon, C. E. (2014). Estrogen signaling and the DNA damage response in hormone dependent breast cancers. *Front. Oncol.* 4:106. doi: 10.3389/fonc.2014.00106

Calvo, J. A., Allocca, M., Fake, K. R., Muthupalani, S., Corrigan, J. J., Bronson, R. T., et al. (2016). Parp1 protects against Aag-dependent alkylation-induced nephrotoxicity in a sex-dependent manner. *Oncotarget* 7, 44950–44965. doi: 10.18632/oncotarget.10440

Cantuti-Castelvetri, I., Lin, M. T., Zheng, K., Keller-McGandy, C. E., Betensky, R. A., Johns, D. R., et al. (2005). Somatic mitochondrial DNA mutations in single neurons and glia. *Neurobiol. Aging* 26, 1343–1355. doi: 10.1016/j.neurobiolaging.2004.11.008

Canugovi, C., Shamanna, R. A., Croteau, D. L., and Bohr, V. A. (2014). Base excision DNA repair levels in mitochondrial lysates of Alzheimer's disease. *Neurobiol. Aging* 35, 1293–1300. doi: 10.1016/j.neurobiolaging.2014.01.004

Cardona-Gómez, G. P., Mendez, P., DonCarlos, L. L., Azcoitia, I., and Garcia-Segura, L. M. (2002). Interactions of estrogen and insulin-like growth factor-I in the brain: molecular mechanisms and functional implications. *J. Steroid Biochem. Mol. Biol.* 83, 211–217. doi: 10.1016/s0960-0760(02)00261-3

Carroll, J. C., Rosario, E. R., and Pike, C. J. (2008). Progesterone blocks estrogen neuroprotection from kainate in middle-aged female rats. *Neurosci. Lett.* 445, 229–232. doi: 10.1016/j.neulet.2008.09.010

Caruso, D., Pesaresi, M., Maschi, O., Giatti, S., Garcia-Segura, L. M., and Melcangi, R. C. (2010). Effect of short-and long-term gonadectomy on neuroactive steroid levels in the central and peripheral nervous system of male and female rats. *J. Neuroendocrinol.* 22, 1137–1147. doi: 10.1111/j.1365-2826.2010.02064.x

Cerbai, F., Lana, D., Nosi, D., Petkova-Kirova, P., Zecchi, S., Brothers, H. M., et al. (2012). The neuron-astrocyte-microglia triad in normal brain ageing and in a model of neuroinflammation in the rat hippocampus. *PLoS One* 7:e45250. doi: 10.1371/journal.pone.0045250

Chakrabarti, S., Munshi, S., Banerjee, K., Thakurta, I. G., Sinha, M., and Bagh, M. B. (2011). Mitochondrial dysfunction during brain aging: role of oxidative stress and modulation by antioxidant supplementation. *Aging Dis.* 2, 242–256.

Chamniansawat, S., and Chongthammakun, S. (2012). A priming role of local estrogen on exogenous estrogen-mediated synaptic plasticity and neuroprotection. *Exp. Mol. Med.* 44, 403–411. doi: 10.3858/emm.2012.44.6.046

Chapman, T. R., Barrientos, R. M., Ahrendsen, J. T., Hoover, J. M., Maier, S. F., and Patterson, S. L. (2012). Aging and infection reduce expression of specific brain-derived neurotrophic factor mRNAs in hippocampus. *Neurobiol. Aging* 33, 832.e1–832.e14. doi: 10.1016/j.neurobiolaging.2011.07.015

Chen, J. Q., Eshete, M., Alworth, W. L., and Yager, J. D. (2004). Binding of MCF-7 cell mitochondrial proteins and recombinant human estrogen receptors α and β to human mitochondrial DNA estrogen response elements. *J. Cell. Biochem.* 93, 358–373. doi: 10.1002/jcb.20178

Christensen, A., and Pike, C. J. (2015). Menopause, obesity and inflammation: interactive risk factors for Alzheimer's disease. *Front. Aging Neurosci.* 7:130. doi: 10.3389/fnagi.2015.00130

Claypool, S. M., and Koehler, C. M. (2012). The complexity of cardiolipin in health and disease. *Trends Biochem. Sci.* 37, 32–41. doi: 10.1016/j.tibs.2011.09.003

Coffey, C. E., Lucke, J. F., Saxton, J. A., Ratcliff, G., Unitas, L. J., Billig, B., et al. (1998). Sex differences in brain aging: a quantitative magnetic resonance imaging study. *Arch. Neurol.* 55, 169–179. doi: 10.1001/archneur.55.2.169

Colciago, A., Casati, L., Negri-Cesi, P., and Celotti, F. (2015). Learning and memory: steroids and epigenetics. *J. Steroid Biochem. Mol. Biol.* 150, 64–85. doi: 10.1016/j.jsbmb.2015.02.008

Coppedè, F., and Migliore, L. (2015). DNA damage in neurodegenerative diseases. *Mutat. Res.* 776, 84–97. doi: 10.1016/j.mrfmmm.2014.11.010

Cotrina, M. L., and Nedergaard, M. (2002). Astrocytes in the aging brain. *J. Neurosci. Res.* 67, 1–10. doi: 10.1002/jnr.10121

Cui, J., Shen, Y., and Li, R. (2013). Estrogen synthesis and signaling pathways during aging: from periphery to brain. *Trends Mol. Med.* 19, 197–209. doi: 10.1016/j.molmed.2012.12.007

Cunningham, R. L., Singh, M., O'Bryant, S. E., Hall, J. R., and Barber, R. C. (2014). Oxidative stress, testosterone, and cognition among Caucasian and Mexican-American men with and without Alzheimer's disease. *J. Alzheimers Dis.* 40, 563–573. doi: 10.3233/JAD-131994

de Boer, J., and Hoeijmakers, J. H. (2000). Nucleotide excision repair and human syndromes. *Carcinogenesis* 21, 453–460. doi: 10.1093/carcin/21.3.453

De Nicola, A. F., Garay, L. I., Meyer, M., Guennoun, R., Sitruk-Ware, R., Schumacher, M., et al. (2017). Neurosteroidogenesis and progesterone anti-inflammatory/neuroprotective effects. *J. Neuroendocrinol.* doi: 10.1111/jne.12502 [Epub ahead of print].

De Nicola, A. F., Gonzalez Deniselle, M. C., Garay, L., Meyer, M., Gargiulo-Monachelli, G., Guennoun, R., et al. (2013). Progesterone protective effects in neurodegeneration and neuroinflammation. *J. Neuroendocrinol.* 25, 1095–1103. doi: 10.1111/jne.12043

De Nicola, A. F., Labombarda, F., Gonzalez Deniselle, M. C., Gonzalez, S. L., Garay, L., Meyer, M., et al. (2009). Progesterone neuroprotection in traumatic CNS injury and motoneuron degeneration. *Front. Neuroendocrinol.* 30, 173–187. doi: 10.1016/j.yfrne.2009.03.001

Demonacos, C. V., Karayanni, N., Hatzoglou, E., Tsiriyiotis, C., Spandidos, D. A., and Sekeris, C. E. (1996). Mitochondrial genes as sites of primary action of steroid hormones. *Steroids* 61, 226–232. doi: 10.1016/0039-128x(96)00019-0

Dietrich, A. K., Humphreys, G. I., and Nardulli, A. M. (2013). 17β-estradiol increases expression of the oxidative stress response and DNA repair protein apurinic endonuclease (Ape1) in the cerebral cortex of female mice following hypoxia. *J. Steroid Biochem. Mol. Biol.* 138, 410–420. doi: 10.1016/j.jsbmb.2013.07.007

Ding, F., Yao, J., Zhao, L., Mao, Z., Chen, S., and Brinton, R. D. (2013). Ovariectomy induces a shift in fuel availability and metabolism in the hippocampus of the female transgenic model of familial Alzheimer's. *PLoS One* 8:e59825. doi: 10.1371/journal.pone.0059825

Dizdaroglu, M., Jaruga, P., Birincioglu, M., and Rodriguez, H. (2002). Free radical-induced damage to DNA: mechanisms and measurement. *Free Radic. Biol. Med.* 32, 1102–1115. doi: 10.1016/S0891-5849(02)00826-2

Do Rego, J. L., Seong, J. Y., Burel, D., Leprince, J., Luu-The, V., Tsutsui, K., et al. (2009). Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. *Front. Neuroendocrinol.* 30, 259–301. doi: 10.1016/j.yfrne.2009.05.006

Dumitriu, D., Rapp, P. R., McEwen, B. S., and Morrison, J. H. (2010). Estrogen and the aging brain: an elixir for the weary cortical network. *Ann. N Y Acad. Sci.* 1204, 104–112. doi: 10.1111/j.1749-6632.2010.05529.x

El-Etr, M., Rame, M., Boucher, C., Ghoumari, A. M., Kumar, N., Liere, P., et al. (2015). Progesterone and nestorone promote myelin regeneration in chronic demyelinating lesions of corpus callosum and cerebral cortex. *Glia* 63, 104–117. doi: 10.1002/glia.22736

Evans, M. D., Butler, J. M., Nicoll, K., Cooke, M. S., and Lunec, J. (2003). 17 β-Oestradiol attenuates nucleotide excision repair. *FEBS Lett.* 535, 153–158. doi: 10.1016/s0014-5793(02)03898-x

Fang, M., Xia, F., Mahalingam, M., Virbasius, C. M., Wajapeyee, N., and Green, M. R. (2013). MEN1 is a melanoma tumor suppressor that preserves genomic integrity by stimulating transcription of genes that promote homologous recombination-directed DNA repair. *Mol. Cell. Biol.* 33, 2635–2647. doi: 10.1128/MCB.00167-13

Fleming, A. M., and Burrows, C. J. (2013). G-quadruplex folds of the human telomere sequence alter the site reactivity and reaction pathway of guanine oxidation compared to duplex DNA. *Chem. Res. Toxicol.* 26, 593–607. doi: 10.1021/tx400028y

Fortress, A. M., Kim, J., Poole, R. L., Gould, T. J., and Frick, K. M. (2014). 17β-Estradiol regulates histone alterations associated with memory consolidation and increases Bdnf promoter acetylation in middle-aged female mice. *Learn. Mem.* 21, 457–467. doi: 10.1101/lm.034033.113

Frick, K. M. (2009). Estrogens and age-related memory decline in rodents: what have we learned and where do we go from here? *Horm. Behav.* 55, 2–23. doi: 10.1016/j.yhbeh.2008.08.015

Gabbita, S. P., Lovell, M. A., and Markesbery, W. R. (1998). Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J. Neurochem.* 71, 2034–2040. doi: 10.1046/j.1471-4159.1998.71052034.x

Gaignard, P., Savouroux, S., Liere, P., Pianos, A., Thérond, P., Schumacher, M., et al. (2015). Effect of sex differences on brain mitochondrial function and its suppression by ovariectomy and in aged mice. *Endocrinology* 156, 2893–2904. doi: 10.1210/en.2014-1913

Gao, X., Smith, G. M., and Chen, J. (2009). Impaired dendritic development and synaptic formation of postnatal-born dentate gyrus granular neurons in the absence of brain-derived neurotrophic factor signaling. *Exp. Neurol.* 215, 178–190. doi: 10.1016/j.expneurol.2008.10.009

Garay, L., Gonzalez Deniselle, M. C., Lima, A., Roig, P., and De Nicola, A. F. (2007). Effects of progesterone in the spinal cord of a mouse model of multiple sclerosis. *J. Steroid Biochem. Mol. Biol.* 107, 228–237. doi: 10.1016/j.jsbmb.2007.03.040

Garay, L., Gonzalez Deniselle, M. C., Meyer, M., Costa, J. J., Lima, A., Roig, P., et al. (2009). Protective effects of progesterone administration on axonal pathology in mice with experimental autoimmune encephalomyelitis. *Brain Res.* 1283, 177–185. doi: 10.1016/j.brainres.2009.04.057

García-Fernández, M., Delgado, G., Puche, J. E., González-Barón, S., and Castilla Cortazar, I. (2008). Low doses of insulin-like growth factor I improve insulin resistance, lipid metabolism, and oxidative damage in aging rats. *Endocrinology* 149, 2433–2442. doi: 10.1210/en.2007-1190

Garcia-Segura, L. M., Arévalo, M. A., and Azcoitia, I. (2010). Interactions of estradiol and insulin-like growth factor-I signalling in the nervous system: new advances. *Prog. Brain Res.* 181, 251–272. doi: 10.1016/s0079-6123(08)81014-x

Garcia-Segura, L. M., Cardona-Gomez, P., Naftolin, F., and Chowen, J. A. (1998). Estradiol upregulates Bcl-2 expression in adult brain neurons. *Neuroreport* 9, 593–597. doi: 10.1097/00001756-199803090-00006

Ghoumari, A. M., Baulieu, E. E., and Schumacher, M. (2005). Progesterone increases oligodendroglial cell proliferation in rat cerebellar slice cultures. *Neuroscience* 135, 47–58. doi: 10.1016/j.neuroscience.2005.05.023

Gibbs, R. B. (1998). Levels of trkA and BDNF mRNA, but not NGF mRNA, fluctuate across the estrous cycle and increase in response to acute hormone replacement. *Brain Res.* 787, 259–268. doi: 10.1016/S0006-8993(97)01511-4

Gillies, G. E., Pienaar, I. S., Vohra, S., and Qamhawi, Z. (2014). Sex differences in Parkinson's disease. *Front. Neuroendocrinol.* 35, 370–384. doi: 10.1016/j.yfrne.2014.02.002

Golden, T. R., Hinerfeld, D. A., and Melov, S. (2002). Oxidative stress and aging: beyond correlation. *Aging Cell* 1, 117–123. doi: 10.1046/j.1474-9728.2002.00015.x

Gould, E., Woolley, C. S., Frankfurt, M., and McEwen, B. S. (1990). Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J. Neurosci.* 10, 1286–1291.

Graham, J. D., and Clarke, C. L. (1997). Physiological action of progesterone in target tissues. *Endocr. Rev.* 18, 502–519. doi: 10.1210/er.18.4.502

Gredilla, R. (2010). DNA damage and base excision repair in mitochondria and their role in aging. *J. Aging Res.* 2011:257093. doi: 10.4061/2011/257093

Gredilla, R., Bohr, V. A., and Stevnsner, T. (2010a). Mitochondrial DNA repair and association with aging—an update. *Exp. Gerontol.* 45, 478–488. doi: 10.1016/j.exger.2010.01.017

Gredilla, R., Garm, C., Holm, R., Bohr, V. A., and Stevnsner, T. (2010b). Differential age-related changes in mitochondrial DNA repair activities in mouse brain regions. *Neurobiol. Aging* 31, 993–1002. doi: 10.1016/j.neurobiolaging.2008.07.004

Gredilla, R., Weissman, L., Yang, J. L., Bohr, V. A., and Stevnsner, T. (2012). Mitochondrial base excision repair in mouse synaptosomes during normal aging and in a model of Alzheimer's disease. *Neurobiol. Aging* 33, 694–707. doi: 10.1016/j.neurobiolaging.2010.06.019

Grimm, A., Friedland, K., and Eckert, A. (2016a). Mitochondrial dysfunction: the missing link between aging and sporadic Alzheimer's disease. *Biogerontology* 17, 281–296. doi: 10.1007/s10522-015-9618-4

Grimm, A., Mensah-Nyagan, A. G., and Eckert, A. (2016b). Alzheimer, mitochondria and gender. *Neurosci. Biobehav. Rev.* 67, 89–101. doi: 10.1016/j.neubiorev.2016.04.012

Grossman, L., and Wei, Q. (1995). DNA repair and epidemiology of basal cell carcinoma. *Clin. Chem.* 41, 1854–1863.

Gyenes, A., Hoyk, Z., Csakvari, E., Siklos, L., and Parducz, A. (2010). 17β-estradiol attenuates injury-induced microglia activation in the oculomotor nucleus. *Neuroscience* 171, 677–682. doi: 10.1016/j.neuroscience.2010.09.033

Habib, P., and Beyer, C. (2015). Regulation of brain microglia by female gonadal steroids. *J. Steroid Biochem. Mol. Biol.* 146, 3–14. doi: 10.1016/j.jsbmb.2014.02.018

Habib, S. L., Yadav, A., Kidane, D., Weiss, R. H., and Liang, S. (2016). Novel protective mechanism of reducing renal cell damage in diabetes: activation AMPK by AICAR increased NRF2/OGG1 proteins and reduced oxidative DNA damage. *Cell Cycle* 15, 3048–3059. doi: 10.1080/15384101.2016.1231259

Hara, Y., Waters, E. M., McEwen, B. S., and Morrison, J. H. (2015). Estrogen effects on cognitive and synaptic health over the lifecourse. *Physiol. Rev.* 95, 785–807. doi: 10.1152/physrev.00036.2014

Hara, Y., Yuk, F., Puri, R., Janssen, W. G., Rapp, P. R., and Morrison, J. H. (2014). Presynaptic mitochondrial morphology in monkey prefrontal cortex correlates with working memory and is improved with estrogen treatment. *Proc. Natl. Acad. Sci. U S A* 111, 486–491. doi: 10.1073/pnas.1311310110

Heldt, S. A., Stanek, L., Chhatwal, J. P., and Ressler, K. J. (2007). Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol. Psychiatry* 12, 656–670. doi: 10.1038/sj.mp.4001957

Hellner, K., Walther, T., Schubert, M., and Albrecht, D. (2005). Angiotensin-(1–7) enhances LTP in the hippocampus through the G-protein-coupled receptor Mas. *Mol. Cell. Neurosci.* 29, 427–435. doi: 10.1016/j.mcn.2005.03.012

Herrick, S. P., Waters, E. M., Drake, C. T., McEwen, B. S., and Milner, T. A. (2006). Extranuclear estrogen receptor β immunoreactivity is on doublecortin-containing cells in the adult and neonatal rat dentate gyrus. *Brain Res.* 1121, 46–58. doi: 10.1016/j.brainres.2006.08.084

Hollensworth, S. B., Shen, C., Sim, J. E., Spitz, D. R., Wilson, G. L., and Ledoux, S. P. (2000). Glial cell type-specific responses to menadione-induced oxidative stress. *Free Radic. Biol. Med.* 28, 1161–1174. doi: 10.1016/s0891-5849(00)00214-8

Huang, R., and Poduslo, S. E. (2006). CYP19 haplotypes increase risk for Alzheimer's disease. *J. Med. Genet.* 43:e42. doi: 10.1136/jmg.2005.039461

Huang, Y. H., and Zhang, Q. H. (2010). Genistein reduced the neural apoptosis in the brain of ovariectomised rats by modulating mitochondrial oxidative stress. *Br. J. Nutr.* 104, 1297–1303. doi: 10.1017/s0007114510002291

Huffman, J., Hoffmann, C., and Taylor, G. T. (2017). Integrating insulin-like growth factor 1 and sex hormones into neuroprotection: implications for diabetes. *World J. Diabetes* 8, 45–55. doi: 10.4239/wjd.v8.i2.45

Hwang, Y. P., and Jeong, H. G. (2010). Ginsenoside Rb1 protects against 6-hydroxydopamine-induced oxidative stress by increasing heme oxygenase-1 expression through an estrogen receptor-related PI3K/Akt/Nrf2-dependent pathway in human dopaminergic cells. *Toxicol. Appl. Pharmacol.* 242, 18–28. doi: 10.1016/j.taap.2009.09.009

Iida, T., Furuta, A., Nishioka, K., Nakabeppu, Y., and Iwaki, T. (2002). Expression of 8-oxoguanine DNA glycosylase is reduced and associated with neurofibrillary tangles in Alzheimer's disease brain. *Acta Neuropathol.* 103, 20–25. doi: 10.1007/s004010100418

Iivonen, S., Corder, E., Lehtovirta, M., Helisalmi, S., Mannermaa, A., Vepsäläinen, S., et al. (2004). Polymorphisms in the CYP19 gene confer increased risk for Alzheimer disease. *Neurology* 62, 1170–1176. doi: 10.1212/01.WNL.0000118208.16939.60

Imam, S. Z., Karahalil, B., Hogue, B. A., Souza-Pinto, N. C., and Bohr, V. A. (2006). Mitochondrial and nuclear DNA-repair capacity of various brain regions in mouse is altered in an age-dependent manner. *Neurobiol. Aging* 27, 1129–1136. doi: 10.1016/j.neurobiolaging.2005.06.002

Irwin, R. W., Yao, J., Ahmed, S. S., Hamilton, R. T., Cadenas, E., and Brinton, R. D. (2011). Medroxyprogesterone acetate antagonizes estrogen up-regulation of brain mitochondrial function. *Endocrinology* 152, 556–567. doi: 10.1210/en.2010-1061

Irwin, R. W., Yao, J., To, J., Hamilton, R. T., Cadenas, E., and Brinton, R. D. (2012). Selective oestrogen receptor modulators differentially potentiate brain mitochondrial function. *J. Neuroendocrinol.* 24, 236–248. doi: 10.1111/j.1365-2826.2011.02251.x

Ishihara, Y., Fujitani, N., Sakurai, H., Takemoto, T., Ikeda-Ishihara, N., Mori-Yasumoto, K., et al. (2016). Effects of sex steroid hormones and their metabolites on neuronal injury caused by oxygen-glucose deprivation/reoxygenation in organotypic hippocampal slice cultures. *Steroids* 113, 71–77. doi: 10.1016/j.steroids.2016.06.004

Iyama, T., and Wilson, D. M. III. (2013). DNA repair mechanisms in dividing and non-dividing cells. *DNA Repair* 12, 620–636. doi: 10.1016/j.dnarep.2013.04.015

Jack, C. R. Jr., Wiste, H. J., Weigand, S. D., Knopman, D. S., Vemuri, P., Mielke, M. M., et al. (2015). Age, sex, and *APOE* ε4 effects on memory, brain structure, and β-amyloid across the adult life span. *JAMA Neurol.* 72, 511–519. doi: 10.1001/jamaneurol.2014.4821

Jacome, L. F., Barateli, K., Buitrago, D., Lema, F., Frankfurt, M., and Luine, V. N. (2016). Gonadal hormones rapidly enhance spatial memory and increase hippocampal spine density in male rats. *Endocrinology* 157, 1357–1362. doi: 10.1210/en.2015-1959

Jayakumar, S., Pal, D., and Sandur, S. K. (2015). Nrf2 facilitates repair of radiation induced DNA damage through homologous recombination repair pathway in a ROS independent manner in cancer cells. *Mutat. Res.* 779, 33–45. doi: 10.1016/j.mrfmmm.2015.06.007

Jeppesen, D. K., Bohr, V. A., and Stevnsner, T. (2011). DNA repair deficiency in neurodegeneration. *Prog. Neurobiol.* 94, 166–200. doi: 10.1016/j.pneurobio.2011.04.013

Jia, J. X., Cui, C. L., Yan, X. S., Zhang, B. F., Song, W., Huo, D. S., et al. (2016). Effects of testosterone on synaptic plasticity mediated by androgen receptors in male SAMP8 mice. *J. Toxicol. Environ. Health Part A* 79, 849–855. doi: 10.1080/15287394.2016.1193113

Jia, J., Kang, L., Li, S., Geng, D., Fan, P., Wang, L., et al. (2013). Amelioratory effects of testosterone treatment on cognitive performance deficits induced by soluble Aβ1–42 oligomers injected into the hippocampus. *Horm. Behav.* 64, 477–486. doi: 10.1016/j.yhbeh.2013.08.002

Jiricny, J. (2006). The multifaceted mismatch-repair system. *Nat. Rev. Mol. Cell Biol.* 7, 335–346. doi: 10.1038/nrm1907

Johri, A., and Beal, M. F. (2012). Mitochondrial dysfunction in neurodegenerative diseases. *J. Pharmacol. Exp. Ther.* 342, 619–630. doi: 10.1124/jpet.112.192138

Kang, D., Kim, S. H., and Hamasaki, N. (2007). Mitochondrial transcription factor A (TFAM): roles in maintenance of mtDNA and cellular functions. *Mitochondrion* 7, 39–44. doi: 10.1016/j.mito.2006.11.017

Katyal, S., Lee, Y., Nitiss, K. C., Downing, S. M., Li, Y., Shimada, M., et al. (2014). Aberrant topoisomerase-1 DNA lesions are pathogenic in neurodegenerative genome instability syndromes. *Nat. Neurosci.* 17, 813–821. doi: 10.1038/nn.3715

Kaur, P., Jodhka, P. K., Underwood, W. A., Bowles, C. A., de Fiebre, N. C., de Fiebre, C. M., et al. (2007). Progesterone increases brain-derived neuroptrophic factor expression and protects against glutamate toxicity in a mitogen-activated protein kinase- and phosphoinositide-3 kinase-dependent manner in cerebral cortical explants. *J. Neurosci. Res.* 85, 2441–2449. doi: 10.1002/jnr.21370

Kelly, J. F., Bienias, J. L., Shah, A., Meeke, K. A., Schneider, J. A., Soriano, E., et al. (2008). Levels of estrogen receptors α and β in frontal cortex of patients with Alzheimer's disease: relationship to Mini-Mental State Examination scores. *Curr. Alzheimer Res.* 5, 45–51. doi: 10.2174/156720508783884611

Kelly, M. J., and Levin, E. R. (2001). Rapid actions of plasma membrane estrogen receptors. *Trends Endocrinol. Metab.* 12, 152–156. doi: 10.1016/s1043-2760(01)00377-0

Kemper, M. F., Stirone, C., Krause, D. N., Duckles, S. P., and Procaccio, V. (2014). Genomic and non-genomic regulation of PGC1 isoforms by estrogen to increase cerebral vascular mitochondrial biogenesis and reactive oxygen species protection. *Eur. J. Pharmacol.* 723, 322–329. doi: 10.1016/j.ejphar.2013.11.009

Kemper, M. F., Zhao, Y., Duckles, S. P., and Krause, D. N. (2013). Endogenous ovarian hormones affect mitochondrial efficiency in cerebral endothelium via distinct regulation of PGC-1 isoforms. *J. Cereb. Blood Flow Metab.* 33, 122–128. doi: 10.1038/jcbfm.2012.159

Kerr, D. S., Bevilaqua, L. R., Bonini, J. S., Rossato, J. I., Köhler, C. A., Medina, J. H., et al. (2005). Angiotensin II blocks memory consolidation through an AT2 receptor-dependent mechanism. *Psychopharmacology* 179, 529–535. doi: 10.1007/s00213-004-2074-5

Kireev, R. A., Vara, E., Vina, J., and Tresguerres, J. A. (2014). Melatonin and oestrogen treatments were able to improve neuroinflammation and apoptotic processes in dentate gyrus of old ovariectomized female rats. *Age* 36:9707. doi: 10.1007/s11357-014-9707-3

Kisby, G. E., Kohama, S. G., Olivas, A., Churchwell, M., Doerge, D., Spangler, E., et al. (2010). Effect of caloric restriction on base-excision repair (BER) in the aging rat brain. *Exp. Gerontol.* 45, 208–216. doi: 10.1016/j.exger.2009.12.003

Kiss, A., Delattre, A. M., Pereira, S. I., Carolino, R. G., Szawka, R. E., Anselmo-Franci, J. A., et al. (2012). 17β-estradiol replacement in young, adult and middle-aged female ovariectomized rats promotes improvement of spatial reference memory and an antidepressant effect and alters monoamines and BDNF levels in memory- and depression-related brain areas. *Behav. Brain Res.* 227, 100–108. doi: 10.1016/j.bbr.2011.10.047

Klinge, C. M. (2017). Estrogens regulate life and death in mitochondria. *J. Bioenerg. Biomembr.* 49, 307–324. doi: 10.1007/s10863-017-9704-1

Kovtun, I. V., Liu, Y., Bjoras, M., Klungland, A., Wilson, S. H., and McMurray, C. T. (2007). OGG1 initiates age-dependent CAG trinucleotide expansion in somatic cells. *Nature* 447, 447–452. doi: 10.1038/nature05778

Kowal, S. L., Dall, T. M., Chakrabarti, R., Storm, M. V., and Jain, A. (2013). The current and projected economic burden of Parkinson's disease in the United States. *Mov. Disord.* 28, 311–318. doi: 10.1002/mds.25292

Kujoth, G. C., Bradshaw, P. C., Haroon, S., and Prolla, T. A. (2007). The role of mitochondrial DNA mutations in mammalian aging. *PLoS Genet.* 3:e24. doi: 10.1371/journal.pgen.0030024

Kulijewicz-Nawrot, M., Verkhratsky, A., Chvátal, A., Syková, E., and Rodríguez, J. J. (2012). Astrocytic cytoskeletal atrophy in the medial prefrontal cortex of a triple transgenic mouse model of Alzheimer's disease. *J. Anat.* 221, 252–262. doi: 10.1111/j.1469-7580.2012.01536.x

Labandeira-Garcia, J. L., Rodríguez-Perez, A. I., Garrido-Gil, P., Rodriguez-Pallares, J., Lanciego, J. L., and Guerra, M. J. (2017). Brain renin-angiotensin system and microglial polarization: implications for aging and neurodegeneration. *Front. Aging Neurosci.* 9:129. doi: 10.3389/fnagi.2017.00129

Labandeira-Garcia, J. L., Rodríguez-Perez, A. I., Valenzuela, R., Costa-Besada, M. A., and Guerra, M. J. (2016). Menopause and Parkinson's disease. Interaction between estrogens and brain renin-angiotensin system in dopaminergic degeneration. *Front. Neuroendocrinol.* 43, 44–59. doi: 10.1016/j.yfrne.2016.09.003

Leclère, R., Torregrosa-Muñumer, R., Kireev, R., García, C., Vara, E., Tresguerres, J. A., et al. (2013). Effect of estrogens on base excision repair in brain and liver mitochondria of aged female rats. *Biogerontology* 14, 383–394. doi: 10.1007/s10522-013-9431-x

Lei, D. L., Long, J. M., Hengemihle, J., O'Neill, J., Manaye, K. F., Ingram, D. K., et al. (2003). Effects of estrogen and raloxifene on neuroglia number and morphology in the hippocampus of aged female mice. *Neuroscience* 121, 659–666. doi: 10.1016/s0306-4522(03)00245-8

Leranth, C., Petnehazy, O., and MacLusky, N. J. (2003). Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *J. Neurosci.* 23, 1588–1592.

Li, L., Chen, J., Sun, S., Zhao, J., Dong, X., and Wang, J. (2017). Effects of estradiol on autophagy and Nrf-2/ARE signals after cerebral ischemia. *Cell. Physiol. Biochem.* 41, 2027–2036. doi: 10.1159/000475433

Li, R., He, P., Cui, J., Staufenbiel, M., Harada, N., and Shen, Y. (2013). Brain endogenous estrogen levels determine responses to estrogen replacement therapy via regulation of BACE1 and NEP in female Alzheimer's transgenic mice. *Mol. Neurobiol.* 47, 857–867. doi: 10.1007/s12035-012-8377-3

Li, S., Kang, L., Zhang, Y., Feng, B., Du, J., and Cui, H. (2015). Detecting the presence of hippocampus membrane androgen receptors in male SAMP8 mice and their induced synaptic plasticity. *Mol. Cell. Endocrinol.* 414, 82–90. doi: 10.1016/j.mce.2015.07.005

Li, M., Masugi-Tokita, M., Takanami, K., Yamada, S., and Kawata, M. (2012). Testosterone has sublayer-specific effects on dendritic spine maturation mediated by BDNF and PSD-95 in pyramidal neurons in the hippocampus CA1 area. *Brain Res.* 1484, 76–84. doi: 10.1016/j.brainres.2012.09.028

Li, M. X., Shan, J. L., Wang, D., He, Y., Zhou, Q., Xia, L., et al. (2012). Human apurinic/apyrimidinic endonuclease 1 translocalizes to mitochondria after photodynamic therapy and protects cells from apoptosis. *Cancer Sci.* 103, 882–888. doi: 10.1111/j.1349-7006.2012.02239.x

Li, R., and Singh, M. (2014). Sex differences in cognitive impairment and Alzheimer's disease. *Front. Neuroendocrinol.* 35, 385–403. doi: 10.1016/j.yfrne.2014.01.002

Lillenes, M. S., Espeseth, T., Støen, M., Lundervold, A. J., Frye, S. A., Rootwelt, H., et al. (2011). DNA base excision repair gene polymorphisms modulate human cognitive performance and decline during normal life span. *Mech. Ageing Dev.* 132, 449–458. doi: 10.1016/j.mad.2011.08.002

Lillenes, M. S., Støen, M., Günther, C. C., Selnes, P., Stenset, V. T., Espeseth, T., et al. (2017). Mitochondrial transcription factor A (TFAM) rs1937 and AP endonuclease 1 (APE1) rs1130409 alleles are associated with reduced cognitive performance. *Neurosci. Lett.* 645, 46–52. doi: 10.1016/j.neulet.2017.02.062

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. *Nature* 362, 709–715. doi: 10.1038/362709a0

Liu, P., and Demple, B. (2010). DNA repair in mammalian mitochondria: much more than we thought? *Environ. Mol. Mutagen.* 51, 417–426. doi: 10.1002/em.20576

Lovell, M. A., and Markesbery, W. R. (2007). Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. *Nucleic Acids Res.* 35, 7497–7504. doi: 10.1093/nar/gkm821

Lovell, M. A., Xie, C., and Markesbery, W. R. (2000). Decreased base excision repair and increased helicase activity in Alzheimer's disease brain. *Brain Res.* 855, 116–123. doi: 10.1016/s0006-8993(99)02335-5

Lu, J. Y., Jin, P., Gao, W., Wang, D. Z., and Sheng, J. Q. (2017). Estrogen enhances mismatch repair by induction of MLH1 expression via estrogen receptor-β. *Oncotarget* 8, 38767–38779. doi: 10.18632/oncotarget.16351

Lu, H., Ma, K., Jin, L., Zhu, H., and Cao, R. (2018). 17β-estradiol rescues damages following traumatic brain injury from molecule to behavior in mice. *J. Cell. Physiol.* 233, 1712–1722. doi: 10.1002/jcp.26083

Lu, T., Pan, Y., Kao, S. Y., Li, C., Kohane, I., Chan, J., et al. (2004). Gene regulation and DNA damage in the ageing human brain. *Nature* 429, 883–891. doi: 10.1038/nature02661

Lu, J., Xu, Y., Hu, W., Gao, Y., Ni, X., Sheng, H., et al. (2014). Exercise ameliorates depression-like behavior and increases hippocampal BDNF level in ovariectomized rats. *Neurosci. Lett.* 573, 13–18. doi: 10.1016/j.neulet.2014.04.053

Luchetti, S., Bossers, K., Van De Bilt, S., Agrapart, V., Morales, R. R., Frajese, G. V., et al. (2011). Neurosteroid biosynthetic pathways changes in prefrontal cortex in Alzheimer's disease. *Neurobiol. Aging* 32, 1964–1976. doi: 10.1016/j.neurobiolaging.2009.12.014

Lynch, A. M., Murphy, K. J., Deighan, B. F., O'Reilly, J. A., Gun'ko, Y. K., Cowley, T. R., et al. (2010). The impact of glial activation in the aging brain. *Aging Dis.* 1, 262–278.

Maclusky, N. J., Hajszan, T., Prange-Kiel, J., and Leranth, C. (2006). Androgen modulation of hippocampal synaptic plasticity. *Neuroscience* 138, 957–965. doi: 10.1016/j.neuroscience.2005.12.054

Maki, P. M., and Resnick, S. M. (2000). Longitudinal effects of estrogen replacement therapy on PET cerebral blood flow and cognition. *Neurobiol. Aging* 21, 373–383. doi: 10.1016/s0197-4580(00)00123-8

Mandal, P. K., Tripathi, M., and Sugunan, S. (2012). Brain oxidative stress: detection and mapping of anti-oxidant marker 'Glutathione' in different brain regions of healthy male/female, MCI and Alzheimer patients using non-invasive magnetic resonance spectroscopy. *Biochem. Biophys. Res. Commun.* 417, 43–48. doi: 10.1016/j.bbrc.2011.11.047

Mangold, C. A., Wronowski, B., Du, M., Masser, D. R., Hadad, N., Bixler, G. V., et al. (2017). Sexually divergent induction of microglial-associated neuroinflammation with hippocampal aging. *J. Neuroinflammation* 14:141. doi: 10.1186/s12974-017-0920-8

Marin-Husstege, M., Muggironi, M., Raban, D., Skoff, R. P., and Casaccia-Bonnefil, P. (2004). Oligodendrocyte progenitor proliferation and maturation is differentially regulated by male and female sex steroid hormones. *Dev. Neurosci.* 26, 245–254. doi: 10.1159/000082141

May, M. (2016). Sex on the brain: unraveling the differences between women and men in neurodegenerative disease. *Nat. Med.* 22, 1370–1372. doi: 10.1038/nm1216-1370

McCullough, L. D., Blizzard, K., Simpson, E. R., Oz, O. K., and Hurn, P. D. (2003). Aromatase cytochrome P450 and extragonadal estrogen play a role in ischemic neuroprotection. *J. Neurosci.* 23, 8701–8705.

McDevitt, M. A., Glidewell-Kenney, C., Jimenez, M. A., Ahearn, P. C., Weiss, J., Jameson, J. L., et al. (2008). New insights into the classical and non-classical actions of estrogen: evidence from estrogen receptor knock-out and knock-in mice. *Mol. Cell. Endocrinol.* 290, 24–30. doi: 10.1016/j.mce.2008.04.003

McEwen, B. S., and Milner, T. A. (2007). Hippocampal formation: shedding light on the influence of sex and stress on the brain. *Brain Res. Rev.* 55, 343–355. doi: 10.1016/j.brainresrev.2007.02.006

McEwen, B. S., and Milner, T. A. (2017). Understanding the broad influence of sex hormones and sex differences in the brain. *J. Neurosci. Res.* 95, 24–39. doi: 10.1002/jnr.23809

McKinnon, P. J. (2016). Topoisomerases and the regulation of neural function. *Nat. Rev. Neurosci.* 17, 673–679. doi: 10.1038/nrn.2016.101

McLachlan, R. I., Tempel, B. L., Miller, M. A., Bicknell, J. N., Bremner, W. J., and Dorsa, D. M. (1991). Androgen receptor gene expression in the rat central nervous system: evidence for two mRNA transcripts. *Mol. Cell. Neurosci.* 2, 117–122. doi: 10.1016/1044-7431(91)90003-7

Melcangi, R. C., Giatti, S., Calabrese, D., Pesaresi, M., Cermenati, G., Mitro, N., et al. (2014). Levels and actions of progesterone and its metabolites in the nervous system during physiological and pathological conditions. *Prog. Neurobiol.* 113, 56–69. doi: 10.1016/j.pneurobio.2013.07.006

Melov, S. (2004). Modeling mitochondrial function in aging neurons. *Trends Neurosci.* 27, 601–606. doi: 10.1016/j.tins.2004.08.004

Mena, M. A., and García de Yébenes, J. (2008). Glial cells as players in parkinsonism: the "good," the "bad," and the "mysterious" glia. *Neuroscientist* 14, 544–560. doi: 10.1177/1073858408322839

Meydan, S., Kus, I., Tas, U., Ogeturk, M., Sancakdar, E., Dabak, D. O., et al. (2010). Effects of testosterone on orchiectomy-induced oxidative damage in the rat hippocampus. *J. Chem. Neuroanat.* 40, 281–285. doi: 10.1016/j.jchemneu.2010.07.006

Miller, V. M., and Harman, S. M. (2017). An update on hormone therapy in postmenopausal women: mini-review for the basic scientist. *Am. J. Physiol. Heart Circ. Physiol.* 313, H1013–H1021. doi: 10.1152/ajpheart.00383.2017

Milner, T. A., Ayoola, K., Drake, C. T., Herrick, S. P., Tabori, N. E., McEwen, B. S., et al. (2005). Ultrastructural localization of estrogen receptor β immunoreactivity in the rat hippocampal formation. *J. Comp. Neurol.* 491, 81–95. doi: 10.1002/cne.20724

Milner, T. A., Lubbers, L. S., Alves, S. E., and McEwen, B. S. (2008). Nuclear and extranuclear estrogen binding sites in the rat forebrain and autonomic medullary areas. *Endocrinology* 149, 3306–3312. doi: 10.1210/en.2008-0307

Mitra, S., Izumi, T., Boldogh, I., Bhakat, K. K., Chattopadhyay, R., and Szczesny, B. (2007). Intracellular trafficking and regulation of mammalian AP-endonuclease 1 (APE1), an essential DNA repair protein. *DNA Repair (Amst)* 6, 461–469. doi: 10.1016/j.dnarep.2006.10.010

Mo, M. S., Li, H. B., Wang, B. Y., Wang, S. L., Zhu, Z. L., and Yu, X. R. (2013). PI3K/Akt and NF-kappaB activation following intravitreal administration of 17β-estradiol: neuroprotection of the rat retina from light-induced apoptosis. *Neuroscience* 228, 1–12. doi: 10.1016/j.neuroscience.2012.10.002

Monteiro-Cardoso, V. F., Oliveira, M. M., Melo, T., Domingues, M. R., Moreira, P. I., Ferreiro, E., et al. (2015). Cardiolipin profile changes are associated to the early synaptic mitochondrial dysfunction in Alzheimer's disease. *J. Alzheimers Dis.* 43, 1375–1392. doi: 10.3233/JAD-141002

Morrison, J. H., Brinton, R. D., Schmidt, P. J., and Gore, A. C. (2006). Estrogen, menopause, and the aging brain: how basic neuroscience can inform hormone therapy in women. *J. Neurosci.* 26, 10332–10348. doi: 10.1523/JNEUROSCI.3369-06.2006

Muslimovic, A., Ismail, I. H., Gao, Y., and Hammarsten, O. (2008). An optimized method for measurement of γ-H2AX in blood mononuclear and cultured cells. *Nat. Protoc.* 3, 1187–1193. doi: 10.1038/nprot.2008.93

Naugle, M. M., Nguyen, L. T., Merceron, T. K., Filardo, E., Janssen, W. G., Morrison, J. H., et al. (2014). G-protein coupled estrogen receptor, estrogen receptor α, and progesterone receptor immunohistochemistry in the hypothalamus of aging female rhesus macaques given long-term estradiol treatment. *J. Exp. Zool. A Ecol. Genet. Physiol.* 321, 399–414. doi: 10.1002/jez.1871

Navarro, A., Lopez-Cepero, J. M., Bandez, M. J., Sanchez-Pino, M. J., Gomez, C., Cadenas, E., et al. (2008). Hippocampal mitochondrial dysfunction in rat aging. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R501–R509. doi: 10.1152/ajpregu.00492.2007

Nilsen, J., and Diaz Brinton, R. (2003). Mechanism of estrogen-mediated neuroprotection: regulation of mitochondrial calcium and Bcl-2 expression. *Proc. Natl. Acad. Sci. U S A* 100, 2842–2847. doi: 10.1073/pnas.0438041100

Nilsen, J., Irwin, R. W., Gallaher, T. K., and Brinton, R. D. (2007). Estradiol *in vivo* regulation of brain mitochondrial proteome. *J. Neurosci.* 27, 14069–14077. doi: 10.1523/JNEUROSCI.4391-07.2007

Nissen, J. C. (2017). Microglial function across the spectrum of age and gender. *Int. J. Mol. Sci.* 18:E561. doi: 10.3390/ijms18030561

Nilsson, S., Mäkelä, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., et al. (2001). Mechanisms of estrogen action. *Physiol. Rev.* 81, 1535–1565. doi: 10.1152/physrev.2001.81.4.1535

Noorbakhsh, F., Baker, G. B., and Power, C. (2014). Allopregnanolone and neuroinflammation: a focus on multiple sclerosis. *Front. Cell. Neurosci.* 8:134. doi: 10.3389/fncel.2014.00134

Oddo, S., Caccamo, A., Shepherd, J. D., Murphy, M. P., Golde, T. E., Kayed, R., et al. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Aβ and synaptic dysfunction. *Neuron* 39, 409–421. doi: 10.1016/S0896-6273(03)00434-3

Olabarria, M., Noristani, H. N., Verkhratsky, A., and Rodríguez, J. J. (2011). Age-dependent decrease in glutamine synthetase expression in the hippocampal astroglia of the triple transgenic Alzheimer's disease mouse model: mechanism for deficient glutamatergic transmission? *Mol. Neurodegener.* 6:55. doi: 10.1186/1750-1326-6-55

Overk, C. R., Lu, P. Y., Wang, Y. T., Choi, J., Shaw, J. W., Thatcher, G. R., et al. (2012). Effects of aromatase inhibition versus gonadectomy on hippocampal complex amyloid pathology in triple transgenic mice. *Neurobiol. Dis.* 45, 479–487. doi: 10.1016/j.nbd.2011.08.035

Pamplona, R. (2008). Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity. *Biochim. Biophys. Acta* 1777, 1249–1262. doi: 10.1016/j.bbabio.2008.07.003

Pan, W., Han, S., Kang, L., Li, S., Du, J., and Cui, H. (2016). Effects of dihydrotestosterone on synaptic plasticity of the hippocampus in mild cognitive impairment male SAMP8 mice. *Exp. Ther. Med.* 12, 1455–1463. doi: 10.3892/etm.2016.3470

Paradies, G., Petrosillo, G., Paradies, V., and Ruggiero, F. M. (2011). Mitochondrial dysfunction in brain aging: role of oxidative stress and cardiolipin. *Neurochem. Int.* 58, 447–457. doi: 10.1016/j.neuint.2010.12.016

Paris, J. J., Walf, A. A., and Frye, C. A. (2011). II. Cognitive performance of middle-aged female rats is influenced by capacity to metabolize progesterone in the prefrontal cortex and hippocampus. *Brain Res.* 1379, 149–163. doi: 10.1016/j.brainres.2010.10.099

Perovic, M., Tesic, V., Mladenovic Djordjevic, A., Smiljanic, K., Loncarevic-Vasiljkovic, N., Ruzdijic, S., et al. (2013). BDNF transcripts, proBDNF and proNGF, in the cortex and hippocampus throughout the life span of the rat. *Age* 35, 2057–2070. doi: 10.1007/s11357-012-9495-6

Petersen, S. L., Intlekofer, K. A., Moura-Conlon, P. J., Brewer, D. N., Del Pino Sans, J., and Lopez, J. A. (2013). Novel progesterone receptors: neural localization and possible functions. *Front. Neurosci.* 7:164. doi: 10.3389/fnins.2013.00164

Pike, C. J., Carroll, J. C., Rosario, E. R., and Barron, A. M. (2009). Protective actions of sex steroid hormones in Alzheimer's disease. *Front. Neuroendocrinol.* 30, 239–258. doi: 10.1016/j.yfrne.2009.04.015

Promislow, D. E. L. (1991). Senescence in natural populations of mammals: a comparative study. *Evolution* 45, 1869–1887. doi: 10.1111/j.1558-5646.1991.tb02693.x

Psarra, A. M., and Sekeris, C. E. (2008). Steroid and thyroid hormone receptors in mitochondria. *IUBMB Life* 60, 210–223. doi: 10.1002/iub.37

Puche, J. E., García-Fernández, M., Muntané, J., Rioja, J., González-Barón, S., and Castilla Cortazar, I. (2008). Low doses of insulin-like growth factor-I induce mitochondrial protection in aging rats. *Endocrinology* 149, 2620–2627. doi: 10.1210/en.2007-1563

Rao, A. K., Dietrich, A. K., Ziegler, Y. S., and Nardulli, A. M. (2011). 17β-Estradiol-mediated increase in Cu/Zn superoxide dismutase expression in the brain: a mechanism to protect neurons from ischemia. *J. Steroid Biochem. Mol. Biol.* 127, 382–389. doi: 10.1016/j.jsbmb.2011.06.008

Rasgon, N. L., Silverman, D., Siddarth, P., Miller, K., Ercoli, L. M., Elman, S., et al. (2005). Estrogen use and brain metabolic change in postmenopausal women. *Neurobiol. Aging* 26, 229–235. doi: 10.1016/j.neurobiolaging.2004.03.003

Regan, J. C., and Partridge, L. (2013). Gender and longevity: why do men die earlier than women? Comparative and experimental evidence. *Best Pract. Res. Clin. Endocrinol. Metab.* 27, 467–479. doi: 10.1016/j.beem.2013.05.016

Rekkas, P. V., Wilson, A. A., Lee, V. W., Yogalingam, P., Sacher, J., Rusjan, P., et al. (2014). Greater monoamine oxidase a binding in perimenopausal age as measured with carbon 11-labeled harmine positron emission tomography. *JAMA Psychiatry* 71, 873–879. doi: 10.1001/jamapsychiatry.2014.250

Rettberg, J. R., Yao, J., and Brinton, R. D. (2014). Estrogen: a master regulator of bioenergetic systems in the brain and body. *Front. Neuroendocrinol.* 35, 8–30. doi: 10.1016/j.yfrne.2013.08.001

Rey, P., Lopez-Real, A., Sanchez-Iglesias, S., Muñoz, A., Soto-Otero, R., and Labandeira-Garcia, J. L. (2007). Angiotensin type-1-receptor antagonists reduce 6-hydroxydopamine toxicity for dopaminergic neurons. *Neurobiol. Aging* 28, 555–567. doi: 10.1016/j.neurobiolaging.2006.02.018

Robertson, A. B., Klungland, A., Rognes, T., and Leiros, I. (2009). DNA repair in mammalian cells: base excision repair: the long and short of it. *Cell. Mol. Life Sci.* 66, 981–993. doi: 10.1007/s00018-009-8736-z

Rocca, W. A., Bower, J. H., Maraganore, D. M., Ahlskog, J. E., Grossardt, B. R., de Andrade, M., et al. (2008). Increased risk of parkinsonism in women who underwent oophorectomy before menopause. *Neurology* 70, 200–209. doi: 10.1212/01.WNL.0000280573.30975.6a

Rodier, F., Coppe, J. P., Patil, C. K., Hoeijmakers, W. A., Muñoz, D. P., Raza, S. R., et al. (2009). Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat. Cell Biol.* 11, 973–979. doi: 10.1038/ncb1909

Rodriguez-Pallares, J., Rey, P., Parga, J. A., Muñoz, A., Guerra, M. J., and Labandeira-Garcia, J. L. (2008). Brain angiotensin enhances dopaminergic cell

death via microglial activation and NADPH-derived ROS. *Neurobiol. Dis.* 31, 58–73. doi: 10.1016/j.nbd.2008.03.003

Rodriguez-Perez, A. I., Valenzuela, R., Villar-Cheda, B., Guerra, M. J., Lanciego, J. L., and Labandeira-Garcia, J. L. (2010). Estrogen and angiotensin interaction in the substantia nigra. Relevance to postmenopausal Parkinson's disease. *Exp. Neurol.* 224, 517–526. doi: 10.1016/j.expneurol.2010.05.015

Rosario, E. R., Chang, L., Head, E. H., Stanczyk, F. Z., and Pike, C. J. (2011). Brain levels of sex steroid hormones in men and women during normal aging and in Alzheimer's disease. *Neurobiol. Aging* 32, 604–613. doi: 10.1016/j.neurobiolaging.2009.04.008

Rosario, E. R., Ramsden, M., and Pike, C. J. (2006). Progestins inhibit the neuroprotective effects of estrogen in rat hippocampus. *Brain Res.* 1099, 206–210. doi: 10.1016/j.brainres.2006.03.127

Rossetti, M. F., Cambiasso, M. J., Holschbach, M. A., and Cabrera, R. (2016). Oestrogens and progestagens: synthesis and action in the brain. *J. Neuroendocrinol.* 28:7. doi: 10.1111/jne.12402

Rossi, D., Brambilla, L., Valori, C. F., Roncoroni, C., Crugnola, A., Yokota, T., et al. (2008). Focal degeneration of astrocytes in amyotrophic lateral sclerosis. *Cell Death Differ.* 15, 1691–1700. doi: 10.1038/cdd.2008.99

Rutten, B. P., Schmitz, C., Gerlach, O. H., Oyen, H. M., de Mesquita, E. B., Steinbusch, H. W., et al. (2007). The aging brain: accumulation of DNA damage or neuron loss? *Cell. Physiol. Biochem.* 28, 91–98. doi: 10.1016/j.neurobiolaging.2005.10.019

Salminen, A., Ojala, J., Kaarniranta, K., Haapasalo, A., Hiltunen, M., and Soininen, H. (2011). Astrocytes in the aging brain express characteristics of senescence-associated secretory phenotype. *Eur. J. Neurosci.* 34, 3–11. doi: 10.1111/j.1460-9568.2011.07738.x

Samarghandian, S., Azimi-Nezhad, M., and Farkhondeh, T. (2016). Preventive effect of carvacrol against oxidative damage in aged rat liver. *Int. J. Vitam. Nutr. Res.* doi: 10.1024/0300-9831/a000393 [Epub ahead of print].

Sanders, L. H., McCoy, J., Hu, X., Mastroberardino, P. G., Dickinson, B. C., Chang, C. J., et al. (2014). Mitochondrial DNA damage: molecular marker of vulnerable nigral neurons in Parkinson's disease. *Neurobiol. Dis.* 70, 214–223. doi: 10.1016/j.nbd.2014.06.014

Sarkey, S., Azcoitia, I., Garcia-Segura, L. M., Garcia-Ovejero, D., and DonCarlos, L. L. (2008). Classical androgen receptors in non-classical sites in the brain. *Horm. Behav.* 53, 753–764. doi: 10.1016/j.yhbeh.2008.02.015

Sárvári, M., Hrabovszky, E., Kallo, I., Solymosi, N., Likó, I., Berchtold, N., et al. (2012). Menopause leads to elevated expression of macrophage-associated genes in the aging frontal cortex: rat and human studies identify strikingly similar changes. *J. Neuroinflammation* 9:264. doi: 10.1186/1742-2094-9-264

Sárvári, M., Hrabovszky, E., Kalló, I., Solymosi, N., Tóth, K., Likó, I., et al. (2011). Estrogens regulate neuroinflammatory genes via estrogen receptors α and β in the frontal cortex of middle-aged female rats. *J. Neuroinflammation* 8:82. doi: 10.1186/1742-2094-8-82

Sárvári, M., Kalló, I., Hrabovszky, E., Solymosi, N., and Liposits, Z. (2014). Ovariectomy and subsequent treatment with estrogen receptor agonists tune the innate immune system of the hippocampus in middle-aged female rats. *PLoS One* 9:e88540. doi: 10.1371/journal.pone.0088540

Saunders-Pullman, R., Gordon-Elliott, J., Parides, M., Fahn, S., Saunders, H. R., and Bressman, S. (1999). The effect of estrogen replacement on early Parkinson's disease. *Neurology* 52, 1417–1421. doi: 10.1212/WNL.52.7.1417

Scarpulla, R. C. (2008). Transcriptional paradigms in mammalian mitochondrial biogenesis and function. *Physiol. Rev.* 88, 611–638. doi: 10.1152/physrev.00025.2007

Scharfman, H. E., and MacLusky, N. J. (2006). Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS. *Front. Neuroendocrinol.* 27, 415–435. doi: 10.1016/j.yfrne.2006.09.004

Schiewer, M. J., and Knudsen, K. E. (2016). Linking DNA damage and hormone signaling pathways in cancer. *Trends Endocrinol. Metab.* 27, 216–225. doi: 10.1016/j.tem.2016.02.004

Schiöth, H. B., Craft, S., Brooks, S. J., Frey, W. H. II., and Benedict, C. (2012). Brain insulin signaling and Alzheimer's disease: current evidence and future directions. *Mol. Neurobiol.* 46, 4–10. doi: 10.1007/s12035-011-8229-6

Schipper, H. M. (1996). Astrocytes, brain aging, and neurodegeneration. *Neurobiol. Aging* 17, 467–480. doi: 10.1016/0197-4580(96)00014-0

Schroots, J. J. F., FernáNdez Ballesteros, R. O., and Rudinger, G. (1999). *Aging in Europe.* Amsterdam; Washington, DC: IOS Press.

Scott, E., Zhang, Q. G., Wang, R., Vadlamudi, R., and Brann, D. (2012). Estrogen neuroprotection and the critical period hypothesis. *Front. Neuroendocrinol.* 33, 85–104. doi: 10.1016/j.yfrne.2011.10.001

Selvamani, A., Sathyan, P., Miranda, R. C., and Sohrabji, F. (2012). An antagomir to microRNA Let7f promotes neuroprotection in an ischemic stroke model. *PLoS One* 7:e32662. doi: 10.1371/journal.pone.0032662

Selvaraj, V., Stocco, D. M., and Tu, L. N. (2015). Minireview: translocator protein (TSPO) and steroidogenesis: a reappraisal. *Mol. Endocrinol.* 29, 490–501. doi: 10.1210/me.2015-1033

Shadyab, A. H., Macera, C. A., Shaffer, R. A., Jain, S., Gallo, L. C., Gass, M. L., et al. (2017). Ages at menarche and menopause and reproductive lifespan as predictors of exceptional longevity in women: the Women's Health Initiative. *Menopause* 24, 35–44. doi: 10.1097/GME.0000000000000710

Shao, C., Xiong, S., Li, G. M., Gu, L., Mao, G., Markesbery, W. R., et al. (2008). Altered 8-oxoguanine glycosylase in mild cognitive impairment and late-stage Alzheimer's disease brain. *Free Radic. Biol. Med.* 45, 813–819. doi: 10.1016/j.freeradbiomed.2008.06.003

Shi, C., Zou, J., Li, G., Ge, Z., Yao, Z., and Xu, J. (2011). Bilobalide protects mitochondrial function in ovariectomized rats by up-regulation of mRNA and protein expression of cytochrome c oxidase subunit I. *J. Mol. Neurosci.* 45, 69–75. doi: 10.1007/s12031-010-9388-z

Shibutani, S., Takeshita, M., and Grollman, A. P. (1991). Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* 349, 431–434. doi: 10.1038/349431a0

Shimada, H., Makizako, H., Doi, T., Yoshida, D., Tsutsumimoto, K., Anan, Y., et al. (2014). A large, cross-sectional observational study of serum BDNF, cognitive function, and mild cognitive impairment in the elderly. *Front. Aging Neurosci.* 6:69. doi: 10.3389/fnagi.2014.00069

Siani, F., Greco, R., Levandis, G., Ghezzi, C., Daviddi, F., Demartini, C., et al. (2017). Influence of estrogen modulation on glia activation in a murine model of Parkinson's disease. *Front. Neurosci.* 11:306. doi: 10.3389/fnins.2017.00306

Simpkins, J. W., Yang, S. H., Sarkar, S. N., and Pearce, V. (2008). Estrogen actions on mitochondria—physiological and pathological implications. *Mol. Cell. Endocrinol.* 290, 51–59. doi: 10.1016/j.mce.2008.04.013

Singh, M., Meyer, E. M., and Simpkins, J. W. (1995). The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. *Endocrinology* 136, 2320–2324. doi: 10.1210/en.136.5.2320

Singh, M., and Su, C. (2013). Progesterone and neuroprotection. *Horm. Behav.* 63, 284–290. doi: 10.1016/j.yhbeh.2012.06.003

Sohrabji, F. (2015). Estrogen-IGF-1 interactions in neuroprotection: ischemic stroke as a case study. *Front. Neuroendocrinol.* 36, 1–14. doi: 10.1016/j.yfrne.2014.05.003

Sohrabji, F., Miranda, R. C., and Toran-Allerand, C. D. (1995). Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci. U S A* 92, 11110–11114. doi: 10.1073/pnas.92.24.11110

Son, S. W., Lee, J. S., Kim, H. G., Kim, D. W., Ahn, Y. C., and Son, C. G. (2016). Testosterone depletion increases the susceptibility of brain tissue to oxidative damage in a restraint stress mouse model. *J. Neurochem.* 136, 106–117. doi: 10.1111/jnc.13371

Sorwell, K. G., Kohama, S. G., and Urbanski, H. F. (2012). Perimenopausal regulation of steroidogenesis in the nonhuman primate. *Neurobiol. Aging* 33, 1487.e1–1487.e13. doi: 10.1016/j.neurobiolaging.2011.05.004

Stirone, C., Duckles, S. P., and Krause, D. N. (2003). Multiple forms of estrogen receptor-α in cerebral blood vessels: regulation by estrogen. *Am. J. Physiol. Endocrinol. Metab.* 284, E184–E192. doi: 10.1152/ajpendo.00165.2002

Sugasawa, K. (2011). Multiple DNA damage recognition factors involved in mammalian nucleotide excision repair. *Biochemistry (Mosc)* 76, 16–23. doi: 10.1134/s0006297911010044

Suzuki, S., Brown, C. M., and Wise, P. M. (2009). Neuroprotective effects of estrogens following ischemic stroke. *Front. Neuroendocrinol.* 30, 201–211. doi: 10.1016/j.yfrne.2009.04.007

Suzuki, T., Masuda, M., Friesen, M. D., and Ohshima, H. (2001). Formation of spiroiminodihydantoin nucleoside by reaction of 8-oxo-7,8-dihydro-2′-

deoxyguanosine with hypochlorous acid or a myeloperoxidase-H_2O_2-Cl^- system. *Chem. Res. Toxicol.* 14, 1163–1169. doi: 10.1021/tx010024z

Swain, U., and Subba Rao, K. (2011). Study of DNA damage via the comet assay and base excision repair activities in rat brain neurons and astrocytes during aging. *Mech. Ageing Dev.* 132, 374–381. doi: 10.1016/j.mad.2011.04.012

Sykora, P., Yang, J. L., Ferrarelli, L. K., Tian, J., Tadokoro, T., Kulkarni, A., et al. (2013). Modulation of DNA base excision repair during neuronal differentiation. *Neurobiol. Aging* 34, 1717–1727. doi: 10.1016/j.neurobiolaging.2012.12.016

Szymczak, S., Kalita, K., Jaworski, J., Mioduszewska, B., Savonenko, A., Markowska, A., et al. (2006). Increased estrogen receptor β expression correlates with decreased spine formation in the rat hippocampus. *Hippocampus* 16, 453–463. doi: 10.1002/hipo.20172

Tang, M. X., Jacobs, D., Stern, Y., Marder, K., Schofield, P., Gurland, B., et al. (1996). Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. *Lancet* 348, 429–432. doi: 10.1016/s0140-6736(96)03356-9

Torika, N., Asraf, K., Roasso, E., Danon, A., and Fleisher-Berkovich, S. (2016). Angiotensin converting enzyme inhibitors ameliorate brain inflammation associated with microglial activation: possible implications for Alzheimer's disease. *J. Neuroimmune. Pharmacol.* 11, 774–785. doi: 10.1007/s11481-016-9703-8

Toro-Urrego, N., Garcia-Segura, L. M., Echeverria, V., and Barreto, G. E. (2016). Testosterone protects mitochondrial function and regulates neuroglobin expression in astrocytic cells exposed to glucose deprivation. *Front. Aging Neurosci.* 8:152. doi: 10.3389/fnagi.2016.00152

Tsutsui, K., Ukena, K., Sakamoto, H., Okuyama, S., and Haraguchi, S. (2011). Biosynthesis, mode of action, and functional significance of neurosteroids in the purkinje cell. *Front. Endocrinol.* 2:61. doi: 10.3389/fendo.2011.00061

Unger, J. W. (1998). Glial reaction in aging and Alzheimer's disease. *Microsc. Res. Tech.* 43, 24–28. doi: 10.1002/(SICI)1097-0029(19981001)43:1<24::AID-JEMT4>3.0.CO;2-P

Urbanska, K., Pannizzo, P., Lassak, A., Gualco, E., Surmacz, E., Croul, S., et al. (2009). Estrogen receptor β-mediated nuclear interaction between IRS-1 and Rad51 inhibits homologous recombination directed DNA repair in medulloblastoma. *J. Cell. Physiol.* 219, 392–401. doi: 10.1002/jcp.21683

Vagnerova, K., Liu, K., Ardeshiri, A., Cheng, J., Murphy, S. J., Hurn, P. D., et al. (2010). Poly (ADP-ribose) polymerase-1 initiated neuronal cell death pathway—do androgens matter? *Neuroscience* 166, 476–481. doi: 10.1016/j.neuroscience.2009.12.041

Veiga, S., Melcangi, R. C., Doncarlos, L. L., Garcia-Segura, L. M., and Azcoitia, I. (2004). Sex hormones and brain aging. *Exp. Gerontol.* 39, 1623–1631. doi: 10.1016/j.exger.2004.05.008

Velarde, M. C. (2014). Mitochondrial and sex steroid hormone crosstalk during aging. *Longev. Healthspan* 3:2. doi: 10.1186/2046-2395-3-2

Verkhratsky, A., Rodriguez, J. J., and Parpura, V. (2014). Neuroglia in ageing and disease. *Cell Tissue Res.* 357, 493–503. doi: 10.1007/s00441-014-1814-z

Vermulst, M., Bielas, J. H., Kujoth, G. C., Ladiges, W. C., Rabinovitch, P. S., Prolla, T. A., et al. (2007). Mitochondrial point mutations do not limit the natural lifespan of mice. *Nat. Genet.* 39, 540–543. doi: 10.1038/ng1988

Villa, A., Vegeto, E., Poletti, A., and Maggi, A. (2016). Estrogens, neuroinflammation, and neurodegeneration. *Endocr. Rev.* 37, 372–402. doi: 10.1210/er.2016-1007

Virbasius, J. V., and Scarpulla, R. C. (1994). Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc. Natl. Acad. Sci. U S A* 91, 1309–1313. doi: 10.1073/pnas.91.4.1309

von Bernhardi, R., Eugenín-von Bernhardi, L., and Eugenin, J. (2015). Microglial cell dysregulation in brain aging and neurodegeneration. *Front. Aging Neurosci.* 7:124. doi: 10.3389/fnagi.2015.00124

Wang, J., Markesbery, W. R., and Lovell, M. A. (2006). Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. *J. Neurochem.* 96, 825–832. doi: 10.1111/j.1471-4159.2005.03615.x

Wang, J., Xiong, S., Xie, C., Markesbery, W. R., and Lovell, M. A. (2005). Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J. Neurochem.* 93, 953–962. doi: 10.1111/j.1471-4159.2005.03053.x

Waters, E. M., Mitterling, K., Spencer, J. L., Mazid, S., McEwen, B. S., and Milner, T. A. (2009). Estrogen receptor α and β specific agonists regulate expression of synaptic proteins in rat hippocampus. *Brain Res.* 1290, 1–11. doi: 10.1016/j.brainres.2009.06.090

Waters, E. M., Yildirim, M., Janssen, W. G., Lou, W. Y., McEwen, B. S., Morrison, J. H., et al. (2011). Estrogen and aging affect the synaptic distribution of estrogen receptor β-immunoreactivity in the CA1 region of female rat hippocampus. *Brain Res.* 1379, 86–97. doi: 10.1016/j.brainres.2010.09.069

Wei, S. M., Baller, E. B., Kohn, P. D., Kippenhan, J. S., Kolachana, B., Soldin, S. J., et al. (2017). Brain-derived neurotrophic factor Val66Met genotype and ovarian steroids interactively modulate working memory-related hippocampal function in women: a multimodal neuroimaging study. *Mol. Psychiatry* doi: 10.1038/mp.2017.72 [Epub ahead of print].

Weissman, L., de Souza-Pinto, N. C., Stevnsner, T., and Bohr, V. A. (2007a). DNA repair, mitochondria, and neurodegeneration. *Neuroscience* 145, 1318–1329. doi: 10.1016/j.neuroscience.2006.08.061

Weissman, L., Jo, D. G., Sørensen, M. M., de Souza-Pinto, N. C., Markesbery, W. R., Mattson, M. P., et al. (2007b). Defective DNA base excision repair in brain from individuals with Alzheimer's disease and amnestic mild cognitive impairment. *Nucleic Acids Res.* 35, 5545–5555. doi: 10.1093/nar/gkm605

Williams, J. S., Lujan, S. A., and Kunkel, T. A. (2016). Processing ribonucleotides incorporated during eukaryotic DNA replication. *Nat. Rev. Mol. Cell Biol.* 17, 350–363. doi: 10.1038/nrm.2016.37

Witty, C. F., Gardella, L. P., Perez, M. C., and Daniel, J. M. (2013). Short-term estradiol administration in aging ovariectomized rats provides lasting benefits for memory and the hippocampus: a role for insulin-like growth factor-I. *Endocrinology* 154, 842–852. doi: 10.1210/en.2012-1698

Woolley, C. S., and McEwen, B. S. (1992). Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *J. Neurosci.* 12, 2549–2554.

Woolley, C. S., and McEwen, B. S. (1993). Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J. Comp. Neurol.* 336, 293–306. doi: 10.1002/cne.903360210

World Health Statistics. (2017). *Mental Health of Older Adults*. Available online at: www.who.int/mediacentre/factsheets/fs381/en/

Yang, J. L., Lin, Y. T., Chuang, P. C., Bohr, V. A., and Mattson, M. P. (2014). BDNF and exercise enhance neuronal DNA repair by stimulating CREB-mediated production of apurinic/apyrimidinic endonuclease 1. *Neuromolecular Med.* 16, 161–174. doi: 10.1007/s12017-013-8270-x

Yang, S. H., Liu, R., Perez, E. J., Wen, Y., Stevens, S. M. Jr., Valencia, T., et al. (2004). Mitochondrial localization of estrogen receptor β. *Proc. Natl. Acad. Sci. U S A* 101, 4130–4135. doi: 10.1073/pnas.0306948101

Yao, J., Chen, S., Cadenas, E., and Brinton, R. D. (2011). Estrogen protection against mitochondrial toxin-induced cell death in hippocampal neurons: antagonism by progesterone. *Brain Res.* 1379, 2–10. doi: 10.1016/j.brainres.2010.11.090

Yao, J., Hamilton, R. T., Cadenas, E., and Brinton, R. D. (2010). Decline in mitochondrial bioenergetics and shift to ketogenic profile in brain during reproductive senescence. *Biochim. Biophys. Acta* 1800, 1121–1126. doi: 10.1016/j.bbagen.2010.06.002

Yao, J., Irwin, R., Chen, S., Hamilton, R., Cadenas, E., and Brinton, R. D. (2012). Ovarian hormone loss induces bioenergetic deficits and mitochondrial β-amyloid. *Neurobiol. Aging* 33, 1507–1521. doi: 10.1016/j.neurobiolaging.2011.03.001

Yao, J., Irwin, R. W., Zhao, L., Nilsen, J., Hamilton, R. T., and Brinton, R. D. (2009). Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U S A* 106, 14670–14675. doi: 10.1073/pnas.0903563106

Yao, J., Zhao, L., Mao, Z., Chen, S., Wong, K. C., To, J., et al. (2013). Potentiation of brain mitochondrial function by S-equol and R/S-equol estrogen receptor β-selective phytoSERM treatments. *Brain Res.* 1514, 128–141. doi: 10.1016/j.brainres.2013.02.021

Yin, F., Yao, J., Sancheti, H., Feng, T., Melcangi, R. C., Morgan, T. E., et al. (2015). The perimenopausal aging transition in the female rat brain: decline in bioenergetic systems and synaptic plasticity. *Neurobiol. Aging* 36, 2282–2295. doi: 10.1016/j.neurobiolaging.2015.03.013

Young, L. J., and Pfaff, D. W. (2014). Sex differences in neurological and psychiatric disorders. *Front. Neuroendocrinol.* 35, 253–254. doi: 10.1016/j.yfrne.2014.05.005

Yu, Y., Cui, Y., Niedernhofer, L. J., and Wang, Y. (2016). Occurrence, biological consequences, and human health relevance of oxidative stress-induced DNA damage. *Chem. Res. Toxicol.* 29, 2008–2039. doi: 10.1021/acs.chemrestox.6b00265

Yu, P., Li, S., Zhang, Z., Wen, X., Quan, W., Tian, Q., et al. (2017). Progesterone-mediated angiogenic activity of endothelial progenitor cell and angiogenesis in traumatic brain injury rats were antagonized by progesterone receptor antagonist. *Cell Prolif.* 50:e12362. doi: 10.1111/cpr.12362

Yue, X., Lu, M., Lancaster, T., Cao, P., Honda, S., Staufenbiel, M., et al. (2005). Brain estrogen deficiency accelerates Aβ plaque formation in an Alzheimer's disease animal model. *Proc. Natl. Acad. Sci. U S A* 102, 19198–19203. doi: 10.1073/pnas.0505203102

Zagni, E., Simoni, L., and Colombo, D. (2016). Sex and gender differences in central nervous system-related disorders. *Neurosci. J.* 2016:2827090. doi: 10.1155/2016/2827090

Zárate, S., Astiz, M., Magnani, N., Imsen, M., Merino, F., Álvarez, S., et al. (2017). Hormone deprivation alters mitochondrial function and lipid profile in the hippocampus. *J. Endocrinol.* 233, 1–14. doi: 10.1530/JOE-16-0451

Zárate, S., and Seilicovich, A. (2010). Estrogen receptors and signaling pathways in lactotropes and somatotropes. *Neuroendocrinology* 92, 215–223. doi: 10.1159/000321683

Zaremba, T., Thomas, H. D., Cole, M., Coulthard, S. A., Plummer, E. R., and Curtin, N. J. (2011). Poly(ADP-ribose) polymerase-1 (PARP-1) pharmacogenetics, activity and expression analysis in cancer patients and healthy volunteers. *Biochem. J.* 436, 671–679. doi: 10.1042/BJ20101723

Zhao, Y., He, L., Zhang, Y., Zhao, J., Liu, Z., Xing, F., et al. (2017). Estrogen receptor α and β regulate actin polymerization and spatial memory through an SRC-1/mTORC2-dependent pathway in the hippocampus of female mice. *J. Steroid Biochem. Mol. Biol.* 174, 96–113. doi: 10.1016/j.jsbmb.2017.08.003

Zhu, C., Wang, S., Wang, B., Du, F., Hu, C., Li, H., et al. (2015). 17β-Estradiol up-regulates Nrf2 via PI3K/AKT and estrogen receptor signaling pathways to suppress light-induced degeneration in rat retina. *Neuroscience* 304, 328–339. doi: 10.1016/j.neuroscience.2015.07.057

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

REVIEW
published: 09 January 2018
doi: 10.3389/fnagi.2017.00439

Sex Hormones and Healthy Psychological Aging in Women

Esperanza Navarro-Pardo[1]*, *Carol A. Holland*[2] *and Antonio Cano*[3]

[1] Department of Developmental and Educational Psychology, Universitat de Valencia, Valencia, Spain, [2] Division of Health Research, Centre for Ageing Research, Lancaster University, Lancaster, United Kingdom, [3] Department of Pediatrics, Obstetrics and Gynecology, Universitat de Valencia, Valencia, Spain

Besides their key role in reproduction, estrogens have effects in several organs in the body, as confirmed by the identification of estrogen receptors (ER) in multiple tissues. Experimental evidence has shown that estrogens have significant impacts on the central nervous system (CNS), and a key question is to what extent the fall in estrogen levels in the blood that occurs with increasing age, particularly around and following the menopause, has an impact on the cognitive function and psychological health of women, specifically regarding mood. This review will consider direct effects of menopausal changes in estrogens on the brain, including cognitive function and mood. Secondary pathways whereby health factors affected by changes in estrogens may interact with CNS functions, such as cardiovascular factors, will be reviewed as well insofar as they also have an impact on cognitive function. Finally, because decline in estrogens may induce changes in the CNS, there is interest in clarifying whether hormone therapy may offer a beneficial balance and the impact of hormone therapy on cognition will also be considered.

Keywords: psychological aging, women, sex hormones, cognition, mood

OPEN ACCESS

Edited by:
Luis Miguel Garcia-Segura, Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
Lydia DonCarlos, Loyola University Chicago, United States
Inigo Azcoitia, Complutense University of Madrid, Spain

***Correspondence:**
Esperanza Navarro-Pardo
esperanza.navarro@uv.es

Received: *15 October 2017*
Accepted: *19 December 2017*
Published: *09 January 2018*

Citation:
Navarro-Pardo E, Holland CA and Cano A (2018) Sex Hormones and Healthy Psychological Aging in Women. Front. Aging Neurosci. 9:439. doi: 10.3389/fnagi.2017.00439

INTRODUCTION

This paper sets out to review evidence on the relationships between changes associated with reductions in sex hormones in women, related to the menopause and psychological impacts, focusing on cognitive and mood changes. Beginning with cognitive effects, we examine the evidence for direct effects of circulating estrogens on neural systems, in combination with the effects of other hormones and factors that may be changing at the same time or affected by the changes in estrogens, such as gonadotropin-releasing hormone (GnRH) and insulin-like growth factor (IGF). We then examine clinical evidence from a variety of methodologies to investigate the relationships of menopausal stages, circulating hormones and hormonal therapy to cognitive changes, and dementia risk. This is followed by a similar discussion of the evidence of hormonal impacts on depression and anxiety. We finish with an examination of cardiovascular effects focusing on the impacts of cardiovascular disease on cognition, exploring the possibility that impacts of sex hormones on cognition may, in some cases, be mediated by both effects on mood and on cardiovascular health.

MENOPAUSE AND COGNITIVE FUNCTIONS

Neuroscience studies have provided evidence that estrogens influence aspects of brain biochemistry and morphology known to be important for cognitive functions (Sherwin, 1997). However, while this evidence is reasonably well-supported in experimental models, relationships to neural functions in the human have been more difficult to determine because of inconsistent evidence on effects of reduced estrogens on measures of attention, concentration, or memory in clinical studies (Schmidt et al., 2013). Thus, one issue of interest is the influence of depletion of estrogens on cognition across and after menopause and, if confirmed, the mechanism through which it occurs. The potential association of menopausal hormonal changes with brain function may therefore be approached in two ways: directly, i.e., effects on neural cells and systems, and indirectly, i.e., effects of hormonal changes on evidenced functions, mainly cognition and mood (Greendale et al., 2011).

In addition, sex hormones have an influence on other systems that have impact on brain functions, specifically the vascular tree, which determines the adequacy of cerebral blood perfusion (Abe et al., 2006; Izumi et al., 2006). Observed evidence of the impact of changes in estrogens on the vascular system are obvious in common menopausal symptoms such as hot flushes, and research in this area suggests that vasomotor symptoms might represent a female-specific risk factor for memory declines during the menopausal transition (Maki, 2015).

DIRECT INFLUENCE OF CIRCULATING ESTROGENS ON NEURAL SYSTEMS

The background supporting the action of estrogens arises from molecular evidence and observations obtained in different experimental models. There is, for example, an abundance of estrogen receptors (ERs) at several locations related to cognition in the CNS. This is particularly observed in areas related to verbal memory and retrieval, working memory, executive function, and control of attention, such as the hippocampus and the prefrontal cortex (Sherwin, 2006; Pompili et al., 2012).

There is a considerable body of knowledge explaining how estradiol exerts neuroprotective actions (Arevalo et al., 2015). The action of estrogens may occur via the increase in the levels of neurotransmitters, which may enhance neuronal growth and formation of synapses (McEwen, 2001; Hesson, 2012). In this context, cell culture experiments have shown that estrogens alter synaptic circuitry in the hypothalamus, hippocampus, and, more recently, neocortex (Hara et al., 2015). Of further importance, recent work has shown that the local production of estradiol by neural cells, as a result of the activation of the widely available aromatase in the brain, may further help in maintaining neuroplasticity (Azcoitia et al., 2017). In contrast, inhibition of aromatase has been shown to interfere with electrophysiological parameters of memory in female mice (Vierk et al., 2012). This is important because the local action in the brain may be poorly related to the circulating levels of the hormone (Giatti et al., 2015). Moreover, the local action of estradiol is different between males and females (Vierk et al., 2012; Melcangi et al., 2016; Ruiz-Palmero et al., 2016).

Observations in humans support the role of estrogens with a special focus on events around menopause. The conception that women have an increased risk of developing cognitive decline and Alzheimer's Dementia (AD) compared to men is widespread in the literature. As suggested above, such increased risk might be due to a reduction in the neuroprotective effects of estrogens on the brain around and following menopause. This view is supported by, for example, findings suggesting that ovariectomy in pre-menopausal women significantly increases risk for the development of memory problems and AD in later life (Ryan et al., 2014). In the same way, other studies have shown that induced hypogonadism, by either surgery or drugs, is accompanied by some detectable decline in cognitive performance (Schmidt et al., 2013).

Together with the above-mentioned observations on the trophic effect of estradiol on synaptic circuitry, classical experiments have shown that this steroid increased the synthesis of choline acetyltransferase (ChAT) in the medial septal nucleus, nucleus basalis of Meynert, and the frontal cortex (Luine, 1985). The potential impact of these findings should be interpreted in terms of the well-established key function of acetylcholine which is a key neurotransmitter in the regulation of surveillance, attention and activation levels, and in memory function, mainly in working and episodic memory (Hasselmo, 2006). The central effects of anticholinergic impacts are generally manifested in behavior with characteristic syndromes such as loss of memory and attention, confused speech and ataxia, confusion, disorientation, and delirium, cognitive impairment or dementia (Campbell et al., 2009).

The alternative hypothesis is that it is not decline in estrogens, but the concomitant increase in GnRH is responsible for neurodegeneration (Skinner et al., 2009). There are GnRH receptors in the human hippocampus, and GnRH is likely to be elevated as a result of the reduction of negative feedback of estrogens after the menopause. To determine whether the elevated level of gonadotropins has an effect on the risk of AD, GnRH analogs (GnRHa) were used in an aged transgenic mouse model. GnRHa produced an acute decline in ovarian hormone output leading to a status similar to menopause. The decrease in LH by GnRHa resulted in an attenuation of amyloid-beta deposition as compared to placebo treated animals. Also, this reduction correlated with improved cognition (Casadesus et al., 2006). Despite the data in support of a role for gonadotropins, most accumulated evidence is concerned with an estrogen-mediated protective effect.

Furthermore, insulin and insulin-like growth factor 1 (IGF1) could play major roles as regulators of growth and regeneration in the CNS. For example, it has been hypothesized that exposure to estradiol may favor the action of IGF1 that, in turn, increases performance of ERα for extended periods, thus favoring protection of hippocampal functions. Specifically, estrogens regulate different insulin-related processes with cognitive correlates (glucose transport, aerobic glycolysis, and

mitochondrial function). Consequently, decline in circulating estrogens during menopause is parallel to a decrease in brain bioenergetics and shift toward a metabolically compromised phenotype (Rettberg et al., 2014).

CLINICAL STUDIES

Memory complaints are a frequent concern in people older than 50 years, but the cognitive issue in menopausal women is much broader and affects cognitive functioning in general. Memory has been the most studied function, perhaps because it is more clearly affected and because has the potential to predict the development of Alzheimer's disease (AD) (Parikh et al., 2014; Mueller et al., 2015). Studies have addressed changes in subtypes of memory such as verbal recall and working memory, processing speed, attention and executive function, reasoning, difficulty in concentrating, and learning processes.

The impact of the reduction of circulating estrogens on cognitive deterioration has been widely investigated at a clinical level. The effects of natural changes in estrogens and the impacts of interventions such as hormone therapy will be considered separately in the following sections.

STUDIES OF THE RELATIONSHIPS BETWEEN ESTROGENS AND COGNITIVE HEALTH IN PERI- AND POST-MENOPAUSAL WOMEN

A range of methodologies have been used to examine this issue. First, longitudinal or cohort studies have observed and measured both cognition and hormonal status and/or menopausal status over a period of time in the same women—that is, a within participants design, so that the same women are assessed at different time points. Second, cross-sectional studies have been conducted where in-depth data on large groups of women are examined at one point in time with comparisons between women of different menopausal status, or correlations within women between hormonal and cognitive status. Finally, intervention studies, such as randomized controlled trials, have examined the impact of either induced estrogen depletion (for example, in women undergoing clinical procedures) or hormonal therapy (HT) on cognition.

COHORT STUDIES

The Kinmen Women-Health Investigation (KIWI) (Fuh et al., 2006) is a longitudinal population-based study of rural women in Taiwan. During a follow-up period of 18 months, 114 out of the 495 women progressed to peri-menopause. Women taking HT or who had a history of hysterectomy were excluded from the study. Measures of verbal and visual memory, verbal fluency and the Trail Making test (both generally accepted as indicators of executive functions) and digit span (with forward span indicating immediate short term memory and backward span indicating working memory) were used to assess cognitive performance. Cognitive scores slightly improved for visual recognition, verbal fluency, Trail making A and B, and forward digit span for the women who stayed pre-menopausal. For those who became peri-menopausal, trail making A and B, and forward digit span also improved. However, given the normal influence of practice effects in healthy participants, improvement is not unusual. The important comparisons here are the differences in longitudinal effect between the women who stayed pre-menopausal and those who became peri-menopausal. The researchers controlled for any age differences, education and baseline performance, and found no differences between the two groups of women at follow-up except in verbal fluency, in which there was a significant reduction for the peri-menopausal women. While carefully constructed to control for cohort effects which may be present in cross-sectional studies, and using a limited time gap between testing (20 months) to ensure women were assessed pre-menopause and around the time menopause was beginning, this meant that this study was conducted over a limited time period which may not have been long enough for any negative effects of reduced estrogens on cognition to be clear. In terms of the women's self-perceived health, the percentage of women with excellent or good health decreased after menopause (70.2 vs. 57%), while those who thought their health was poor increased from 2.6 to 4.4%. The authors indicated that the average duration of peri-menopause was nearly 4 years.

The 14 year longitudinal Penn Ovarian Aging Study (POAS) (Epperson et al., 2013) examined women who were pre-menopausal and aged 35–47 at the beginning of the study, with approximately yearly assessments. By the 14th assessment, most women were post-menopausal, with menopausal stages assessed at each visit, using standard menstrually defined menopausal staging categories (Gracia et al., 2005). Differences across menopausal stages were adjusted for age, ethnicity, BMI, education and baseline task performance, and significant reductions in both immediate and delayed recall measures were found. Using menopausal stages as opposed to time as the repeated measures factor enabled these researchers to reveal differences in cognitive effects at different stages of menopausal progression, such that immediate recall declined in the post-menopausal stage but decline in delayed verbal recall occurred in early transition. There were impacts of menopausal stage on measures of processing speed (digit symbol substitution test) and sensorimotor processing speed (symbol copy task) but this was only significant in the unadjusted models, suggesting that age, education, ethnicity, and BMI (as a proxy for health factors such as hypertension, diabetes, and vascular disease) were more important predictors of processing speed than menopausal stage. In the unadjusted model, higher estradiol was associated with better memory performance, but not once data were adjusted for the covariates of age, education, ethnicity, and BMI.

Another longitudinal study, the Seattle Midlife Women's Health Study (SMWHS), explored memory functioning and found that it was more closely related to perceived health, depressed mood or stress than to peri-menopause or age (Woods et al., 2000).

CROSS-SECTIONAL OBSERVATIONAL STUDIES

The Study of Women's Health Across the Nation (SWAN) (Santoro and Sutton-Tyrrell, 2011) is a multi-center, multi-ethnic longitudinal study designed to characterize the physiological and psychosocial changes that occur during the menopausal transition and to observe their effects on subsequent health and risk factors for age-related diseases. In the study, a total of 3,302 women were enrolled at seven clinical sites between 1996 and 1997. At the time of enrollment, women were pre-menopausal, not taking hormones and between 42 and 52 years of age. Participants self-identified as African-American (28%), Caucasian (47%), Chinese (8%), Hispanic (8%), or Japanese (9%). SWAN has a multi-disciplinary focus and thus has repeated measures of bone health, cardiovascular risk factors, psychosocial factors, and ovarian hormones.

Luetters et al. (2007) performed a cross-sectional analysis of a consistent cohort of 1,657 women in the SWAN cohort for whom menopausal status was assessed together with hormonal levels, estradiol and follicle stimulating hormone (FSH). The sample of women was stratified according to menopause stage, and their cognitive function was evaluated. Given that previous studies have been equivocal in terms of whether natural estrogen decline through menopausal stages is related to cognitive decline, this cross-sectional study sought to determine the extent to which menstrually defined menopausal stages were related to cognitive function, but also whether menopausal symptoms, such as hot flushes or poor sleep, mediated any relationship. The study also examined the extent to which cognitive difficulties are related to levels of estradiol or FSH. Researchers assessed immediate and delayed verbal recall, working memory, and processing speed and found no relationships with menopausal stage or hormone levels once sociodemographic factors, self-perceived health, and possible menopausal symptoms were adjusted for. This did not change when either estradiol or FSH levels were included in the analysis. The authors discussed possible limitations of using menstrually defined menopause stages in such analyses, given that there is not a monotonic decline in estradiol levels across stages. For example, there are significant periods in the peri-menopausal stage with heightened estradiol compared with pre- and post-menopausal women (Santoro et al., 1996).

Taken together with the results of other well-controlled studies including those in which many more measures of hormones in the same women were taken across pre- to post-menopause in the longitudinal studies discussed above, findings from the SWAN study suggest that there is no direct effect of the menopause or circulating estrogens on cognition for the measures used. The only clear effect in a well-controlled study was the effect of a decline in verbal fluency in Fuh et al. (2006). Most studies have focused on verbal memory (immediate and delayed) and processing speed, which is appropriate given commonly found age effects on both, but also evidence of specific ERs in the hippocampus. However, few studies have focused on executive function, which is surprising given the evidence for ERs in the prefrontal cortex. Of the tests conducted by the studies reviewed, only verbal fluency and the Trail Making test tap executive function. However, the executive functioning component of trails is calculated by subtracting time taken to complete test A from time taken to complete test B, to subtract psychomotor time from the extra time it takes a participant to alternate between numbers and letters, that is, to inhibit prepotent associates and update one's place in a sequence, the executive components. The Fuh et al. (2006) study did not do this. As such, it seems that further research on the role of menopausal stages and estrogens in effect on executive function, controlling for appropriate covariates, is still needed.

INTERVENTIONS: THE IMPACT OF HT ON COGNITION

Hormone therapy consists predominantly of estrogens, since menopausal symptoms essentially derive from the effect of withdrawal of estrogens at different target tissues. Nevertheless, the normal ovarian cycle includes the regular secretion of progesterone during the luteal phase. Progesterone has a neutralizing effect over endometrial proliferation; for this reason progestogens are added to HT formulations in order to protect the endometrium. So, guidelines recommend that women with a uterus use estrogens plus progestogens, which may be combined in different forms. And because of that, the impact of HT on health should also consider the specific effects of both estrogens and progestogens in comparison to effects of only estrogens.

Observational studies and, more recently, randomized controlled trials have provided current clinical knowledge. The specific form of HT, including hormone preparations, either estrogens alone or combined with progestogens, and the route of the medication, either oral or transdermal have also been investigated. A significant intervention study is the Women's Health Initiative Memory Study (Coker et al., 2010). Conceived as an ancillary study of the Women's Health Initiative randomized controlled trial, the memory study aimed to evaluate the effect of replacement with estrogens plus progestogens on the incidence of dementia or mild cognitive impairment compared with placebo (Shumaker et al., 2003). At the early termination of the study after an average follow up of 4.05 years, 66% of women were diagnosed with probable dementia in the treatment group vs. 34% in the placebo (95% confidence interval; $P = 0.01$). The hazard ratio was 2.05. The incidence of mild cognitive impairment did not differ between groups. This data was corroborated by an MRI study in which regional brain volumes were measured in a subset of 1,403 women, including hippocampal and frontal regions by magnetic resonance at an average of 3.0 years post-trial (Resnick et al., 2009). The use of hormones, both CEE alone or associated with medroxyprogesterone acetate (MPA), was associated with greater brain atrophy, the effects being more evident in women who already demonstrated some cognitive deficits before initiating HT (Resnick et al., 2009). A related study, the Women's Health Initiative Study of Cognitive Aging analyzed the impact of HT on working, verbal and figural memory, speed of processing, attention, executive function, spatial reasoning, and motor performance. They found that the

combined estrogens + progesterone formulation had a negative impact on verbal memory but only after long-term HT, with no other cognitive impacts shown. Estrogens alone were associated with lower spatial processing but this effect diminished after a longer duration of therapy (Resnick et al., 2004).

There has been debate on whether such unfavorable impacts might be due to the relatively advanced age, 65 years or older, of participants in the Women's Health Initiative Memory Study. An interpretation of a diminution in the responsiveness of neurons to estrogens with increasing age or the incapacity of the hormone to reverse neural loss and/or dysfunction, which may have occurred during the interim between menopause and the beginning of treatment, cannot be discarded. This effect was examined in the Kronos Early Estrogen Prevention Study (KEEPS), another randomized controlled trial that had an ancillary study, the KEEPS-Cog. Women in this study were younger, 52.6 years average age, and 1.4 years since menopause. A total of 693 women were randomized to daily oral estrogens (CEE) or transdermal estradiol, in both cases associated with micronized progesterone (12 days per month), or placebo. The primary outcome was the effect on the Modified Mini-Mental State examination. Again, HT was not associated with clear benefit, although in contrast to the results in the Women's Health Initiative Memory Study, no harm was found this time (Gleason et al., 2015).

The Women's Health Initiative Memory Study of Younger Women (Coker et al., 2010) had a similar purpose. Cognition was assessed in women who had enrolled in the Women's Health Initiative study when they were 50–55 years of age. When 7.2 years had elapsed since the end of the trial, women were assessed by telephone, their mean age being 67.2 at that moment. As for the KEEPS-Cog, no substantial difference was found between women receiving hormones and the placebo-treated controls (Espeland et al., 2013).

Studies which explicitly examined the impact of years since menopause on the cognitive effects of HT with estradiol are those by Dunkin et al. (2005, 2006). Dunkin et al. (2006) reviewed the literature on the basis of age of the women involved in HT trials and determined that those studies where the women were over the age of 65 have generally failed to find any cognitive benefit, or have found a cognitive disadvantage of HT, whereas those studies using younger participants have been more likely to find positive effects. Indeed, in Dunkin et al.'s randomized placebo-controlled design (Dunkin et al., 2005), these researchers found that recently post-menopausal women benefitted more in terms of executive functioning than women with a longer time since menopause, from a 10 week transdermal estradiol intervention. Some of the latter group actually showed decline in executive function. While these studies showed a significant relationship between years since menopause and cognitive impact of estradiol therapy, with younger women showing a greater positive effect, they did not attempt to disentangle the age and years since menopause confound. Nevertheless, evidence from the Cache County longitudinal study examining HT (Zandi et al., 2002) found that the normally found increased risk of AD in women in comparison to men was not found for women who had begun HT at earlier ages and had used it for around 10 years. The current average age of the women in this study was 73 years, and current users of HT who had not begun it at earlier stages did not show reduced risk of AD.

This accumulation of evidence has led some researchers to propose a critical period for any HT-related neuroprotection (Resnick and Henderson, 2002; Schneider, 2004). Genazzini et al. (2007) suggest a mechanism whereby aging affects ERs, and ER co-activators such as growth factors, neurotransmitters, and neuromodulators. For example, they refer to increased expression of Brain Derived Neurotrophic Factor (BDNF) in young rats in response to estrogens, but decreased BDNF expression in older rats. Given that BDNF has been related to maintenance of brain plasticity in the aging brain in animals and humans, this illustrates the issue. A review by Maki (2013) examines a range of study designs to collate findings on this issue, conducting meta-analyses where appropriate. She concluded that observational studies and neuroimaging studies provide reliable evidence that early use of HT is protective of cognitive function and later use is not, or is detrimental. RCTs showed a benefit or early use of estrogens but not for estrogens + progesterone, regardless of timing and determined that overall there is evidence of both cognitive benefit and reduced risk of AD where the HT is taken in the first 5 years after the last menstrual period when the regimen is not continuous estrogens + progesterone. However, this author also highlighted the possibility that the relationship maybe related to the health of the woman's brain as opposed to their reproductive age, given that data from the Women's Health Initiative Memory Study showed the greatest hippocampal loss in women with initially low cognition scores (Resnick et al., 2009).

Some research has pointed out that, despite the evidence obtained in randomized studies, duration of therapy may have been missed as a variable. Most studies only followed participants for a few years. In response to this uncertainty, the duration of HT was used as a variable in a French cohort assessed for cognitive function. HT was found be associated with better performance in some cognitive domains, such as verbal fluency, visual memory, and psychomotor speed, but only if treatments lasted over 10 years (Ryan et al., 2009). However, if data were adjusted by age, education level, age at menopause, depressive symptoms, etc., none of the effects of HT remained significant. This conclusion is further supported in another recent study in Finland (Imtiaz et al., 2017); in this cohort study, women had received HT from zero (never received it) to more than 5 years and the overall findings provided no strong evidence for a protective effect of estradiol-based HT against cognitive decline.

A further study by Erickson et al. (2007) examined this issue explicitly in relation to cognitive assessments and regional atrophy using detailed voxel based morphometry of MRI scans, enabling analysis of systematic variation of gray and white matter in significant brain regions. They compared HT treatment duration groups of women, balanced for numbers taking opposed or unopposed estrogens, on a test of executive function, the Wisconsin Card Sorting Task, performance on which has been previously shown to be related to prefrontal cortex gray matter. They also used a test of general cognition, the Modified Mini-Mental State Examination. The duration groups were (i) never used HT (ii) up to 10 years (iii) 11–15 years (iv) 16+ years,

with a mean age of 69.61 years. Given that both physical exercise and estrogens have similar actions on neurotrophic growth and vascularization, and BDNF actions are affected both by estrogens and exercise in the hippocampus, with humans showing the greatest concentration of BDNF receptors in the frontal lobes, Erickson et al. (2007) hypothesized that estrogens and exercise may interact to positively affect human brain tissue in the frontal lobes and also cognition. They found that HT duration up to 10 years was associated with better performance on the Wisconsin Card Sorting Task and with spared gray matter in the prefrontal cortex, compared with other groups, adjusting for age, age at menopause, socio-economic status and education. However, HT duration longer than 10 years was associated with greater prefrontal deterioration and greater decline in the measures of executive function. Importantly, physical fitness level (measured by VO_2 max) interacted with duration such that higher fitness levels ameliorated the negative effects of longer duration of HT and enhanced positive effects of shorter durations in both performance and brain morphometry. This evidence for interactions between estrogen therapy and exercise/fitness on cognitive and brain health is important, and supports the role of healthy lifestyle interventions alongside HT. However, the effects were specific to executive function and did not occur for the general cognition measure.

To summarize, and in consistence with the conclusion from other groups (Henderson and Popat, 2011), the most solid evidence seems to favor the notion that HT is not indicated for treatment of cognitive decline or dementia, and that the inconsistency in results may be related to the control of confounding variables, the timeline (onset and duration) of the use of HT and to the treatment regimen. Maki (2013) suggests that there is tentative support for beneficial effects for hysterectomised women who are treated with estrogens alone, and Erickson et al.'s (2007) suggests durations longer than 10 years are not beneficial but that HT benefit or negative effects interact with physical fitness and health. The concept of a critical period of treatment in early post-menopause seems well-established as at least not a disadvantage for cognition. However, studies are still insufficient to be clear on which circumstances, to which women, and for how long HT should be prescribed for maximum cognitive benefit and/or minimum cognitive negative effect. Studies where the underlying brain health or physical fitness of the women is considered as a variable, seem a useful way to move forward.

MOOD AND COGNITION

The influence of mood, particularly depressed mood and anxiety, on cognition is supported by considerable evidence at all ages, but a greater impact of depression on cognition is recognized for older people. Indeed, higher levels of depressive and anxiety symptoms are directly related to poorer cognitive performance because both disorders are often accompanied by attention and concentration deficit symptoms. There is considerable support for depression as a risk factor for cognitive impairment, with Chung et al. (2015) finding an association between lifetime history of major depression and amyloid beta deposition in individuals with mild cognitive impairment (MCI). In addition to the influence of depression on cognition, depression is commonly implicated as a significant risk factor for dementia in older adults (da Silva et al., 2013) to the extent that it may be a prodromal symptom of some kinds of dementia (Panza et al., 2010). That is, depression can be both a risk factor for dementia and an early indicator of incipient dementia (Fiske et al., 2009). Both anxiety and depression affect cognitive performance at all ages, with long term anxiety being a risk factor for depression. However, stress also has a long-term negative effect on cognition, particularly executive function. Stress affects similar brain structures and cognitive functions (particularly memory) as advancing age, e.g., the hippocampus or the frontal lobes, with effects of psychological stress mediated by the effects of the action of stress hormones such as glucocorticoids (Bunce et al., 2008).

The correlation of depressed mood with nearly every indicator of memory functioning, for example, in the longitudinal studies discussed above (Woods et al., 2000), raises important questions and opens the door to indirect actions of estrogens on cognition through their well-known relationship with mood states. The influence of ovarian function on mood states has been widely claimed in the literature for a considerable time. The issue is interesting in itself, but also in terms of its effects on cognition in later life. That is, mood state could act as an indirect effect of changes in sex hormones on cognition with increasing age. Thus, cognitive complaints during peri-menopause may at least partially result from the effect of peri-menopausal anxiety or depressive symptoms on the brain (Greendale et al., 2011).

Research has demonstrated that the lifetime prevalence rate of mood disorders is significantly greater in women than in men, approximately two times more frequent (Walf and Frye, 2006; Ter-Horst et al., 2009). Data suggests that estrogens, or their absence, are strongly implicated in the regulation of mood and behavior, as well as in the pathobiology of mood disorders (Halbreich and Kahn, 2001). So the interaction of estrogens with the serotonergic systems (Amin et al., 2006) has been taken to propose estradiol as a protective agent against mood changes related to serotonin withdrawal (Wharton et al., 2012). Due to the correlation between depression and serotonin, estrogen treatment can have beneficial effects to buffer changes in mood.

Clinical observation also shows that estrogens play an essential role in the emergence and course of mood disorders in women. Gender differences in mood disorders are present throughout the lifespan, but periods of hormonal fluctuations or estrogens instability (i.e., pre-menstrually, post-partum, peri-menopausally) are related to increased vulnerability to mood disorders among susceptible women (Halbreich and Kahn, 2001).

Mood disorders are prevalent during the menopausal transition, with prevalence figures of 16.5% for depressed mood in women during midlife (Prairie et al., 2015). The SWAN study found that women were two to four times more likely to suffer a depressive episode during the menopausal transition than during the pre-menopausal period (Bromberger et al., 2011). Other investigators have reported similar findings (Soares, 2010). The known interaction of estrogens with serotonin and other monoamines provides biochemical rationality to the clinical

findings (Warnock et al., 2017). Mood problems have been found to increase in those with a history of mood disorders, but can also occur *de novo* as a consequence of the hormonal changes. In most cases, the period of vulnerability to mood problems (Alexander et al., 2007) subsides when hormonal levels stabilize and women enter full menopause. It is understood that it is hormonal fluctuations that more directly determine the mood changes (Wharton et al., 2012).

HT AND MOOD

The clear hormonal influence in mood fluctuations has prompted the postulation of HT as a useful remedy. One recent central study is the previously mentioned KEEPS-Cog, a multicenter randomized trial that, in addition to cognitive effects, assessed the impact on mood, including depression and anxiety. Oral estrogens (CEE), but not transdermal estradiol, effectively reduced scores of depression and anxiety over the 48 months of treatment (Gleason et al., 2015). The reasons for the difference between the treatments remain elusive, although it is possible that the maintained levels of circulating estradiol downregulates ER in the concerned brain regions. This effect of estrogens in KEEPS-Cog further confirms previous studies of different sizes and relevance, as shown in several reviews (Schmidt et al., 2000; Soares et al., 2001; Schiff et al., 2005). Fischer et al. (2014) reviewed five RCTs which investigated the impact of HT on mood, all of which showed a benefit of treatment. Three of the studies involved only estradiol, whereas two found effects for both estrogens alone or with different amounts of progesterone (MPA) and a further study found reductions in depression and anxiety following 3 months of transdermal estradiol with or without norethisterone. Thus, although there is a suggestion of positive effects on mood of HT, the mechanisms need further investigation, with remaining questions being the extent to which mood improvements are direct effects, or indirect via effects of HT on other distressing menopausal symptoms such as sleep disturbance or hot flushes. Furthermore, the complex relationship of depression with cognition also suggests that the influence of HT on depression as a possible mediator of any observed influence on cognition could also be examined in future research, rather than as separate effects.

INDIRECT IMPACTS ON COGNITION VIA EFFECTS OF SEX HORMONE REDUCTION ON CARDIOVASCULAR HEALTH

Cardiovascular diseases represent an important health issue all over the world because they are the primary cause of death for both men and women. Morbidity and mortality rates are higher in men before menopause, whereas after this age, these rates converge.

As reviewed in Navarro-Pardo et al. (2017), estrogens act to improve lipid profile, promote vasodilatation and antioxidant activities, supporting vascular health. By contrast, the menopause leads to an overturn of all these effects, increasing cardiovascular risk. Vascular deterioration is an important issue that should be considered when assessing the impact of the menopause on cognition, for example, the vascular changes associated with vasomotor symptoms that take place during and after menopause. The SWAN study evaluated whether the decrease in cognitive processing speed observed during peri-menopause might be influenced by the existence of hot flushes. Moreover, anxiety and depressive symptoms were also considered in the research. Only a small effect was found, related to anxiety and depressive symptoms, but there was no interaction with vasomotor symptoms (Luetters et al., 2007).

A possible long-term effect has also been raised. The hypothesis that the menopause might accelerate atherosclerosis and consequently increase future cardiovascular risk, has a correlate in terms of cognitive function. Vascular factors are involved in roughly half of all dementia cases, probably because they show the cumulative impact of very different factors along lifespan (Bowler, 2005; Sonnen et al., 2007; Gorelick et al., 2011). Outside of dementia, vascular factors are also associated with cognitive change in otherwise healthy older people. Going back to early longitudinal studies, Wilkie and Eisdorfer (1971) pointed out that "The presence of large numbers of aged with cardiovascular illness suggests that the basis for the cognitive decline associated with aging after maturity should be considered secondary to some pathologic process, and not merely as a "normal" aging process" (p962) and longitudinal studies such as Okonkwo et al. (2011) demonstrated that cardiovascular indices such as variability in blood pressure and cardiac output predicted declines in attention, executive function and psychomotor function over 36 months, but basic resting blood pressure measurements did not. Likewise, Singh-Manoux et al. (2003) demonstrated prediction of poor cognitive function from occurrence of angina pectoris, myocardial infarction, all coronary heart disease and intermittent claudication, 11 years prior to the cognitive measures.

The notion of a critical period for positive cognitive effect or avoidance of a negative impact on cognition or increase in dementia risk is mirrored in investigations of HT where HT is shown to be beneficial in the early peri- and post-menopausal period, but not in older women or those who may already have advanced atherosclerosis (Hodis et al., 2016; Savolainen-Peltonen et al., 2016). These parallel findings further suggest the role of estrogens effects on the cardiovascular system as a mediator in the impact on cognitive function health and changes.

Thus, the relationship between cardiovascular health and cognitive health is well-supported, but studies examining the impact of HT on cardiovascular health as a mediator of any beneficial impact on cognition are needed.

CONCLUSIONS

The presence of ERs has been confirmed in different brain locations related to cognition, mainly to verbal memory and retrieval, working memory, executive function and attention, such as the hippocampus and the prefrontal cortex, leading to the rationale that changes in levels of estrogens would produce direct effects in the brain and accordingly in cognitive function. Furthermore, the interaction of estrogens with serotonergic

pathways also provides a focus for explaining peri-menopausal mood changes.

Menopausal withdrawal of estrogens could also have a specific impact on the vascular tree and thereby have a secondary effect on cognition such as memory declines via vasomotor symptoms.

A range of methodologies have been used to study these effects, such as longitudinal, cross-sectional, and intervention studies, including randomized controlled trials. In reviewing several of these studies, it is concluded that menopause effects on cognitive functioning are not clear because of the series of confounding variables found to have an effect. When confounds such as age are controlled, the most reliable effects are for a measure related to executive function, verbal fluency.

Research on hormonal therapy (HT) in menopausal women has also been reviewed. Differences in HT regarding therapy timeline (onset and duration), composition, manner of administration, and interaction effects with other variables (such as age differences, education, and baseline performance) had significant impacts and made direct comparisons amongst the studies difficult.

It was concluded that HT does not have a clear impact on cognition, including episodic memory or executive functions and is not recommended as a treatment or prevention pathway for cognitive decline or dementia in older women. There is not enough evidence to support the existence of a window of opportunity in the immediate post-menopause, but an HT neutral effect (on cognition) extends for a reasonable number of years post-menopause. The suggestions of beneficial effects on cognitive function in the first few years after menopause may imply some risk reduction regarding cognitive decline and dementias. Although there are useful developments, for example, the interaction of HT impacts with the benefits of physical exercise, late-life cognitive consequences are still poorly addressed.

On another front, mood disorders are twice as frequent in women than in men, and estrogens changes are probably implicated given that differences appear from menarche through reproductive age, including periods of estrogenic instability. An important component of well-being, self-perceived health, seems to change significantly from pre- to post-menopause. Estrogens interact with the serotonergic system so that estradiol could have a protective effect against mood changes, which could implicate the reduction in sex hormones post-menopausally in related episodes of low mood and depression. Related to this depression-serotonin correlation, HT could have a beneficial effect on mood disorders, although the mechanisms need further investigation. Depressed mood and anxiety have a negative influence on cognition, particularly on memory functioning, particularly so for older people. Depression is a well-known risk factor or prodromal symptom for cognitive impairment and dementia.

Cardiovascular effects of reducing estrogen, including increased cardiovascular risk, seem to act as a secondary pathway by which reductions in this sex hormone has an impact on cognition, for example, given that vascular effects are implicated in at least half of all dementia cases and in many other aging processes.

AUTHOR CONTRIBUTIONS

EN-P, CH, and AC: have contributed equally to this article. All authors have read and approved the final manuscript.

REFERENCES

Abe, T., Bereczki, D., Takahashi, Y., Tashiro, M., Iwata, R., and Itoh, M. (2006). Medial frontal cortex perfusion abnormalities as evaluated by positron emission tomography in women with climacteric symptoms. *Menopause* 13, 891–901. doi: 10.1097/01.gme.0000227852.82303.d7

Alexander, J. L., Dennerstein, L., Woods, N. F., Kotz, K., Halbreich, U., Burt, V., et al. (2007). Neurobehavioral impact of menopause on mood. *Expert Rev. Neurother.* 7(Suppl. 11), S81–S91. doi: 10.1586/14737175.7.11s.S81

Amin, Z., Gueorguieva, R., Cappiello, A., Czarkowsk, K., Stiklus, S., Anderson, G., et al. (2006). Estradiol and tryptophan depletion interact to modulate cognition in menopausal women. *Neuropsychofarmacology* 31, 2489–2497. doi: 10.1038/sj.npp.1301114

Arevalo, M. A., Azcoitia, I., and Garcia-Segura, L. (2015). The neuroprotective actions of oestradiol and oestrogen receptors. *Nat. Rev. Neurosci.* 16, 17–29. doi: 10.1038/nrn3856

Azcoitia, I., Arevalo, M. A., and Garcia-Segura, L. M. (2017). Neural-derived estradiol regulates brain plasticity. *J. Chem. Neuroanat.* doi: 10.1016/j.jchemneu.2017.04.004. [Epub ahead of print].

Bowler, J. (2005). Vascular cognitive impairment. *J. Neurol. Neurosurg. Psychiatry* 76, 35–44. doi: 10.1136/jnnp.2005.082313

Bromberger, J. T., Kravitz, H. M., Chang, Y. F., Cyranowski, J. M., Brown, C., and Matthews, K. (2011). Major depression during and after the menopausal transition: Study of Women's Health Across the Nation (SWAN). *Psychol. Med.* 41, 1879–1888. doi: 10.1017/S003329171100016X

Bunce, D., Tzur, M., Rarachurn, A., Gain, F., and Bond, F. W. (2008). Mental health and cognitive function in adults aged 18 to 92 years. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 63, P67–P74. doi: 10.1093/geronb/63.2.P67

Campbell, N., Boustani, M., Limbil, T., Ott, C., Fox, C., Maidment, I., et al. (2009). The cognitive impact of anticholinergics: a clinical review. *Clin. Interv. Aging* 4, 225–233. doi: 10.2147/CIA.S5358

Casadesus, G., Webber, K., Atwood, C., Pappolla, M., Perry, G., Bowen, R., et al. (2006). Luteinizing hormone modulates cognition and amyloid-β deposition in Alzheimer APP transgenic mice. *Biochim. Biophys. Acta* 1762, 447–452. doi: 10.1016/j.bbadis.2006.01.008

Chung, J., Plitman, E., Nakajima, S., Chow, T., Chakravarty, M., Caravaggio, F., et al. (2015). Lifetime history of depression predicts increased amyloid-β accumulation in patients with mild cognitive impairment. *J. Alzheimers Dis.* 45, 907–919. doi: 10.3233/JAD-142931

Coker, L. H., Espeland, M. A., Rapp, S. R., Legault, C., Resnick, S. M., Hogan, P., et al. (2010). Postmenopausal hormone therapy and cognitive outcomes: the Women's Health Initiative Memory Study (WHIMS). *J. Steroid Biochem. Mol. Biol.* 118, 304–310. doi: 10.1016/j.jsbmb.2009.11.007

da Silva, J., Gonçalves-Pereira, M., Xavier, M., and Mukaetova-Ladinska, E. (2013). Affective disorders and risk of developing dementia: systematic review. *Br. J. Psychol.* 202, 177–186. doi: 10.1192/bjp.bp.111.101931

Dunkin, J., Ragson, N., Wagner-Steh, K., David, S. Altshuler, L., and Rapkin, A. (2005). Reproductive events modify the effects of estrogen replacement therapy on cognition in healthy postmenopausal women. *Psychoneuroendocrinology* 30, 284–296. doi: 10.1016/j.psyneuen.2004.09.002

Dunkin, J., Ragson, N., Zeller, M., Wagner-Steh, K., David, S., Altshulter, L., et al. (2006). Estrogne replacement and cognition in postmenopausal women: effect of years since menopause on response to treatment. *Drug Dev. Res.* 66, 150–159. doi: 10.1002/ddr.20054

Epperson, C. N., Sammel, M. D., and Freeman, E. W. (2013). Menopause effects of verbal memory: findings from a longitudinal community cohort. *J. Clin. Endocrinol. Metab.* 98, 3829–3838. doi: 10.1210/jc.2013-1808

Erickson, K. I., Colcombe, S. J., Elavsky, S., McAuley, E., Korol, D. L., Scalf, P. E., et al. (2007). Interactive effects of fitness and hormone treatment on brain health in postmenopausal women. *Neurobiol. Aging* 28, 179–185. doi: 10.1016/j.neurobiolaging.2005.11.016

Espeland, M. A., Shumaker, S. A., Leng, I., Manson, J. E., Brown, C. M., LeBlanc, E. S., et al. (2013). Long-term effects on cognitive function of postmenopausal hormone therapy prescribed to women aged 50 to 55 years. *JAMA Intern. Med.* 173, 1429–1436. doi: 10.1001/jamainternmed.2013.7727

Fischer, B., Gleason, C., and Asthana, S. (2014). Effects of hormone therapy on cognition and mood. *Fertil. Steril.* 101, 898–904. doi: 10.1016/j.fertnstert.2014.02.025

Fiske, A., Wetherel, J., and Gatz, M. (2009). Depression in older adults. *Annu. Rev. Clin. Psychol.* 5, 363–389. doi: 10.1146/annurev.clinpsy.032408.153621

Fuh, J. L., Wang, S. J., Lee, S. J., Lu, S. R., and Juang, K. D. (2006). A longitudinal study of cognition change during early menopausal transition in a rural community. *Maturitas* 53, 447–453. doi: 10.1016/j.maturitas.2005.07.009

Genazzini, A. R., Pluchino, N., Luisi, S., and Luisi, M. (2007). Estrogen, cognition and female ageing. *Hum. Reprod. Update* 13, 175–187. doi: 10.1093/humupd/dml042

Giatti, S., Garcia-Segura, L. M., and Melcangi, R. C. (2015). New steps forward in the neuroactive steroid field. *J. Steroid Biochem. Mol. Biol.* 153, 127–134. doi: 10.1016/j.jsbmb.2015.03.002

Gleason, C. E., Dowling, N. M., Wharton, W., Manson, J. E., Miller, V. M., Atwood, C. S., et al. (2015). Effects of hormone therapy on cognition and mood in recently postmenopausal women: findings from the randomized, controlled KEEPS-Cognitive and Affective Study. *PLoS Med.* 12:e1001833. doi: 10.1371/journal.pmed.1001833

Gorelick, P., Scuteri, A., Black, S., Decarli, C., Greenberg, S., Iadecola, C., et al. (2011). Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 42, 2672–2713. doi: 10.1161/STR.0b013e3182299496

Gracia, C. R., Samuel, M. D., Freeman, E. W., Lin, H., Langan, E., Kapoor, S., et al. (2005). Defining menopause status: creation of a new definition to identify the ealry changes of the menopause transition. *Menopause* 12, 128–135. doi: 10.1097/00042192-200512020-00005

Greendale, G. A., Derby, C. A., and Maki, P. M. (2011). Perimenopause and cognition. *Obstet. Gynecol. Clin. North Am.* 38, 519–535. doi: 10.1016/j.ogc.2011.05.007

Halbreich, U., and Kahn, L. (2001). Role of estrogen in the aetiology and treatment of mood disorders. *CNS Drugs* 15, 797–817. doi: 10.2165/00023210-200115100-00005

Hara, Y., Waters, E. M., McEwen, B. S., and Morrison, J. H. (2015). Estrogen effects on cognitive and synaptic health over the lifecourse. *Physiol. Rev.* 95, 785–807. doi: 10.1152/physrev.00036.2014

Hasselmo, M. (2006). The role of acetylcholine in learning and memory. *Curr. Opin. Neurobiol.* 16, 710–715. doi: 10.1016/j.conb.2006.09.002

Henderson, V. W., and Popat, R. A. (2011). Effects of endogenous and exogenous estrogen exposures in midlife and late-life women on episodic memory and executive functions. *Neuroscience* 191, 129–138. doi: 10.1016/j.neuroscience.2011.05.059

Hesson, J. (2012). Cumulative estrogen exposure and prospective memory in older women. *Brain Cogn.* 80, 89–95. doi: 10.1016/j.bandc.2012.05.001

Hodis, H. N., Mack, W. J., Henderson, V. W., Shoupe, D., Budoff, M. J., Hwang-Levine, J., et al. (2016). Vascular effects of early versus late postmenopausal treatment with estradiol. *N. Engl. J. Med.* 374, 1221–1231. doi: 10.1056/NEJMoa1505241

Imtiaz, B., Taipale, H., Tanskanen, A., Tiihonen, M., Kivipelto, M., Heikkinen, A., et al. (2017). Risk of Alzheimer's disease among users of postmenopausal hormone therapy: a nationwide case-control study. *Maturitas* 98, 7–13. doi: 10.1016/j.maturitas.2017.01.002

Izumi, S., Muano, T., Mori, A., Kika, G., and Okuwaki, S. (2006). Common carotid artery stiffness, cardiovascular function and lipid metabolism after menopause. *Life Sci.* 78, 1696–1701. doi: 10.1016/j.lfs.2005.08.006

Luetters, C., Huang, M. H., Seeman, T., Buckwalter, G., Meyer, P., Avis, N. E., et al. (2007). Menopause transition stage and endogenous estradiol and follicle-stimulating hormone levels are not related to cognitive performance: cross-sectional results from the study of women's health across the nation (SWAN). *J. Womens Health* 16, 331–344. doi: 10.1089/jwh.2006.0057

Luine, V. (1985). Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. *Exp. Neurol.* 89, 484–490. doi: 10.1016/0014-4886(85)90108-6

Maki, P. (2013). The critical window hypothesis of hormone therapy and cognition: a scientific update on clinical studies. *Menopause* 20, 695–709. doi: 10.1097/GME.0b013e3182960cf8

Maki, P. (2015). Verbal memory and menopause. *Maturitas* 82, 288–290. doi: 10.1016/j.maturitas.2015.07.023

McEwen, B. (2001). Estrogens effects on the brain: multiple sites and molecular mechanisms. *J. Appl. Physiol.* 91, 2785–2801. doi: 10.1152/jappl.2001.91.6.2785

Melcangi, R. C., Giatti, S., and Garcia-Segura, L. M. (2016). Levels and actions of neuroactive steroids in the nervous system under physiological and pathological conditions: sex-specific features. *Neurosci. Biobehav. Rev.* 67, 25–40. doi: 10.1016/j.neubiorev.2015.09.023

Mueller, K. D., Koscik, R. L., LaRue, A., Clark, L. R., Hermann, B., Johnson, S. C., et al. (2015). Verbal fluency and early memory decline: results from the Wisconsin Registry for Alzheimer's prevention. *Arch. Clin. Neuropsychol.* 30, 448–457. doi: 10.1093/arclin/acv030

Navarro-Pardo, E., Mikkola, T., Simoncini, T., Millan, M., Julia, M., and Cano, A. (2017). "The impact of estrogen decline on other noncommunicable diseases," in *Menopause: A Comprehensive Approach*, ed A. Cano (Zug: Springer International), 159–178.

Okonkwo, O. C., Cohen, R. A., Gunstad, J., and Athena Poppas, M. D. (2011). Cardiac output, blood pressure variability, and cognitive decline in geriatric cardiac patients. *J. Cardiopulm. Rehabil. Prev.* 31, 290–297. doi: 10.1097/HCR.0b013e318220a817

Panza, F., Frisardi, V., Capurso, C., D'Introno, A., Colacicco, A. M., Imbimbo, B. P., et al. (2010). Late-life depression, mild cognitive impairment, and dementia: possible continuum? *Am. J. Geriatr. Psychiatry* 18, 98–116. doi: 10.1097/JGP.0b013e3181b0fa13

Parikh, M., Hynan, L. S., Weiner, M. F., Lacritz, L., Ringe, W., and Cullum, C. M. (2014). Single neuropsychological test scores associated with rate of cognitive decline in early Alzheimer disease. *Clin. Neuropsychol.* 28, 926–940. doi: 10.1080/13854046.2014.944937

Pompili, A., Arnone, B., and Gasbarri, A. (2012). Estrogens and memory in physiological and neuropathological conditions. *Psychoneuroendocrinology* 37, 1379–1396. doi: 10.1016/j.psyneuen.2012.01.007

Prairie, B. A., Wisniewski, S. R., Luther, J., Hess, R., Thurston, R. C., Wisner, K. L., et al. (2015). Symptoms of depressed mood, disturbed sleep, and sexual problems in midlife women: cross-sectional data from the Study of Women's Health Across the Nation. *J. Womens Health* 24, 119–126. doi: 10.1089/jwh.2014.4798

Resnick, S., Coker, L., Maki, P., Rapp, S., Espeland, M., and Shumaker, S. (2004). The Women's Health Initiative Study of Cognitive Aging (WHISCA): a randomised clinical trial of the effects of hormone therapy on age-associated cognitive decline. *Clin. Trials* 1, 440–450. doi: 10.1191/1740774504cn040oa

Resnick, S. M., Espeland, M. A., Jaramillo, S. A., Hirsch, C., Stefanick, M. L., Murray, A. M., et al. (2009). Postmenopausal hormone therapy and regional brain volumes: the WHIMS-MRI Study. *Neurology* 72, 135–142. doi: 10.1212/01.wnl.0000339037.76336.cf

Resnick, S., and Henderson, V. (2002). Hormone therapy and risk of Alzheimer's disease: a critical time. *JAMA* 288, 2170–2172. doi: 10.1001/jama.288.17.2170

Rettberg, J. R., Yao, J., and Brinton, R. D. (2014). Estrogen: a master regulator of bioenergetic systems in the brain and body. *Front. Neuroendocrinol.* 35, 8–30. doi: 10.1016/j.yfrne.2013.08.001

Ruiz-Palmero, I., Ortiz-Rodriguez, A., Melcangi, R. C., Caruso, D., Garcia-Segura, L. M., Rune, G. M., et al. (2016). Oestradiol synthesized by female neurons generates sex differences in neuritogenesis. *Sci. Rep.* 6:31891. doi: 10.1038/srep31891

Ryan, J., Carrière, I., Scali, J., Dartigues, J., Tzourio, C., Poncet, M., et al. (2009). Characteristics of hormone therapy, cognitive function, and dementia: the prospective 3C study. *Neurology* 73, 1729–1737. doi: 10.1212/WNL.0b013e3181c34b0c

Ryan, J., Scali, J., Carrière, I., Amieva, H., Rouaud, O., Berr, C., et al. (2014). Impact of a premature menopause on cognitive function in later life. *BJOG* 121, 1729–1739. doi: 10.1111/1471-0528.12828

Santoro, N., Brown, J. R., Adel, T., and Skurnick, J. H. (1996). Characterization of reproductive hormonal dynamics in the perimenopause. *J. Clin. Endocrinol. Metab.* 81, 1495–1501.

Santoro, N., and Sutton-Tyrrell, K. (2011). The SWAN song: study of women's health across the nation's recurring themes. *Obstet. Gynecol. Clin. North Am.* 38, 417–423. doi: 10.1016/j.ogc.2011.05.001

Savolainen-Peltonen, H., Tuomikoski, P., Korhonen, P., Hoti, F., Vattulainen, P., Gissler, M., et al. (2016). Cardiac death risk in relation to the age at initiation or the progestin component of hormone therapies. *J. Clin. Endocrinol. Metab.* 101, 2794–2801. doi: 10.1210/jc.2015-4149

Schiff, R., Bulpitt, C., Wesnes, K., and Rajkumar, C. (2005). Short-term transdermal estradiol therapy, cognition and depressive symptoms in healthy older women. A randomised placebo controlled pilot cross-over study. *Psychoneuroendocrinology* 30, 309–315. doi: 10.1016/j.psyneuen.2004.08.007

Schmidt, P. J., Keenan, P. A., Schenkel, L. A., Berlin, K., Gibson, C., and Rubinow, D. R. (2013). Cognitive performance in healthy women during induced hypogonadism and ovarian steroid addback. *Arch. Womens Ment. Health* 16, 47–58. doi: 10.1007/s00737-012-0316-9

Schmidt, P. J., Nieman, L., Danaceau, M. A., Tobin, M. B., Roca, C. A., Murphy, J. H., et al. (2000). Estrogen replacement in perimenopause-related depression: a preliminary report. *Am. J. Obstet. Gynecol.* 183, 414–420. doi: 10.1067/mob.2000.106004

Schneider, L. (2004). Estrogen and dementia: insights from the Women's Health Initiative Memory Study. *JAMA* 291, 3005–3007. doi: 10.1001/jama.291.24.3005

Sherwin, B. (1997). Estrogen effects on cognition in menopausal women. *Neurology* 48(5 Suppl. 7), 21S−26S. doi: 10.1212/WNL.48.5_Suppl_7.21S

Sherwin, B. (2006). Estrogen and cognitive aging in women. *Neuroscience* 138, 1021–1026. doi: 10.1016/j.neuroscience.2005.07.051

Shumaker, S. A., Legault, C., Rapp, S. R., Thal, L., Wallace, R. B., Ockene, J. K., et al. (2003). Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA* 289, 2651–2662. doi: 10.1001/jama.289.20.2651

Singh-Manoux, A., Britton, A. R., and Marmot, M. (2003). Vascular disease and cognitive function: evidence from the Whitehall II Study. *JAGS* 51, 1445–1450. doi: 10.1046/j.1532-5415.2003.51464.x

Skinner, D. C., Albertson, A. J., Navratil, A., Smith, A., Mignot, M., Talbott, H., et al. (2009). Effects of gonadotrophin-releasing hormone outside the hypothalamic-pituitary-reproductive axis. *J. Neuroendocrinol.* 21, 282–292. doi: 10.1111/j.1365-2826.2009.01842.x

Soares, C. (2010). Can depression be a menopause-associated risk? *BMC Med.* 8:79. doi: 10.1186/1741-7015-8-79

Soares, C. N., Almeida, O. P., Joffe, H., and Cohen, L. S. (2001). Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebo-controlled trial. *Arch. Gen. Psychiatry* 58, 529–534. doi: 10.1001/archpsyc.58.6.529

Sonnen, J. A., Larson, E. B., Crane, P. K., Haneuse, S., Li, G., Schellenberg, G. D., et al. (2007). Pathological correlates of dementia in a longitudinal, population-based sample of aging. *Ann. Neurol.* 62, 406–413. doi: 10.1002/ana.21208

Ter-Horst, G. J., Wichmann, R., Gerrits, M., Westenbroek, C., and Lin, Y. (2009). Sex differences in stress responses: focus on ovarian hormones. *Physiol. Behav.* 97, 239–249. doi: 10.1016/j.physbeh.2009.02.036

Vierk, R., Glassmeier, G., Zhou, L., Brandt, N., Fester, L., Dudzinski, D., et al. (2012). Aromatase inhibition abolishes LTP generation in female but not in male mice. *J. Neurosci.* 32, 8116–8126. doi: 10.1523/JNEUROSCI.5319-11.2012

Walf, A. A., and Frye, C. A. (2006). A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. *Neuropsychopharmacology* 31, 1097–1111. doi: 10.1038/sj.npp.1301067

Warnock, J. K., Cohen, L. J., Blumenthal, H., and Hammond, J. E. (2017). Hormone-related migraine headaches and mood disorders: treatment with estrogen stabilization. *Pharmacotherapy* 37, 120–128. doi: 10.1002/phar.1876

Wharton, W., Gleason, C. E., Olson, S. R., Carlsson, C. M., and Asthana, S. (2012). Neurobiological underpinnings of the estrogen–mood relationship. *Curr. Psychiatry Rev.* 8, 247–256. doi: 10.2174/157340012800792957

Wilkie, F., and Eisdorfer, C. (1971). Intelligence and blood pressure in the aged. *Science* 172, 959–962. doi: 10.1126/science.172.3986.959

Woods, N. F., Mitchel, E. S., and Adams, C. (2000). Memory functioning among midlife women: observations from the Seattle Midlife Women's Health Study. *Menopause* 7, 257–265. doi: 10.1097/00042192-200007040-00008

Zandi, P., Carlson, M., Plassman, B., Welsh-Bohmer, K., Mayer, L., Steffens, D., et al. (2002). Hormone replacement therapy and incidence of Alzheimer's deisease in older women: the Cache County study. *JAMA* 288, 2123–2129. doi: 10.1001/jama.288.17.2123

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Molecular Neuroscience

ORIGINAL RESEARCH
published: 15 November 2017
doi: 10.3389/fnmol.2017.00384

Adrenomedullin Contributes to Age-Related Memory Loss in Mice and Is Elevated in Aging Human Brains

Ignacio M. Larrayoz[1†], Hilda Ferrero[2†], Eva Martisova[2], Francisco J. Gil-Bea[2], María J. Ramírez[2] and Alfredo Martínez[1]*

[1]Oncology Area, Center for Biomedical Research of La Rioja (CIBIR), Logroño, Spain, [2]Department of Pharmacology and Toxicology, University of Navarra, Pamplona, Spain

Memory decline is common in elderly individuals and is the hallmark of Alzheimer's disease (AD). Memory failure follows the loss of synaptic contacts in the cerebral cortex and hippocampus, caused in part by cytoskeleton disruption. Adrenomedullin (AM) and its gene-related peptide, proadrenomedullin N-terminal 20 peptide (PAMP), are microtubule-associated proteins (MAP) whose expression has been identified as a potential biomarker for predicting progression from predementia to clinical AD. Here we analyze the connection between AM levels and memory preservation. Mice lacking neuronal AM and PAMP (knockout, KO) and their wild type (WT) littermates were subjected, at different ages, to the novel object recognition test and the contextual fear conditioned test. Aged KO mice have significantly better retention memory than their WT counterparts. This feature was more prominent in females than in males. Prefrontal cortex and hippocampus samples from these animals were subjected to Western blotting for phospho-Tau and acetylated tubulin. Aged female KO mice had significantly less accumulation of phospho-Tau than their WT littermates. In addition, protein extracts from the frontal cortex of non-demented mature (65.10 ± 3.86 years) and aged (77.14 ± 2.77 years) human donors were analyzed by Western blotting. Aged human brains had significantly higher levels of AM and lower levels of acetylated tubulin than younger donors. These observations suggest that drugs or interventions that reduce AM/PAMP expression may constitute a new avenue to prevent memory decline during normal aging and in patients suffering moderate AD in high risk of rapid cognitive decline.

Keywords: adrenomedullin, normal aging, Alzheimer's disease, memory loss, microtubules, p-Tau

OPEN ACCESS

Edited by:
Luis Miguel Garcia-Segura, Consejo Superior de Investigaciones Científicas (CSIC), Spain

Reviewed by:
Jorge Valero, Achucarro Basque Center for Neuroscience/Ikerbasque Basque Foundation for Science, Spain
Yolanda Diz-Chaves, Institute of Biomedical Research of Vigo (IBIV), The Biomedical Research Centre (CINBIO), Spain

***Correspondence:**
Alfredo Martínez
amartinezr@riojasalud.es

†These authors have contributed equally to this work.

***Received:** 04 August 2017*
***Accepted:** 03 November 2017*
***Published:** 15 November 2017*

Citation:
Larrayoz IM, Ferrero H, Martisova E, Gil-Bea FJ, Ramírez MJ and Martínez A (2017) Adrenomedullin Contributes to Age-Related Memory Loss in Mice and Is Elevated in Aging Human Brains. Front. Mol. Neurosci. 10:384. doi: 10.3389/fnmol.2017.00384

INTRODUCTION

Memory loss is a common characteristic of normal aging (Leal et al., 2017) which gets greatly accelerated in some neurodegenerative diseases. Alzheimer's disease (AD) is the most frequent cause of memory loss and other dementia symptoms among elderly patients (Hickman et al., 2016). AD is an irreversible degenerative pathology of the brain characterized by

Abbreviations: AD, Alzheimer's disease; AM, adrenomedullin; APP, amyloid precursor protein; CFH, complement factor H; CNS, central nervous system; KO, knockout; MAP, microtubule associated protein; NORT, novel object recognition test; PAMP, proadrenomedullin N-terminal 20 peptide; PHF, paired helical filaments; SNP, single nucleotide polymorphism; WT, wild type.

progressive deterioration of cognitive functions, affecting mainly neurons in the hippocampus and the cerebral cortex. The histological hallmarks of AD include senile plaques, made up by accumulations of β-amyloid (Aβ) peptide, and neurofibrillary tangles, which are deposits of Tau, a microtubule associated protein (MAP), which gets abnormally phosphorylated (pTau; Hernandez et al., 2013). Pathological accumulation of pTau, which compromises synaptic transmission and neuronal viability by contributing to cytoskeleton collapse, correlates better with the severity of cognitive decline in AD than senile plaques (Nelson et al., 2012).

The causes of memory loss during normal aging are not completely understood. Atrophy of some brain areas has been shown in normal aging (Pini et al., 2016) and changes in intrinsic neural electrical excitability associated with oxidative stress have been hypothesized as potential causes (Hermann et al., 2014). Subtle perturbations in stabilization of neuronal cytoskeleton, reminiscent of those occurring during AD neurodegeneration, may also be an important underlying cause of age-associated neuronal dysfunction and cognitive decline (Savva et al., 2009). In this line, modifications on pTau expression and status (tauopathies) are also typical of normal aging (Delacourte et al., 2002) and their distribution pattern correlates with memory capabilities (Guillozet et al., 2003).

Some interventions have been proposed to delay aging-related cognitive deficits and thus improve the quality of life in older people. Some of these interventions include mental stimulation and physical activity (Collette and Salmon, 2014) but a better knowledge of the mechanisms underlying the loss of memory may help in devising novel pharmaceutical approaches.

In the search for predictive blood biomarkers of AD cognitive decline, some studies have found that mid-regional proadrenomedullin is elevated in the plasma of AD patients and that the concentration of this peptide could have predictive value in the progression from predementia to clinical AD (Buerger et al., 2011; Henriksen et al., 2014), although a recent study on a Swedish population found no correlation (Holm et al., 2017). The proadrenomedullin gene, *adm*, codes for a 185 amino acid preprohormone which, after post-translational modifications, generates two biologically active peptides: proadrenomedullin N-terminal 20 peptide (PAMP) and adrenomedullin (AM). Both peptides are amidated at their carboxy terminus and their 3-dimensional structure is based on a central α-helix (Pérez-Castells et al., 2012). Expression of these peptides is widespread and several functions have been ascribed to them, including vasodilatation, bronchodilatation, angiogenesis, hormone secretion regulation, growth modulation and antimicrobial activities, among others (López and Martínez, 2002). In the central nervous system (CNS), AM is expressed throughout the whole brain and spinal cord (Serrano et al., 2000) where it acts as a neuromodulator through mechanisms dependent and independent of NMDA receptors (Xu and Krukoff, 2004). It has been shown that plasma levels of AM increase with normal aging (Kato et al., 2002).

Knockout studies have shown that total abrogation of *adm* results in embryo lethality (Caron and Smithies, 2001). To circumvent this problem, we generated a conditional knockout model where *adm* was eliminated just from neurons by using Cre/loxP technology, and the physiological consequences of such manipulation have been published (Fernández et al., 2008, 2010; Hurtado et al., 2010).

An intriguing finding from our laboratory showed that both AM and PAMP decorate the microtubules in a variety of cell types, including neurons (Sackett et al., 2008). Yeast-2-hybrid analysis demonstrated that AM binds to several MAPs, whereas PAMP binds directly to tubulin and kinesin. Cell physiology studies point to a direct involvement of PAMP in regulating microtubule dynamics and kinesin speed (Larráyoz and Martínez, 2012). Downregulation of *adm* expression, through either gene knockdown or targeted knockout, results in a massive hyperpolymerization of the tubulin cytoskeleton, an increase on Glu- and acetylated-tubulin, a reduction of kinesin velocity, and the apparition of actin filopodia in CNS stem/progenitor cells (Sackett et al., 2008; Vergaño-Vera et al., 2010). In addition, AM immunoreactivity increases in the brain of AD patients (Ferrero et al., 2017) and in mouse models of AD where it seems to be associated with activated astrocytes in the vicinity of amyloid plaques (Fernandez et al., 2016).

Taking all this into consideration we decided to investigate the potential connection between *adm* gene products and normal aging memory loss in a mouse model and the expression of AM in human brains.

MATERIALS AND METHODS

Knockout (KO) Mice Lacking Neuronal AM

Conditional knockout (KO) mice where AM was eliminated from neurons have been previously described (Fernández et al., 2008). KO and wild type (WT) littermates of both sexes were allowed free access to food and water under standard laboratory conditions, with light/dark cycles of 12/12 h, and a constant temperature of 24ºC. All procedures were carried out in accordance with the European Communities Council Directive (86/609/CEE) on animal experiments and with approval from the ethical committees on animal welfare of our institutions (OEBA-CIBIR and University of Navarra protocol 054-12).

Behavioral Tests

Novel Object Recognition Test (NORT)

Test was performed as previously described (Gerenu et al., 2013) in young (3 months old) and old (18 months old) mice. In short, animals (n = 8 per group) were first familiarized with the arena (65 × 65 × 45 cm) for 30 min and after 1 day mice were allowed to explore two identical objects during 5 min (training). Retention was assessed 24 h post-training, when one object was replaced by a novel one. Retention score is expressed as discrimination index (percentage of time exploring the novel object to the total time of object exploration).

Contextual Fear Conditioned Test

After 24 h of rest, all mice were subjected to a fear conditioned test. The behavioral procedure involved three phases: habituation, training, and testing. Mice were habituated for 5 min to the context, which consisted in a soundproof box with white walls, light, and a background noise produced by a fan. The training phase was conducted 24 h later, where mice were placed in the same context and allowed to explore for 2 min prior to a 2-s footshock (0.3 mA) stimulus. After 30 s mice were returned to their home cage. 24 h later mice were placed back in the conditioning box and allowed to explore the context for 2 min, during which freezing time was recorded (contextual long-term memory). Freezing behavior was defined as an absence of cage displacement. Freezing scores were expressed as percentages of total freezing time. The conditioning procedure was carried out in a StartFear system (Panlab S.L., Barcelona, Spain) that allows movement recording by a high-sensitivity Weight Transducer system and data analysis by the built-in FREEZING and STARTLE software.

Human Brain Tissue

Brain tissues were obtained from the Oxford Project to Investigate Memory and Ageing (OPTIMA, see www.medsci.ox.ac.uk/optima). Subjects for this study constituted a randomly selected subset of the participants, now part of the Thomas Willis Oxford Brain Collection within the Brains for Dementia Research Initiative (BDR). At death, informed consent had been obtained from the patients' next-of-kin before collection of brains and the study was approved by the UK National Research Ethics Service. All cases were selected based on clinic-pathological consensus diagnoses. A total of 12 individuals were included in the study. They included six mature donors (3 men, 3 women, age at death = 65.10 ± 3.86 years; post mortem delay = 34.10 ± 4.91 h) and six older donors (3 men, 3 women, age at death = 77.14 ± 2.77 years; post mortem delay = 49.42 ± 6.82 h). These participants were classified as normal controls, did not have dementia or other neurological diseases, did not meet CERAD criteria for AD diagnosis, and were staged at Braak 0-II. Frontal (Brodmann Area, BA10) cortices were dissected free of meninges. To partially mitigate the possible effects of cause of death on neurochemical determinations, brain pH was measured as an index of acidosis associated with terminal coma. Brain pH is used as an indication of tissue quality in post-mortem research, with pH > 6.1 considered acceptable (Bahn et al., 2001; Lewis, 2002). All the tissue used fulfilled this condition. All subsequent analyses were performed blind to clinical information.

Western Blotting

Two days after completing behavioral testing, all mice were euthanized and prefrontal cortex and hippocampus were dissected out. Mouse and human samples were homogenized in RIPA buffer (Thermo Scientific, Rockford, IL, USA) containing protease (EDTA-free complete, Roche, Basilea, Switzerland) and phosphatase (PhosStop, Roche) inhibitors. Homogenates were centrifuged for 30 min at 15,000× *g* and the supernatants collected. Protein concentration was determined by the BCA kit (Pierce, Rockford, IL, USA), with bovine serum albumin as standard, using a spectrophotometer (POLARstar Omega, BMG Labtech, Ortenberg, Germany). Then, 25 μg of each sample were mixed with 4× sample buffer (Invitrogen, Carlsbad, CA, USA) and heated for 10 min at 70°C. Samples were run on 4%–12% SDS–polyacrylamide gels. Seeblue plus 2 Prestained Standards (Invitrogen) were used as molecular weight markers. Proteins were transferred onto 0.2-μm nitrocellulose membranes (Amersham GE HealthCare, Pittsburgh, PA, USA). Membranes were incubated overnight at 4°C with primary antibodies followed by peroxidase-labeled secondary antibodies (**Table 1**). Immunoreactive bands were visualized using enhanced chemiluminescence and quantified by an image analyzer (Quantity One, Bio-Rad, Hercules, CA, USA). Membranes were stripped with Restore PLUS Western Blot Stripping Buffer (Thermo Scientific). β-Actin was used as an internal loading control. Results were calculated as the percentage of optical density (OD) values of the WT.

Statistical Analysis

Data were analyzed by SPSS for Windows, release 15.0. Normalcy and homoscedasticity were checked by Shapiro–Wilks's and Levene's tests, respectively. Normally distributed data were analyzed by Student's *t*-test or two-way analysis of variance

TABLE 1 | Antibodies and conditions used in this study.

Target	Species	Dilution	Reference
Adrenomedullin	Rabbit polyclonal	1:500	Novus NBP1-19731
AT8	Mouse monoclonal	1:1000	Thermo Scientific MN1020
PHF1	Mouse monoclonal	1:1000	Gift from Peter Davies
TAU	Mouse monoclonal	1:1000	Cell Signaling 4019
Acetylated tubulin	Mouse monoclonal	1:15,000	Sigma T7451
β-Actin	Mouse monoclonal	1:10,000	Sigma AC74
Secondary peroxidase-labeled antibodies			
Specificity	**Host**	**Dilution**	**Reference**
Anti-mouse	Goat	1:5000	Dako P0447
Anti-rabbit	Goat	1:5000	Dako P0448

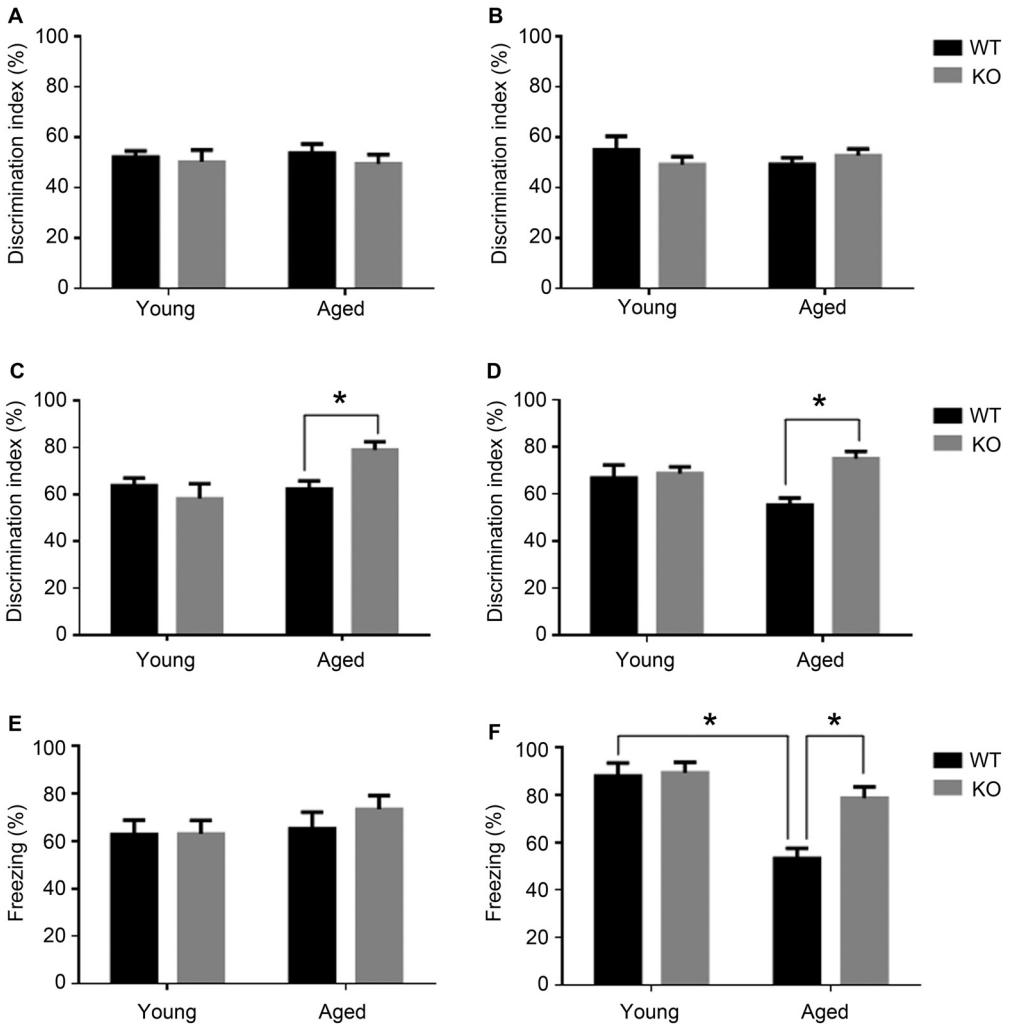


FIGURE 1 | Cognitive phenotype in animals lacking neuronal *adm* as tested in the novel object recognition test (NORT, **A–D**) and fear conditioning **(E,F)**. Behavioral NORT data are shown as percentage of discrimination index (time exploring new object/total time of exploration *100) in male **(A,C)** and female **(B,D)**, young (3 months) and aged (18 months) mice. Discrimination index was measured with two identical objects on day 2 **(A,B)** and after introducing the novel object on day 3 **(C,D)**. Fear conditioning data are shown as percentage of freezing over the 2 min test in male **(E)** and female **(F)** young and aged mice. Two-way analysis of variance (ANOVA) (age × genotype), $*p < 0.05$ interaction. WT, wild type; KO, *adm* knockout.

(ANOVA) (genotype × age) followed by Tukey's *post hoc* test. *P* values lower than 0.05 were considered statistically significant.

RESULTS

Mouse Experiments

The conditional AM KO animals and their WT littermates were tested in two different cognitive paradigms: the novel object recognition test (NORT) and the fear conditioning test. Animals included were either young (3 months) or aged (18 months) mice. Each age group was composed by KO ($n = 8$) and WT ($n = 8$) littermates.

First of all, to ensure that WT and KO mice had no differences in general locomotor activity or other psychological/physiological traits that may interfere with memory determination, their investigative activity was recorded on the first and second day of the experiment. On the first day, in the first 30 min, WT mice traveled 18871 ± 2362 cm whereas KO mice walked for 16486 ± 1666 cm (*t* test, $p > 0.05$). On the second day (training), when mice were presented with two identical objects, all groups of mice had a ratio close to 50% and there were no significant differences among them (*t* test, $p > 0.05$; **Figures 1A,B**). These results show that genotype does not influence mice ability to move freely or to show curiosity when presented with different objects.

When presented with the novel object, young animals had no significant differences in recognition memory between those carrying *adm* and the knockouts (**Figures 1C,D**).

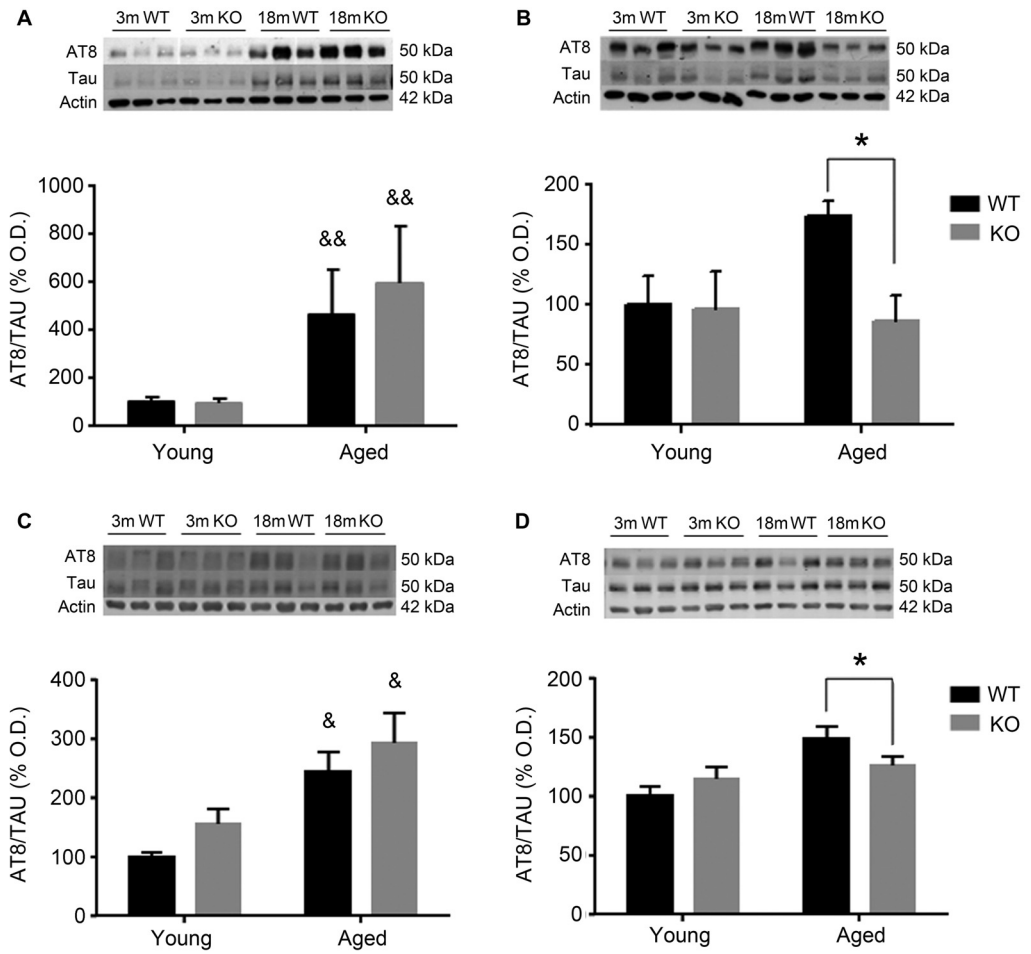


FIGURE 2 | Expression of pTAU (using the AT8 antibody) in the frontal cortex **(A,B)** and the hippocampus **(C,D)** of male **(A,C)** and female **(B,D)**, young (3 months, 3 m) and aged (18 months, 18 m), control wild type (WT) and *adm* knockout (KO) mice. Two-way ANOVA: $^{*}p < 0.05$ interaction, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ main effect of age. Panels show percentage of optical density (OD) values of control and representative pictures of the blotting. β-Actin is used as internal loading control.

Recognition memory was significantly facilitated in aged *adm* KO animals, both in males (**Figure 1C**, two way ANOVA, significant interaction $F = 12.44$, $p < 0.05$, Tukey's multiple comparisons test, $p < 0.05$) and females (**Figure 1D**, two way ANOVA, significant interaction $F = 11.11$, $p < 0.05$, Tukey's multiple comparisons test, $p < 0.05$).

In the fear conditioning test (contextual learning), first we measured the freezing levels during training and found they were around 40% for both genotypes (t test, $p > 0.05$) during the first 2.5 min of their stay in the chamber. We also tested foot shock sensitivity by measuring the freezing levels immediately following the foot shock. These levels were close to 80% for both genotypes (t test, $p > 0.05$) for the 30 s following the shock, indicating that the genotype has no influence in regular freezing behavior or in foot pain sensitivity.

During the memory test period, male mice showed no statistically significant differences in freezing behavior irrespective of age or genotype (**Figure 1E**). In contrast, older female WT mice presented an age-related memory loss as demonstrated by a statistically significant reduction in freezing time when compared with younger females of the same genotype (**Figure 1F**, two way ANOVA, significant interaction $F = 9.02$, $p < 0.05$, Tukey's multiple comparisons test, $p < 0.05$). Interestingly, old female mice lacking neuronal *adm* showed better memory than their WT littermates (Tukey's multiple comparisons test $p < 0.05$).

Altogether, both cognitive tests point to a memory protection phenotype in animals lacking neuronal *adm*.

To investigate the possible mechanism underlying these behavioral observations, changes in pTau expression were checked in the mouse prefrontal cortex and hippocampus using two different antibodies: AT8 (**Figure 2**) and PHF1 (**Figure 3**). In male mice, there was an increased pTau/Tau ratio associated to aging that was not significantly affected by deleting the *adm* gene (two way ANOVA, main effect

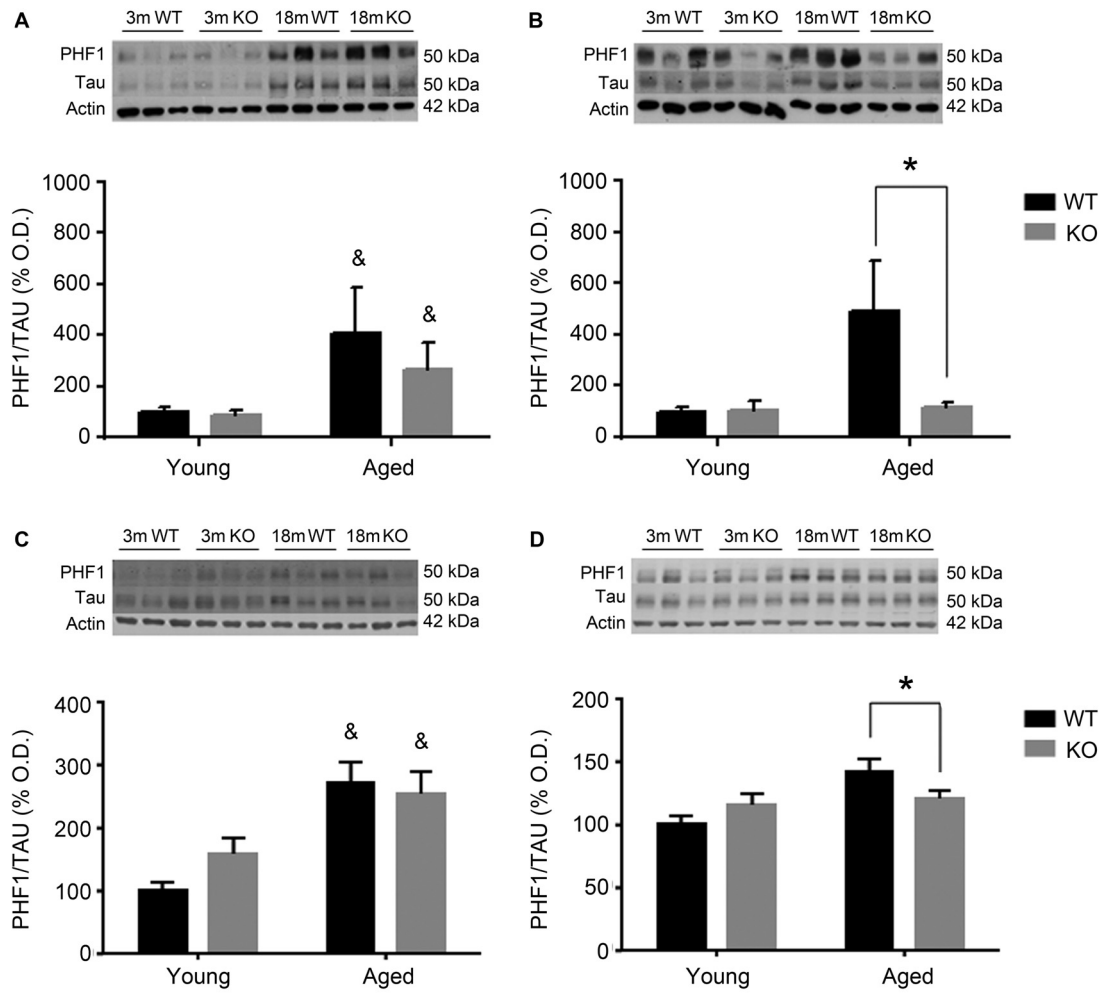


FIGURE 3 | Expression of pTAU (using the PHF1 antibody) in the frontal cortex **(A,B)** and the hippocampus **(C,D)** of male **(A,C)** and female **(B,D)**, young (3 months, 3 m) and aged (18 months, 18 m), control (WT) and *adm* knockout (KO) mice. Two-way ANOVA: $^{*}p < 0.05$ interaction, $^{\&}p < 0.05$ main effect of age. Panels show percentage of OD values of control and representative pictures of the blotting. β-Actin is used as internal loading control. β-Actin and total TAU are the same as in **Figure 2** and are repeated here to allow a direct comparison with the total protein and loading controls.

of age, $F = 21.50$, $p < 0.01$, **Figures 2A,C**). However, in female mice, a significant interaction between genotype and age was found (**Figures 2B,D**), and the increased expression of pTAU associated to aging was counteracted in mice lacking the *adm* gene (two way ANOVA, significant interaction $F = 16.81$, $p < 0.05$, Tukey's multiple comparisons test, $p < 0.05$).

For the other antibody, PHF1 (**Figure 3**), results were perfectly parallel to those observed with AT8. There was an increased pTau/Tau ratio associated to aging in males that was not significantly affected by deleting the *adm* gene (two way ANOVA, main effect of age, $F = 28.81$, $p < 0.05$, **Figures 3A,C**). In females, a significant interaction between genotype and age was found (**Figures 3B,D**), and the increased expression of pTAU associated to aging was counteracted in mice lacking the *adm* gene (two way ANOVA, significant interaction $F = 18.24$, $p < 0.05$ Tukey's multiple comparisons test, $p < 0.05$).

To further study cytoskeleton stability, tubulin acetylation was checked in the frontal cortex and hippocampus of these animals. Aged male mice showed a decrease in the expression of acetylated tubulin that was independent of the genotype (two way ANOVA, main effect of age, $F = 39.23$, $p < 0.01$, **Figures 4A,C**). No effect associated to either age or genotype was found in female mice (**Figures 4B,D**).

Human Specimens

To test whether similar changes happen in humans, frontal cortex protein extracts were obtained from mature and aged human donors. Western blots for AM showed a significant ($p < 0.05$) increase in the relative levels of this peptide in aged

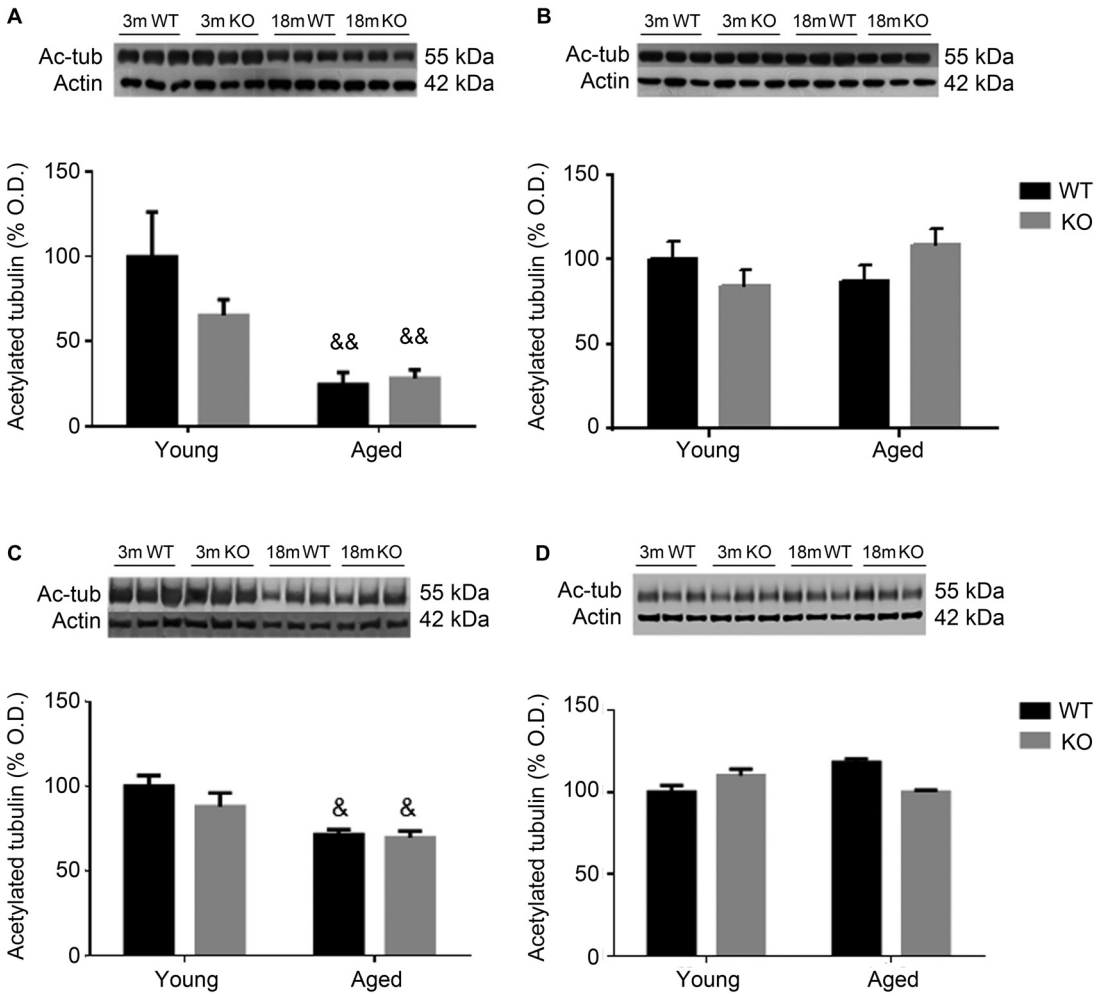


FIGURE 4 | Expression of acetylated tubulin (Ac-tub) in the frontal cortex **(A,B)** and hippocampus **(C,D)** of male **(A,C)** and female **(B,D)**, young (3 months, 3 m) and aged (18 months, 18 m), control (WT) and *adm* knockout (KO) mice. Two-way ANOVA: $^{\&}p < 0.05$ main effect of age, and $^{\&\&}p < 0.01$ main effect of age. Panels show percentage of OD values of control and representative pictures of the blotting. β-Actin is used as internal loading control.

brains, which was more than double the levels observed in younger samples (**Figure 5A**). In contrast, the relative levels of acetylated tubulin were significantly ($p < 0.05$) reduced by aging (**Figure 5B**).

DISCUSSION

In this study we have shown that aged mice that lack neuronal AM have better contextual and recognition memory than their WT littermates. In parallel, the brain cortex and hippocampus of these mice have a lower accumulation of p-Tau, suggesting that p-Tau may be the link between lack of AM and memory preservation, although we cannot rule out other alternative molecular pathways. In addition, we also showed that older human individuals present higher levels of AM and lower levels of acetylated tubulin in their brains than younger controls.

Previous studies had found that plasma AM increases with age (Kato et al., 2002), but a description of the levels of AM in the aging brain was lacking. Here we have demonstrated that normal aging is accompanied by an increase of AM protein expression, at least in the frontal cortex. Previous reports have indicated that elevated AM levels occur in the brain of AD patients (Ferrero et al., 2017) and that plasma levels of the related peptide, mid-regional proadrenomedullin, may constitute an early marker for progression to AD (Buerger et al., 2011; Henriksen et al., 2014). Obviously, patients in their path to develop AD should have a larger AM increase than normally aging individuals. These correlation studies provide important information by identifying potential markers to detect early cases of rapidly declining individuals, but they do not help in understanding the underlying biochemical mechanisms. Our mouse study provides a first insight into the potential mechanisms linking AM levels to contextual

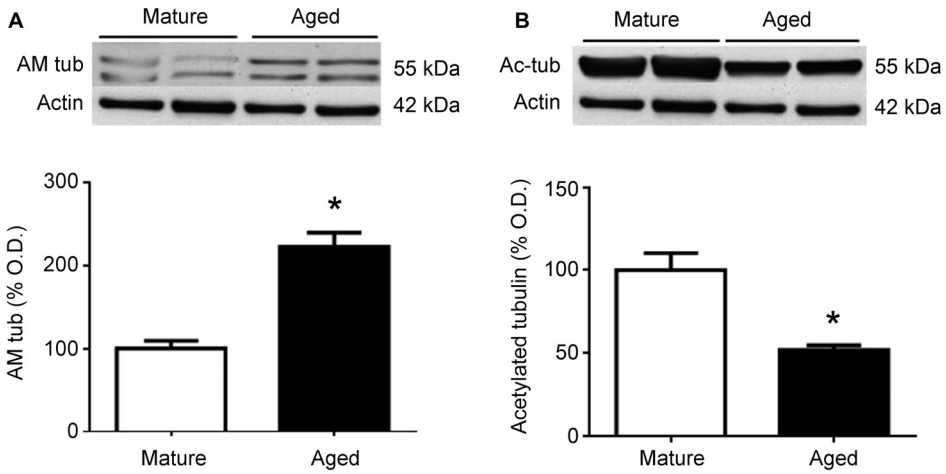


FIGURE 5 | Expression of adrenomedullin (AM) **(A)** and acetylated tubulin **(B)** in the frontal cortex of mature and aged human donors. The band of immunoreactive tubulin-associated AM (~55 kDa) is more intense in the aged brain whereas acetylated tubulin is significantly lower in aged individuals. Student's *t* test, $*p < 0.05$. β-Actin is used as internal loading control. β-Actin blot in **(B)** is the same as in **(A)** and is repeated here to allow a direct comparison with the loading controls.

memory loss, through the regulation of p-Tau, especially among females. Although a protein-protein contact between Tau and AM was not detected by yeast-2-hybrid experiments (Sackett et al., 2008), these two proteins are MAPs and are located in close proximity on the microtubule surface, so a direct physical interaction cannot be excluded. Alternatively, AM could modulate any of the kinases that phosphorylate Tau (Tell et al., 2016). The exact biochemical mechanism linking AM to Tau phosphorylation should be addressed in future studies.

Two different antibodies were used in this study to asses Tau phosphorylation. AT8 is a phospho-specific antibody that recognizes phosphorylation in Ser202 and Thr205 (Goedert et al., 1995), whereas the PHF1 antibody recognizes phosphorylation on Ser396 and Ser404 of the Tau protein (Otvos et al., 1994). All these phosphorylation sites have been involved in pathological findings (Mondragón-Rodríguez et al., 2014). PHF-1 is generally considered a later marker in the dynamic sequence of Tau phosphorylation events during the evolution of neurofilaments in AD, when compared to AT8 (Oh et al., 2010). Having very similar results with both antibodies indicates that we are detecting highly phosphorylated forms of Tau, which are the hallmarks of AD and other tauopathies related to memory loss (Tepper et al., 2014).

The levels of acetylated tubulin, a post-translational modification of α-tubulin which results in microtubule stability (Strzyz, 2016), were also studied as another potential mechanism of AM-mediated memory preservation. Aged human donors and male mice had lower levels than their younger counterparts, although this did not happen with female mice. In these animals we did not see any difference between genotypes, although we expected them based on previous studies (Sackett et al., 2008). Perhaps this loss of hyperpolymerized microtubules in the neurons occurs late in the aging process, resulting in synaptic disconnection, and is not so prevalent in females, where Tau phosphorylation may be more relevant. Of course, another possibility is that female hormones may have a neuroprotective effect in this context. Other α-tubulin modifications have been also reported to increase in neurodegenerative diseases, including AD (Vu et al., 2017), suggesting an important contribution of the cytoskeleton to the pathogenesis of memory loss.

An intriguing feature is the clear sexual dimorphism we observed in the behavioral and biochemical studies. Our fear conditioning results suggest that aged female mice are more prone to contextual memory loss than their male counterparts, a condition that was compensated by the lack of AM expression in neurons. Albeit using different memory tests, these sexual differences have been also reported in humans where women have an increased risk for memory disorders relative to men later in life (Jacobs et al., 2016; Reed et al., 2017). It seems that this risk depends on sex steroids, whose decline after menopause correlates with more pronounced alterations in hippocampal connectivity (Jacobs et al., 2016). In addition, the physiological effects of AM have shown to be also sex-dependent with females being more susceptible to changes in the levels of AM expression (Martínez-Herrero et al., 2016).

We have previously found that lack of AM has an impact on locomotor activity (Fernández et al., 2008) and pain perception (Fernández et al., 2010) in young mice. These characteristics may have an influence in the way the KO mice respond to the memory tests. To address these issues we performed an open field test in old mice before the NORT and found no differences in motility, as previously shown for mice of this age (Fernández et al., 2008). Since we do not observe any memory differences in younger mice and the older mice do not have locomotor activity differences, we can rule out the influence of this feature on the results of the NORT. Also, to ensure that the different sensitivity to pain did not influence the fear conditioning results, we measured the

freezing behavior of mice of either genotype before and after the foot shock. In both cases, the differences were not statistically significant, indicating that this is not an issue for this specific test.

Previous studies have shown that neuronal AM may have a neuroprotective effect, especially in the context of stroke (Hurtado et al., 2010) and exposure to hypobaric environments (Fernández et al., 2008). Nevertheless, we cannot generalize and say that AM is always neuroprotective. For instance, high levels of circulating AM correlate with increased neurological severity in stroke patients (Serrano-Ponz et al., 2016) and endothelial AM seems to have the reverse effect on stroke and brain damage than neuronal AM (Ochoa-Callejero et al., 2016). Until we acquire a more complete understanding on the effects of AM in the CNS, our present data need to be interpreted on their own, indicating that lower levels of AM are beneficial for memory preservation, especially in females.

AM has been shown to bind to complement factor H (CFH) in the blood stream, and each molecule influences the physiological activities of the other (Pio et al., 2001). CFH is also present at high levels in the CNS (Serrano et al., 2003). Although there are no data on the evolution of brain CFH with age, some studies point to a correlation between lower levels of circulating CFH and more severe cognitive impairment during the development of AD, both in patients (Gezen-Ak et al., 2013) and in mouse models (Wang et al., 2016). It would be interesting to investigate the potential impact of CFH on AM-mediated memory loss during normal aging.

Our data suggest that reducing AM/PAMP levels may constitute a novel path to preventing/delaying memory loss. A few years ago, a particular single nucleotide polymorphism (SNP) close to the *adm* gene was found to be responsible for a natural reduction in the circulating levels of AM (Cheung et al., 2011) and to correlate with cancer susceptibility (Martínez-Herrero and Martínez, 2013). Therefore, it would be interesting to test whether carriers of this SNP are more protected from developing memory impairment. Also, several physiological inhibitors of AM have been proposed for clinical development, including a monoclonal antibody (Martínez et al., 1996), the peptide fragment AM_{22-52} (Ishikawa et al., 2003), and a number of small molecules that target either AM or PAMP (Martínez et al., 2004; Roldós et al., 2012). Therefore some of these inhibitors may be used for the pharmacological prevention of age-related memory loss.

In conclusion, normal aging results in higher expression of AM in the brain and AM ablation prevents Tau phosphorylation in female mice and favors memory preservation in advanced age. These observations suggest that a pharmacological inhibition of *adm* gene products may constitute a novel approach to preventing memory loss in normal aging and in patients suffering moderate AD in high risk of rapid cognitive decline.

AUTHOR CONTRIBUTIONS

AM and MJR conceived of the idea for the study. IML, HF, EM and FJG-B performed experiments and analyzed data. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed intellectually to the revision of the manuscript and approved the final version of the manuscript.

FUNDING

IML and AM are funded by Fundación Rioja Salud (FRS). HF is a recipient of a fellowship from Spain's Ministerio de Educación, cultura y Deporte (FPU).

REFERENCES

Bahn, S., Augood, S. J., Ryan, M., Standaert, D. G., Starkey, M., and Emson, P. C. (2001). Gene expression profiling in the post-mortem human brain—no cause for dismay. *J. Chem. Neuroanat.* 22, 79–94. doi: 10.1016/s0891-0618(01)00099-0

Buerger, K., Uspenskaya, O., Hartmann, O., Hansson, O., Minthon, L., Blennow, K., et al. (2011). Prediction of Alzheimer's disease using midregional proadrenomedullin and midregional proatrial natriuretic peptide: a retrospective analysis of 134 patients with mild cognitive impairment. *J. Clin. Psychiatry* 72, 556–563. doi: 10.4088/JCP.09m05872oli

Caron, K. M., and Smithies, O. (2001). Extreme hydrops fetalis and cardiovascular abnormalities in mice lacking a functional Adrenomedullin gene. *Proc. Natl. Acad. Sci. U S A* 98, 615–619. doi: 10.1073/pnas.021548898

Cheung, B. M., Ong, K. L., Tso, A. W., Leung, R. Y., Cherny, S. S., Sham, P. C., et al. (2011). Plasma adrenomedullin level is related to a single nucleotide polymorphism in the adrenomedullin gene. *Eur. J. Endocrinol.* 165, 571–577. doi: 10.1530/EJE-11-0513

Collette, F., and Salmon, E. (2014). The effect of normal and pathological aging on cognition. *Rev. Med. Liege* 69, 265–269. Available online at: http://hdl.handle.net/2268/170920

Delacourte, A., Sergeant, N., Wattez, A., Maurage, C. A., Lebert, F., Pasquier, F., et al. (2002). Tau aggregation in the hippocampal formation: an ageing or a pathological process? *Exp. Gerontol.* 37, 1291–1296. doi: 10.1016/s0531-5565(02)00141-9

Fernandez, A. P., Masa, J. S., Guedan, M. A., Futch, H. S., and Martínez-Murillo, R. (2016). Adrenomedullin expression in Alzheimer's brain. *Curr. Alzheimer Res.* 13, 428–438. doi: 10.2174/1567205013666160229112725

Fernández, A. P., Serrano, J., Martínez-Murillo, R., and Martínez, A. (2010). Lack of adrenomedullin in the central nervous system results in apparently paradoxical alterations on pain sensitivity. *Endocrinology* 151, 4908–4915. doi: 10.1210/en.2010-0121

Fernández, A. P., Serrano, J., Tessarollo, L., Cuttitta, F., and Martínez, A. (2008). Lack of adrenomedullin in the mouse brain results in behavioral changes, anxiety and lower survival under stress conditions. *Proc. Natl. Acad. Sci. U S A* 105, 12581–12586. doi: 10.1073/pnas.0803174105

Ferrero, H., Larrayoz, I. M., Martisova, E., Solas, M., Howlett, D. R., Francis, P. T., et al. (2017). Increased levels of brain adrenomedullin in the neuropathology of Alzheimer's disease. *Mol. Neurobiol.* doi: 10.1007/s12035-017-0700-6 [Epub ahead of print].

Gerenu, G., Dobarro, M., Ramirez, M. J., and Gil-Bea, F. J. (2013). Early cognitive stimulation compensates for memory and pathological changes in Tg2576 mice. *Biochim. Biophys. Acta* 1832, 837–847. doi: 10.1016/j.bbadis.2013.02.018

Gezen-Ak, D., Dursun, E., Hanagasi, H., Bilgiç, B., Lohman, E., Araz, Ö. S., et al. (2013). BDNF, TNFα, HSP90, CFH, and IL-10 serum levels in patients with early or late onset alzheimer's disease or mild cognitive impairment. *J. Alzheimers Dis.* 37, 185–195. doi: 10.3233/JAD-130497

Goedert, M., Jakes, R., and Vanmechelen, E. (1995). Monoclonal antibody AT8 recognises tau protein phosphorylated at both serine 202 and threonine 205. *Neurosci. Lett.* 189, 167–169. doi: 10.1016/0304-3940(95)11484-e

Guillozet, A. L., Weintraub, S., Mash, D. C., and Mesulam, M. M. (2003). Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch. Neurol.* 60, 729–736. doi: 10.1001/archneur.60.5.729

Henriksen, K., O'Bryant, S. E., Hampel, H., Trojanowski, J. Q., Montine, T. J., Jeromin, A., et al. (2014). The future of blood-based biomarkers for Alzheimer's disease. *Alzheimers Dement.* 10, 115–131. doi: 10.1016/j.jalz.2013.01.013

Hermann, P. M., Watson, S. N., and Wildering, W. C. (2014). Phospholipase A2—nexus of aging, oxidative stress, neuronal excitability, and functional decline of the aging nervous system? Insights from a snail model system of neuronal aging and age-associated memory impairment. *Front. Genet.* 5:419. doi: 10.3389/fgene.2014.00419

Hernandez, F., Lucas, J. J., and Avila, J. (2013). GSK3 and tau: two convergence points in Alzheimer's disease. *J. Alzheimers Dis.* 33, S141–S144. doi: 10.3233/JAD-2012-129025

Hickman, R. A., Faustin, A., and Wisniewski, T. (2016). Alzheimer disease and its growing epidemic: risk factors, biomarkers, and the urgent need for therapeutics. *Neurol. Clin.* 34, 941–953. doi: 10.1016/j.ncl.2016.06.009

Holm, H., Nägga, K., Nilsson, E. D., Ricci, F., Melander, O., Hansson, O., et al. (2017). Biomarkers of microvascular endothelial dysfunction predict incident dementia: a population-based prospective study. *J. Intern. Med.* 282, 94–101. doi: 10.1111/joim.12621

Hurtado, O., Serrano, J., Sobrado, M., Fernández, A. P., Lizasoain, I., Martínez-Murillo, R., et al. (2010). Lack of adrenomedullin, but not complement factor H, results in larger infarct size and more extensive brain damage in a focal ischemia model. *Neuroscience* 171, 885–892. doi: 10.1016/j.neuroscience.2010.09.021

Ishikawa, T., Chen, J., Wang, J., Okada, F., Sugiyama, T., Kobayashi, T., et al. (2003). Adrenomedullin antagonist suppresses *in vivo* growth of human pancreatic cancer cells in SCID mice by suppressing angiogenesis. *Oncogene* 22, 1238–1242. doi: 10.1038/sj.onc.1206207

Jacobs, E. G., Weiss, B. K., Makris, N., Whitfield-Gabrieli, S., Buka, S. L., Klibanski, A., et al. (2016). Impact of sex and menopausal status on episodic memory circuitry in early midlife. *J. Neurosci.* 36, 10163–10173. doi: 10.1523/JNEUROSCI.0951-16.2016

Kato, J., Kitamura, K., Uemura, T., Kuwasako, K., Kita, T., Kangawa, K., et al. (2002). Plasma levels of adrenomedullin and atrial and brain natriuretic peptides in the general population: their relations to age and pulse pressure. *Hypertens. Res.* 25, 887–892. doi: 10.1291/hypres.25.887

Larráyoz, I. M., and Martínez, A. (2012). Proadrenomedullin N-terminal 20 peptide increases kinesin's velocity both *in vitro* and *in vivo*. *Endocrinology* 153, 1734–1742. doi: 10.1210/en.2011-1685

Leal, S. L., Noche, J. A., Murray, E. A., and Yassa, M. A. (2017). Age-related individual variability in memory performance is associated with amygdala-hippocampal circuit function and emotional pattern separation. *Neurobiol. Aging* 49, 9–19. doi: 10.1016/j.neurobiolaging.2016.08.018

Lewis, D. A. (2002). The human brain revisited: opportunities and challenges in postmortem studies of psychiatric disorders. *Neuropsychopharmacology* 26, 143–154. doi: 10.1016/s0893-133x(01)00393-1

López, J., and Martínez, A. (2002). Cell and molecular biology of the multifunctional peptide, adrenomedullin. *Int. Rev. Cytol.* 221, 1–92. doi: 10.1016/s0074-7696(02)21010-4

Martínez-Herrero, S., Larrayoz, I. M., Narro-Íñiguez, J., Villanueva-Millán, M. J., Recio-Fernández, E., Pérez-Matute, P., et al. (2016). Lack of adrenomedullin results in microbiota changes and aggravates azoxymethane and dextran sulfate sodium-induced colitis in mice. *Front. Physiol.* 7:595 . doi: 10.3389/fphys.2016.00595

Martínez-Herrero, S., and Martínez, A. (2013). Cancer protection elicited by a single nucleotide polymorphism close to the adrenomedullin gene. *J. Clin. Endocrinol. Metab.* 98, E807–E810. doi: 10.1210/jc.2012-4193

Martínez, A., Julián, M., Bregonzio, C., Notari, L., Moody, T. W., and Cuttitta, F. (2004). Identification of vasoactive nonpeptidic positive and negative modulators of adrenomedullin using a neutralizing antibody-based screening strategy. *Endocrinology* 145, 3858–3865. doi: 10.1210/en.2003-1251

Martínez, A., Weaver, C., López, J., Bhathena, S. J., Elsasser, T. H., Miller, M. J., et al. (1996). Regulation of insulin secretion and blood glucose metabolism by adrenomedullin. *Endocrinology* 137, 2626–2632. doi: 10.1210/en.137.6.2626

Mondragón-Rodríguez, S., Perry, G., Luna-Muñoz, J., Acevedo-Aquino, M. C., and Williams, S. (2014). Phosphorylation of tau protein at sites Ser(396–404) is one of the earliest events in Alzheimer's disease and Down syndrome. *Neuropathol. Appl. Neurobiol.* 40, 121–135. doi: 10.1111/nan.12084

Nelson, P. T., Alafuzoff, I., Bigio, E. H., Bouras, C., Braak, H., Cairns, N. J., et al. (2012). Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *J. Neuropathol. Exp. Neurol.* 71, 362–381. doi: 10.1097/NEN.0b013e31825018f7

Ochoa-Callejero, L., Pozo-Rodrigálvarez, A., Martínez-Murillo, R., and Martínez, A. (2016). Lack of adrenomedullin in mouse endothelial cells results in defective angiogenesis, enhanced vascular permeability, less metastasis, and more brain damage. *Sci. Rep.* 6:33495. doi: 10.1038/srep33495

Oh, K. J., Perez, S. E., Lagalwar, S., Vana, L., Binder, L., and Mufson, E. J. (2010). Staging of Alzheimer's pathology in triple transgenic mice: a light and electron microscopic analysis. *Int. J. Alzheimers Dis.* 2010:780102. doi: 10.4061/2010/780102

Otvos, L. Jr., Feiner, L., Lang, E., Szendrei, G. I., Goedert, M., and Lee, V. M. (1994). Monoclonal antibody PHF-1 recognizes tau protein phosphorylated at serine residues 396 and 404. *J. Neurosci. Res.* 39, 669–673. doi: 10.1002/jnr.490390607

Pérez-Castells, J., Martín-Santamaría, S., Nieto, L., Ramos, A., Martínez, A., Pascual-Teresa, B., et al. (2012). Structure of micelle-bound adrenomedullin: a first step toward the analysis of its interactions with receptors and small molecules. *Biopolymers* 97, 45–53. doi: 10.1002/bip.21700

Pini, L., Pievani, M., Bocchetta, M., Altomare, D., Bosco, P., Cavedo, E., et al. (2016). Brain atrophy in Alzheimer's disease and aging. *Ageing Res. Rev.* 30, 25–48. doi: 10.1016/j.arr.2016.01.002

Pio, R., Martinez, A., Unsworth, E. J., Kowalak, J. A., Bengoechea, J. A., Zipfel, P. F., et al. (2001). Complement factor H is a serum-binding protein for adrenomedullin and the resulting complex modulates the bioactivities of both partners. *J. Biol. Chem.* 276, 12292–12300. doi: 10.1074/jbc.M007822200

Reed, J. L., Gallagher, N. M., Sullivan, M., Callicott, J. H., and Green, A. E. (2017). Sex differences in verbal working memory performance emerge at very high loads of common neuroimaging tasks. *Brain Cogn.* 113, 56–64. doi: 10.1016/j.bandc.2017.01.001

Roldós, V., Carbajo, R. J., Schott, A. K., Pineda-Lucena, A., Ochoa-Callejero, L., Martínez, A., et al. (2012). Identification of first proadrenomedullin N-terminal 20 peptide (PAMP) modulator by means of virtual screening and NMR interaction experiments. *Eur. J. Med. Chem.* 55, 262–272. doi: 10.1016/j.ejmech.2012.07.031

Sackett, D. L., Ozbun, L., Zudaire, E., Wessner, L., Chirgwin, J. M., Cuttitta, F., et al. (2008). Intracellular proadrenomedullin-derived peptides decorate the microtubules and contribute to cytoskeleton function. *Endocrinology* 149, 2888–2898. doi: 10.1210/en.2007-1763

Savva, G. M., Wharton, S. B., Ince, P. G., Forster, G., Matthews, F. E., and Brayne, C. (2009). Age, neuropathology, and dementia. *N. Engl. J. Med.* 360, 2302–2309. doi: 10.1056/NEJMoa0806142

Serrano, J., Encinas, J. M., Fernández, A. P., Castro-Blanco, S., Alonso, D., Fernández-Vizarra, P., et al. (2003). Distribution of immunoreactivity for the adrenomedullin binding protein, complement factor H, in the rat brain. *Neuroscience* 116, 947–962. doi: 10.1016/s0306-4522(02)00773-x

Serrano, J., Uttenthal, L. O., Martínez, A., Fernández, A. P., Martínez de Velasco, J., Alonso, D., et al. (2000). Distribution of adrenomedullin-like immunoreactivity in the rat central nervous system by light and electron microscopy. *Brain Res.* 853, 245–268. doi: 10.1016/s0006-8993(99)02273-8

Serrano-Ponz, M., Rodrigo-Gasqué, C., Siles, E., Martínez-Lara, E., Ochoa-Callejero, L., and Martínez, A. (2016). Temporal profiles of blood pressure, circulating nitric oxide and adrenomedullin as predictors of clinical outcome in acute ischemic stroke patients. *Mol. Med. Rep.* 13, 3724–3734. doi: 10.3892/mmr.2016.5001

Strzyz, P. (2016). Post-translational modifications: extension of the tubulin code. *Nat. Rev. Mol. Cell Biol.* 17:609. doi: 10.1038/nrm.2016.117

Tell, V., Hilbrich, I., Holzer, M., Totzke, F., Schachtele, C., Slynko, I., et al. (2016). Drug development of small-molecule inhibitors of AD-relevant kinases as novel perspective multitargeted approach. *Curr. Alzheimer Res.* 13, 1330–1336. doi: 10.2174/1567205013666160615091821

Tepper, K., Biernat, J., Kumar, S., Wegmann, S., Timm, T., Hübschmann, S., et al. (2014). Oligomer formation of tau protein hyperphosphorylated in cells. *J. Biol. Chem.* 289, 34389–34407. doi: 10.1074/jbc.M114.611368

Vergaño-Vera, E., Fernández, A. P., Hurtado-Chong, A., Vicario-Abejón, C., and Martínez, A. (2010). Lack of adrenomedullin affects growth and differentiation of adult neural stem/progenitor cells. *Cell Tissue Res.* 340, 1–11. doi: 10.1007/s00441-010-0934-3

Vu, H. T., Akatsu, H., Hashizume, Y., Setou, M., and Ikegami, K. (2017). Increase in α-tubulin modifications in the neuronal processes of hippocampal neurons in both kainic acid-induced epileptic seizure and Alzheimer's disease. *Sci. Rep.* 7:40205. doi: 10.1038/srep40205

Wang, D., Di, X., Fu, L., Li, Y., Han, X., Wu, H., et al. (2016). Analysis of serum β-amyloid peptides, α2-macroglobulin, complement factor H, and clusterin levels in APP/PS1 transgenic mice during progression of Alzheimer's disease. *Neuroreport* 27, 1114–1119. doi: 10.1097/WNR.0000000000000661

Xu, Y., and Krukoff, T. L. (2004). Adrenomedullin in the rostral ventrolateral medulla increases arterial pressure and heart rate: roles of glutamate and nitric oxide. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R729–R734. doi: 10.1152/ajpregu.00188.2004

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Molecular Neuroscience

ORIGINAL RESEARCH
published: 24 July 2017
doi: 10.3389/fnmol.2017.00233

Memory Training Program Decreases the Circulating Level of Cortisol and Pro-inflammatory Cytokines in Healthy Older Adults

Mirko Pesce, Raffaella Tatangelo, Irene La Fratta, Alessia Rizzuto, Giovanna Campagna, Cinzia Turli, Alessio Ferrone, Sara Franceschelli, Lorenza Speranza, Maria C. Verrocchio, Maria A. De Lutiis, Mario Felaco and Alfredo Grilli*

School of Medicine and Health Science, University G. D'Annunzio, Chieti, Italy

Aging cognitive decline has been associated to impairment of the Hypothalamus Pituitary Adrenals (HPA) axis activity and a higher level of the systemic inflammation. However, little is known about the molecules driving this process at peripheral level. In addition, the cognitive function is to some extent modifiable with Memory Training (MT) programs, even among older adults and beyond. The study aims to evaluate whether MT could contribute to ameliorate cognitive performance and modulate the HPA axis activity as well the low level inflammation in the aging phenotype. Whether the phosphatase WIP-1, a negative regulator for inflammation, is involved in this process was also investigated. We recruited 31 young adults (19–28, years of age) and 62 older adults aged over 60. Thirty-two older adults were submitted to 6-months of MT program (EG), and 28 older adults were no treated and used as Control Group (CG). Global cognitive functioning (MMSE score), verbal and visual memory, and attention were assessed at baseline (T0) and after 6-months (T1). At the same time, plasmatic level of Cortisol (C), IL-1β, IL-18, IL-6, and the expression of WIP-1 mRNA and protein in *ex vivo* Peripheral Blood Mononuclear Cells were analyzed in young adults at T0, as well in older adults at T0 and T1. Together, the results suggest that MT improves the global cognitive functionality, verbal and visual memory, as well as the level of attention. At the same time we observed a decrease of the plasmatic level of C, of the cytokines, and an increase of the expression of mRNA and protein of WIP-1. The analysis of correlations highlighted that the level of the mRNA of WIP-1 was positively associated to the MMSE score, and negatively to the C and cytokine levels. In conclusion, we purpose the MT as tool that could help support successful aging through the improving of memory, attention and global cognitive function performance. Furthermore, this approach could participate to maintain lower the peripheral levels of the C and pro-inflammatory cytokines. The WIP-1 as a potential new target of the pathophysiology of aging is theorized.

Keywords: Memory Training, cortisol, inflammaging, WIP-1, pro-inflammatory cytokines

OPEN ACCESS

Edited by:
Julie A. Chowen, Hospital Infantil Universitario Niño Jesús, Spain

Reviewed by:
Mariano Ruiz-Gayo, CEU San Pablo University, Spain
Vicente Barrios, Hospital Infantil Universitario Niño Jesús, Spain

****Correspondence:***
Raffaella Tatangelo
tatangeloraffaella@gmail.com

Received: *07 February 2017*
Accepted: *06 July 2017*
Published: *24 July 2017*

Citation:
Pesce M, Tatangelo R, La Fratta I, Rizzuto A, Campagna G, Turli C, Ferrone A, Franceschelli S, Speranza L, Verrocchio MC, De Lutiis MA, Felaco M and Grilli A (2017) Memory Training Program Decreases the Circulating Level of Cortisol and Pro-inflammatory Cytokines in Healthy Older Adults. Front. Mol. Neurosci. 10:233. doi: 10.3389/fnmol.2017.00233

INTRODUCTION

During adulthood, the cognitive performance and immunity system begins to decline concurrently prior to the third decade of life suggesting that the mechanisms underlying these processes are inter-related (Rosano et al., 2012). There is comparable evidence indicating that increases in the level of peripheral markers of inflammation are associated with age-related declines in the health of the brain (Franceschi, 2007). Such evidence is parallel to longstanding findings from neurocognitive studies in populations of older adults living in a community, that indicate in a consistent manner an association between higher inflammatory levels and lower cognitive levels and a higher risk of cognitive impairment over time (Gorelick et al., 2014).

The systemic inflammation related to aging involves multiple organs and represents a common mechanism leading to multiple chronic diseases such as dementia, diabetes, atherosclerosis, and arthritis, which has a negative impact on the health span of the elderly (Maggio et al., 2005; Franceschi, 2007; Green et al., 2011). These findings supported the 'inflammaging theory' that hypothesizes a global reduction in the capacity to cope with a variety of stressors and purposes a concomitant progressive cytokines dysregulation as the major characteristics of the aging process (Franceschi and Campisi, 2014).

The modification of the Hypothalamus Pituitary Adrenal (HPA) axis activity represents another hallmark of the aging phenotype (Ferrari and Magri, 2008). Several findings suggest that a higher basal endogenous cortisol (C) concentration, a downstream effect of the increase in basal tonus of the HPA axis activation, is age-related (Deuschle et al., 1997; Giordano et al., 2005). The enhanced HPA axis activity could be explained by a decrease of the hippocampal inhibitory function that leads to an increase of the release of C (De Kloet et al., 1998). Consistently, the aging related higher plasma levels of C have predicted a smaller hippocampal volume, faster hippocampal atrophy (Lupien et al., 1998; O'Hara et al., 2007), that are associated to memory decline, and to general poor cognitive performance (Lupien et al., 2007; Lee et al., 2008; Tatomir et al., 2014; Geerlings et al., 2015; Vogel et al., 2016).

The aging systemic higher exposure to C could be associated with the accelerated features of low-grade inflammation that characterizes older adults. The HPA axis activity exerts a pivotal role for the homeostasis of the immune system. The circulating level of C and those of several cytokines have been concurrently associated with a decreased hippocampal volume and cognitive impairment (Bauer, 2008; Sudheimer et al., 2014; Metti et al., 2015; Chesnokova et al., 2016; Jin et al., 2016). The Glucocorticoids (GCs) and pro-inflammatory cytokines are not independent of each other and they interact on multiple levels. In particular, the GCs that were classically viewed as anti-inflammatory could have a dual role, inducing pro-inflammatory effects. Their administration results in an increased systemic trafficking of lymphocytes and monocytes (Bowers et al., 2008), and the exertion of pro-inflammatory actions through synergistic interactions with inflammatory cytokines, such as IL-1β and IL-6 (Langlais et al., 2008; Ding et al., 2010; Busillo et al., 2011).

The mechanisms underlying low-grade systemic inflammation in several organs in the absence of overt infection that was observed during aging are still speculative, and little is known about the "master" molecule involved in this process. The Wild type p53-Induced Phosphatase-1 (WIP-1) is a phosphatase whose expression is induced in response to many types of cellular stress, as γ or UV radiation in a p53-dependent manner (Fiscella et al., 1997). This protein has been associated with cellular senescence (Salminen and Kaarniranta, 2011), and its expression has been showed to decrease during age (Wong et al., 2009). More recently, WIP-1 has been recognized as an intrinsic negative regulator of inflammatory response in the CNS (Tan et al., 2013), and also at the peripheral level where it acts as a negative regulator for neutrophil migration and pro-inflammatory cytokines production through various pathways, including the feedback regulation of the NF-κB signaling (Sun et al., 2014). It could be hypothesized that WIP-1 could be associated with sterile inflammation that underlines the aging process.

The above mentioned age-related modulations and variations are dynamic and not irreversible processes. The ways that allow modulation of brain plasticity in animal and human using cognitive or physical training have resulted to be of great interest (Curlik and Shors, 2013). Several studies on rodents have shown that Environmental Enrichment (EE) – modification of the environment to enhance the animal physical and psychological well-being – (Baumans, 2005), has been shown to slow down neuronal aging (Kempermann et al., 2002), improve cognition and memory (Jankowsky et al., 2005; Hannan, 2014). Some EE stimuli are able to affect C (Simpson and Kelly, 2011), cytokines and various immune components at a peripheral level, suggesting that this could be a potential mechanism of action in how it could modulate the brain function (Singhal et al., 2014). To this end, within cognitive training procedures, Memory Training (MT) based research generally reveals an improvement in memory performance in older adults through the utilization of mnemonic strategies. However, even cognitive training was successfully included within EE stimuli. To our knowledge, neither study has been carried out to determine whether it reduces the endogenous C levels and the behavioral sequence of chronic inflammatory conditions that characterize the aging phenotype.

Considering the above mentioned in aggregate, we hypothesize that the MT could be considered as a treatment that similar to other stimuli of the EE, could enhance the cognitive and physical well-being of older adults. It is for this reason that this study wants to investigate whether a MT program acts on the plasmatic level of C, the pro-inflammatory cytokines IL-6, IL-1β, and L-18, and the expression of the WIP-1 in *ex vivo* Peripheral Blood Mononuclear Cells (PBMCs) of apparently healthy elderly (older adults, 65–74 years; De Beni, 2009), relating to their degree of cognitive abilities. We chose these cytokines because all of them had been previously associated with specific features of aging phenotype (Franceschi, 2007), and also their higher levels had been successfully suggested as a marker of poorer cognitive performance in healthy subjects

TABLE 1 | Exclusion criteria of all participants.

Exclusion criteria
• Body Mass Index (<20 and >33 kg/m^2)
• Erythrocyte Sedimentation Rate (<20 mm/hr for males; <30 mm/hr for females)
• C Reactive Protein (<5 mg/L)
• Hematological and Biochemical Analyses Values within Normal Range
• Unusual Dietary Habits (e.g., vegetarians)
• Recent infections, allergies, present or past autoimmune disorders
• Diagnosis of or treatment for pathological diseases (cancer, diabetes, insufficient renal and hepatic performance, mal-absorption and chronic inflammatory pathologies)
• Current medication (including herbal remedies or vitamins)
• Smoking
• Alcohol consumption (1 week the sample collection, >30 g/d for men and >20 g/d for woman)

for the IL-6 and the IL-1β (Yirmiya and Goshen, 2011), and in a clinical population for the IL-18 (Bossù et al., 2008; Zhang et al., 2013).

Our results provide important evidence that MT could contribute to the decreasing of the C level, restore the WIP-1 expression and reduce the value of the pro-inflammatory cytokines at a peripheral level, participating in "holding off" the systemic inflammatory condition that characterizes healthy older adults. We first involved WIP-1 deficiency in the pathophysiology of aging.

MATERIALS AND METHODS

Participants

Participants of the study were 62 apparent healthy older adult volunteers (29 female, age range 65–74 years of age), recruited with the use of posters, pamphlets and word of mouth at recreational clubs. Thirty-one apparent healthy young adults (16 female, age 19–28 years of age), were also recruited by word of mouth and with pamphlets at University G. d'Annunzio, CH-PE, Italy. The exclusion criteria regarding all participants were summarized in **Table 1**.

Briefly, subjects with the BMI < 20 and >33 k/m^2 were excluded (Travison et al., 2007; Mangnus et al., 2016). Subjects with unusual dietary habits (e.g., vegetarians) were also excluded (Sutliffe et al., 2015). Hematological and biochemical tests needed to be within the normal range. Negative serologies for the HIV and Hepatitis C viruses were verified. To assess the inflammatory status, all recruited subjects underwent the same laboratory blood tests: Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) were measured as non-specific markers for inflammation and were utilized as exclusion criteria. The upper limit of the ESR is 20 mm/hr, for males, and 30 mm/hr for females (Sox and Liang, 1986; Caswell, 1993). To avoid the inclusion of subjects in an acute phase of inflammation (e.g., infection, trauma and tissue necrosis, malignancies, and autoimmune disorders), the subjects with CRP levels > 5 mg/L were also excluded (Gabay and Kashner, 1999; Ablij and Meinders, 2002; Biasucci, 2004).

Habitual smokers were excluded because this factor has already been significantly marked as a modulator of C level and strong pro-inflammatory (Mendelson et al., 2008; Messner and Bernhard, 2014). The participants were invited not to consume alcohol starting 1 week before the sample collection, in order to avoid any effects on C and the systemic inflammation levels (Sher et al., 2007; Morris et al., 2015).

Subjects who had current infections, allergies, or a present and past history of autoimmune disorders, and those on current medication (including herbal remedies or vitamins) such as anti-inflammatory, antiviral agents or immunosuppressive medication that could directly or indirectly affect the systemic inflammatory state were excluded.

Older adults reaching the "exclusion criteria" adapted from the SENIEUR protocol for demographic suitability was excluded from the study (Ligthart et al., 1984). The exclusion criteria for older adults included factors thought to influence the relationship between the cognitive function and chronic inflammation such as the presence of dementia, and depression. Volunteers were invited to a preliminary screening session based on a full medical history and examination including, anthropometric measurements, the assessment of dietary habits, tobacco and alcohol consumption, and screening for cognitive impairment using the Mini-Mental State Examination (MMSE, Folstein et al., 1975), and the Geriatric Depression Scale (GDS), that provides a screening measure of depressive symptoms in adults over 55 years (Yesavage et al., 1982). All participants had their cognitive functions assessed using a neurocognitive battery of tests as described to follow. The neurocognitive battery was designed in order to evaluate the memory and attention which are frequently affected in older persons. The subjects with depression (score ≥ 11 on the GDS) and subjects with dementia (score ≤ 24 on the MMSE) were excluded.

A total of 94 consecutive older adults (39 female) were invited to participate in the study. Ten subjects (4 female) were excluded as they were smokers; 3 male subjects were excluded because they had elevated ESR values, one female subject for an elevated CRP value. Eight male and 10 female were excluded as they were on medication. In the end, 62 consecutive subjects (of which 66% subjects contacted) were included in the study. After having signed the consent, 34 subjects were randomly assigned to an Experimental Group (EG) (16 female) and 28 to a Control Group (CG) (13 female) before the cognitive screening assessment.

A total of 43 consecutive young adults (20 female) were invited to participate in the study. Eight subjects (2 female) were excluded as they were smokers, four (2 female) were excluded as they were on medication. In the end, 31 consecutive subjects (of which 74% of subjects contacted) were included in the study.

The study procedures were described in detail to all participants, a minimum reflection time of 24 h was given before written informed consent was obtained. Participants not having the capacity to consent to research participation and non-Italian-speaking participants (because the inclusion of these participants would have meant not to be able to use standardized assessment techniques) were also excluded from the study.

TABLE 2 | Characteristics of older adults at baseline.

Characteristics	Control Group ($n = 28$) (mean ± SD)	Experimental Group (n = 34) (mean ± SD)	*p*-value
Age (years)	69.0 ± 8.0	70.6 ± 7.0	0.428[a]
Gender (F)	28 (13)	34 (16)	0.730[b]
Education (years)	9.4 ± 1.7	9.6 ± 1.8	0.681[a]
BMI (Kg/m^2)	26.8 ± 2.7	27.0 ± 3.0	0.824[a]
Plasma CRP (mg/l)	1.7 ± 0.6	1.6 ± 0.6	0.530[a]

[a]*Unpaired Student t-test;* [b]*Chi-squared test; BMI, Body Mass Index; CRP, C-Reactive Protein.*

The study was conducted according to the principles expressed in the Declaration of Helsinki and subsequent revisions and was approved by the Ethics Committee of G. d'Annunzio University, Chieti, Italy.

Characteristic of the Sample

Demographic data of the subjects studied are listed in **Table 2**, together with BMI value and plasmatic level of CRP.

The CG included 28 subjects (13 women; age, mean ± SD, 69.0 ± 8.0 years). The EG included 34 subjects (16 women; age, mean ± SD, 70.6 ± 7.0 years).

The two groups were not significantly different at the baseline (T0) in terms of age ($p = 0.428$), male/female ratio ($p = 0.730$), BMI ($p = 0.841$), education ($p = 0.681$), and plasma CRP ($p = 0.530$). They did not significantly differ for any neuropsychological scores obtained at the beginning in which all scores were within the normal range. At this period all participants were considered healthy and defined as not suffering from any recent episode that could condition the basal inflammatory level and cognitive performance (i.e., infection and malignancy).

Neuropsychological Test Battery

Cognitive functioning in CG and EG was measured using a comprehensive neuropsychological test battery at baseline (T0) and after 6 months (T1), 1 day before blood sample collection. For all subjects and controls, the same test administration order was used. The battery was comprised of tests with strong psychometric properties and assessed the following cognitive domains:

Global Assessment of Cognitive Function. MMSE (Folstein et al., 1975) is the use a psychometric test used to quantify the global cognitive functioning and the cognitive change in population-based longitudinal studies. MMSE consists of a series of questions with the aim of quantifying the global cognitive functioning on a 0–30 scale.

The following instruments were administered (in this fixed order), by a psychologist for the overall assessment of each participant, of which had a duration of approximately 90 min, with breaks provided, as requested by participants:

(1) Global Assessment of Cognitive Function. MMSE (Folstein et al., 1975) is the use a psychometric test used to quantify the global cognitive functioning and the cognitive change in population-based longitudinal studies. MMSE consists of a series of questions with the aim of quantifying the global cognitive functioning on a 0–30 scale.

(2) The GDS was created specifically for screening depressive symptoms among older populations and has been widely utilized in both clinical and research settings. The GDS-30 comprises 30 questions regarding high and low mood, lack of energy, anxiety, and social withdrawal. Assesses depressive symptoms by means of 30 questions to be answered with 'yes' or 'no'. Each question is weighted with 1 point for a positive answer. The sum of these points defines the final score, ranging from 0 to 30 points. The GDS-30 asks how the person feels currently and how the person has felt during the previous week (Yesavage et al., 1982).

(3) The Rey-Osterrieth Complex Figure Test was used to assess visuo-spatial constructional functions, visuographic memory, and some aspects of planning (Reedy et al., 2013). The subject was asked to make a copy of a stimulus drawing comprised of a complex design, which was then scored on 18 identifiable details with a maximum score of 36 points possible and a higher score indicating better performance. The subject then drew the figure from memory 5 min later, and again 30 min later.

(4) Memory. The ability to retain and recall verbal information was measured using the Rey Auditory Verbal Learning Test (Lezak, 1995). Participants read a 15-item word list five times and were asked to repeat as many words as they could remember for each time. After 50 times, participants were presented an interference list. Participants were then asked to recall words from the original list. Participants were also asked to recall words from the original list 20 min later.

(5) Attentive Matrices Test (Attentive Test) is a valid instrument to measure selective and sustained attention (Spinnler and Tognoni, 1987). It consists of 3 numeric matrices (10 columns of 13 numbers from 0 to 9). The participants are required to check specific target numbers in 45 s for each matrix. There are 1 target in the first matrix, 2 in the second one, and 3 in the third one. The score is assigned giving 1 point for each target correctly found in the 3 matrices (max 60 for all the 3 matrices). The time to complete the 3 matrices is considered as scores too.

(6) Trail Making Test (TMT) provides information on visual search, speed of processing, mental flexibility, selective visual attention and ability to shifting (Reitan, 1958). It consists of two parts (A and B), both consisting of 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1–25, and the subject should draw lines to connect the numbers in ascending order. In Part B, the circles include both numbers (1–13) and letters (A–L) and the subject should draw lines to connect alternatively numbers and letters in ascending order (i.e., 1-A-2-B-3-C). The subject should be instructed to connect the circles as quickly as possible, without lifting the pen or pencil from the paper. Errors should be pointed out immediately and subjects should be allowed to correct it. Time required to complete each of the two parts is recorded, considered as scores of the test.

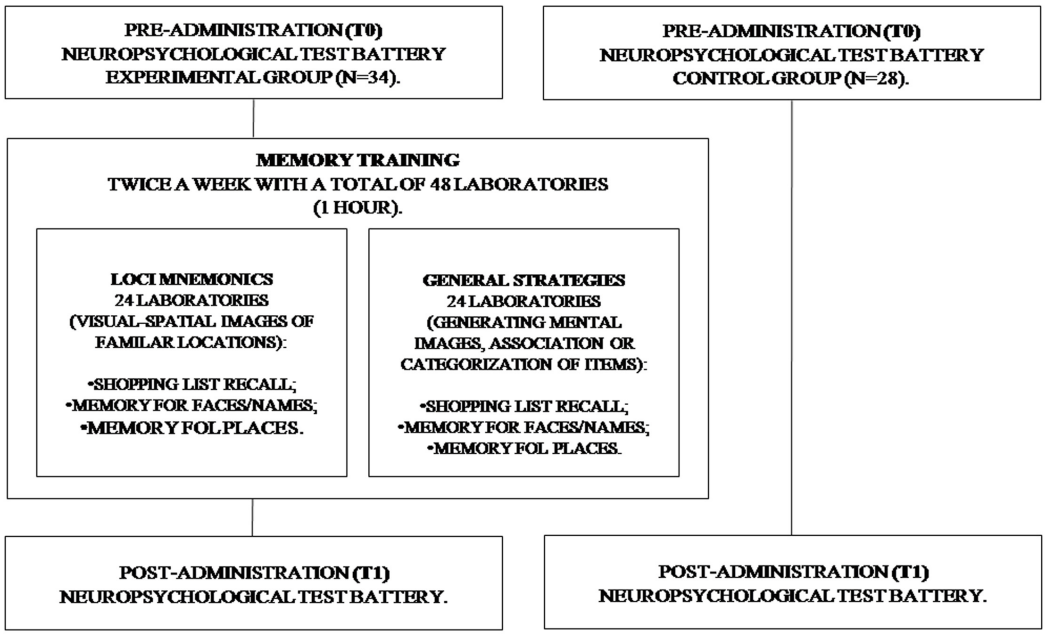


FIGURE 1 | Flow chart of the Memory Training Program.

Memory Training

The participants of EG were submitted to MT from T0 to T1. The MT is based on the concept that repeated practice within a specific domain results in gains in both cognitive and behavioral efficiency of the targeted domain, as well as subsequently transferring improvements to untrained domains.

The intervention was conducted in several laboratories. All sessions lasted 1 h and were held twice a week, with a total of 48 laboratories (**Figure 1**).

During MT, two different mnemonics were used. To assess the EG visual mnemonic (Loci) and general strategies (Strategic) were used (Cavallini et al., 2003). The Loci mnemonics is based on participants that generate sequences of visuo-spatial images of familiar locations, and further produce an image that is more complex, with the initial scene and the name/object to remember. The second training was characterized by the use of disparate strategies in different situations and each of them required a special strategy (generating mental images, association or categorization of items).

Older adults are particularly sensitive to the setting and emotional conditions. For this reason, we used ecological tasks to reproduce daily activities (Baltes and Baltes, 1990).

Story recall. All participants had 5 min to study a short story composed of 22 units. They then had to write what they remembered. Performance was assessed according to the correct number of units they could remember.

Shopping list recall. A shopping list was read to all the participants and they had 5 min to remember and subsequently put it in writing. Performance was evaluated according to the correct number of products which were remembered.

Memory for faces/names. Twelve photographs, each coupled with a name, on a computer screen was presented to all participants for 30 s. The same faces, were then shown without names and participants had to remember the names. Performance was assessed according to the correct face/names associations made.

Memory for places. A map of an Italian city with the name and the position of 10 monuments was presented to all participants for 5 min. They then had to remember and to write the name and the position of the monuments on a blank map. Performance was evaluated according to the correct names and positions which were remembered (Cavallini et al., 2003).

Cell Sources and Cultures

Blood samples were collected at T0 and T1. Veni-puncture was performed in the morning between 08.00 and 10.00 am. in order to avoid the effect of diurnal variation, and peripheral blood samples were collected in 4 ml endotoxin-free Heparin tubes (Vacutainer, Becton Dickinson, NJ, United States). Tubes were kept at room temperature and transported to the laboratory for processing within 1 h of collection. The plasma was obtained by blood centrifugation as described previously and was kept frozen at −20°C (Pesce et al., 2014).

The PBMCs were isolated as described previously and used freshly for the evaluation of the WIP-1 mRNA and protein expressions and for the study of NF-kB signaling (Speranza et al., 2013). Briefly, the PBMCs were isolated by density-gradient centrifugation through Ficoll-Hypaque (Pharmacia), suspended (10^6/ml) in RPMI 1640 medium (Sigma-Aldrich, St Louis, MO, United States), containing L-glutamine 1% and antibiotics (penicillin 100 U/ml-streptomycin 100 ug/ml) with

10% heat-inactivated fetal calf serum (Sigma, CA, United States), seeded in polypropylene tubes (Falcon, BD, Lincoln Park, NJ, United States) and incubated at 37°C in a 95% humidified 5% CO_2 cell culture incubator. Cell viability in each culture was assessed by Trypan blue die exclusion. All solutions were prepared using pyrogen-free water and sterile polypropylene plastic-ware and were free of detectable LPS (<0.1 EU/ml), as determined by the Limulus amoebocyte lysate assay (sensitivity limit 12 pg/ml; Associates of Cape Cod, Falmouth, MA, United States). All reagents used were tested before use for Mycoplasma contamination (minimum detection level 0.1 μg/ml) (Whittaker Bioproducts, Walkersville, MD, United States) and found negative. The same batches of serum and medium were used in all experiments. After 24 h incubation, samples were centrifuged at 400 × *g* for 10 min at room temperature, supernatants were collected and stored at −80°C pending assay (Pesce et al., 2013). The PBMCs yield per mL of blood was approximately 1×10^6 cells.

RNA Extraction and the Quantitative Real-Time Polymerase Chain Reaction (qPCR) System

Total RNA was extracted from PBMCs of all subjects using the TRIzol reagent (Invitrogen, Life Technologies, Paisley, United Kingdom) according to the manufacturer's protocol. The RNA concentration and purity were determined by measuring absorbencies at 260 and 280 nm; a 260:280 ratio of 1.9 being considered acceptable for analysis. The RNA concentration was estimated by measuring the absorbance at 260 nm using a Bio-Photometer (Eppendorf AG, Hamburg, Germany), and RNA samples were kept frozen at –80°C until use. Purified RNA was electrophoresed on a 1% agarose gel to assess the integrity of the purified RNA. The RNA yield was 8.2 ± 2.7 μg/10^6 cells (mean ± SD).

Before Reverse Transcription, residual genomic DNA was removed from Total RNA using DNaseI (Thermo Scientific). The reaction mix was as follows: 1 μg of Total RNA, 1 μl of 10x Reaction Buffer, 1 U of DNaseI and water up to 10 μl. The mix was incubated at 37°C for 30 min and stopped by adding 1 μl of EDTA 50 mM and incubating at 65°C for 10 min. A High Capacity cDNA Reverse Transcription kit (Applied Biosystem) was used for reverse transcription of mRNAs. The reaction mix was prepared as follows: 2 μg of total RNA, 2 μl of 10x RT Buffer, 0.8 μl of dNTP, 2 μl of 10x Random Hexamer, 50 U of MultiScribe Reverse Transcriptase, 1 μl of RNase Inhibitor and water up to 20 μl. cDNA synthesis was performed in a thermal block cycler (Bio-rad) following these steps: priming at 25°C for 10 min, transcription at 37°C for 120 min and enzyme inactivation at 85°C for 5 min. All cDNA samples were stored at −20°C pending qPCR analysis.

A qPCR assay was carried out in an Eppendorf Mastercycler EP Realplex (Eppendorf AG). Briefly, preliminary PCR reactions were run to optimize the concentration and ratio of each primer set. For all the cDNA templates 2 μL was used in a 20 μL qPCR amplification system of the SYBR Green Real Master Mix Kit. Primers for human WIP-1 gene and GAPDH as reference gene were designed using GeneWorks software (IntelliGenetix, Inc., Mountain View, CA, United States).

The primer pairs used were as follows: WIP-1 (NM003620) forward-5′-TTTTTATATTGTTTTTAGGTTATT-3′ and reverse -5′-ATCTATATAAACTTTTAACTCAATC-3′; GAPDH (NM00-2046) forward-5′-ACCACCATGGAGAAGGC-3′ and reverse-5′-GGCATGGACTGTGGTCATGA-3′. The amplification procedures and data computation followed were similar to those described above (Patruno et al., 2012). In this study stability of GAPDH did not vary in the cells whatever the conditions. Thus, the relative expression of WIP-1 was normalized to GAPDH using the ΔC_q method [relative expression $= 2^{-\Delta Cq}$, where $\Delta C_q = C_{q(WIP1)} - C_{q(GAPDH)}$].

Western Blotting

Peripheral Blood Mononuclear Cells were washed once in cold phosphate-buffered saline (PBS; 0.5 mol/L sodium phosphate, pH 7.5), harvested by gentle scraping, and used to prepare total or nuclear protein extracts. Total protein extracts were prepared as described previously by treating cells with lysis buffer for 30 min at 4°C (Felaco et al., 2000). Nuclear extracts were prepared as previously described (Speranza et al., 2009). The protein concentrations of the extracts were determined using the Bradford method (Bio-Rad protein assay, Hercules, CA, United States).

For Western blotting (WB) analysis, 50 μg of protein per lane was separated on a 4-12% NuPAGE gradient gel (Gibco Invitrogen), electrotransferred onto a nitrocellulose membrane and blocked with 10% skimmed milk in PBS containing 0.1% Tween-20 (Franceschelli et al., 2011). Blots were probed and incubated overnight at 4°C with polyclonal rabbit IgG anti-WIP1, and pNF-κB (p-p65^{Ser536}) (Santa Cruz Biotechnology, Santa Cruz, CA, United States), all at 0.2 μg mL^{-1} in Tris-buffered saline (TBS)/0.1% Tween-20. A mouse antihuman monoclonal antibody recognizing human β-actin (A5441; Sigma–Aldrich) was used as the control in all experiments. A rabbit antihuman antibody for β-tubulin was used as the control in experiments with nuclear extract proteins (Santa Cruz Biotechnology, Santa Cruz, CA, United States). Three microgram of human recombinant the WIP-1 protein was used as positive control (Lifespan Biosciences, Seattle, WA, United States). Fifty microgram of nuclear extract proteins from PBMCs previously treated with the LPS (10 μg/mL) and INFγ (20 ng/mL) was used as positive control for the detection of p-p65^{Ser536} (Pesce et al., 2014).

Blots were then washed and incubated for 1 h with goat antirabbit-horseradish peroxidase (Pierce Biotechnology, Rockford, IL, United States) diluted 1:10000 in TBS/0.1% Tween-20. Immunoblot signals were developed using Super Signal Ultra chemiluminescence detection reagents (Pierce Biotechnology). The blot images were analyzed by a gel analysis software package (Gel Doc 1000; Bio-Rad, Milan, Italy). Data are expressed as the mean ± SD intensity of optical density.

Role of WIP-1 in Regulating NF-kB Signaling

In order to detect the role of the WIP-1 in regulating NF-κB signaling, the PBMCs were isolated and cultured during the study, as above described. The cultured cells were pre-treated or not treated with the WIP-1 inhibitor GSK2830371 for 1 h, and later the cells were stimulated with the LPS and INFγ. The LPS was used at 10 μg/mL, IFNγ at 20 ng/mL, and GSK2830371 at 10 μM (Sigma–Aldrich, St Louis, MO, United States) (Pesce et al., 2014).

Plasma Sampling and Measurement of C, CRP, IL-1β, IL-18, and IL-6 Levels, and *In Vitro* Production of Cytokines

Plasma was obtained with blood centrifugation at 1500 × *g* for 7 min and kept frozen at −20°C from all participants. The amount of plasma circulating IL-1β, IL-18, IL-6, C, and the CRP was assayed with the use of specific ELISA development systems (Pierce, Rockford, IL, United States) in all individuals. The amount of IL-1β, IL-18, IL-6 from the culture supernatants of the PBMCs was assayed with the use of specific ELISA development systems (Pierce, Rockford, IL, United States) in older adults after 6 months of being on a MT program. The experiments were performed according to the manufacturer's instructions.

Plates were scanned using a specialized Charge Coupled Device cooled tool. The integrated density values of the spots of known standards were used to generate a standard curve. Density values for unknown samples were determined by using the standard curve for each subject in order to calculate the real values in pg/mL. All steps were performed in duplicate and at room temperature. The IL-1β assay sensitivity was ≤1.0 pg/ml, for IL-18 it was ≤12.5 pg/mL, for IL-6 it was ≤1 pg/ml, for C it was ≤56.7 pg/mL, and for CRP ≤ 10.0 pg/ml. The values below the assay sensitivity have been excluded. The intra- and inter-assay reproducibility was >90%. Duplicate values that differed from the mean by more than 10% were considered suspect and were repeated.

Statistical Analysis

The normal distributions of data were tested by the Skewness and Kurtosis measurement. All the variables considered, resulted distributed in a normal manner. The results were reported separately for each group: young adults, total older adults, older adults Control Group (CG) and older adults Experimental Group (EG). The Unpaired Student *t*-test was used to assess significant differences between the young group and total older adults. The unpaired Student *t*-test and Chi-squared test were applied to evaluate significant differences between the CG and the EG. The same statistical test was applied to evaluate the differences for biological variables. Pearson's (r) co-efficient correlation was applied to assess the strength of the relationship between the basal levels of age, BMI, WIP-1, C, IL-1β, IL-18, IL-6, CRP and of the MMSE scores in older adult subjects. In order to assess the multi-collinearity of the variables, the diagnostic factors, Tolerance and Variance Inflation Factor (VIF) were considered. Accordingly, the MMSE scores were used as an dependent variable in a multiple regression analysis, whereas Age, as well as C, WIP-1, IL-1β, IL-18, and IL-6 were used as independent ones. The differences pre- and post-cognitive training were analyzed by the Paired samples *t*-test. Statistical analyses were performed using the SPSS 19.0 statistic (SPSS Inc., Chicago, IL, United States) for Windows (IBM). Results are described as means ± SD for each assessment performed in triplicate. All statistical tests were two-tailed and were evaluated at an alpha level of 0.05.

RESULTS

The Relationship between Global Cognitive Functioning and Biological Variables in Older Adults

Table 3 shows the bivariate correlations at T0 between Age, BMI, the plasmatic level of C, CRP, the pro-inflammatory cytokines IL-1β, IL-18, IL-6, the mRNA expression of the WIP-1 from *ex vivo* PBMCs, and MMSE score of all older adults ($n = 62$).

The Age parameter correlated significantly as well as negatively with the WIP-1 mRNA expression ($r = -0.368$, $p < 0.01$), whereas it correlated positively with the IL-1β ($r = 0.320$, $p < 0.05$), the IL-18 ($r = 0.371$, $p < 0.01$), and the IL-6 ($r = 0.327$, $p < 0.05$). The BMI did not correlate with any variable considered, except for the IL-6 ($r = 0.254$, $p < 0.05$).

The MMSE score correlated positively to the WIP-1 ($r = 0.322$; $p < 0.01$), and negatively to C ($r = 0.340$; $p < 0.01$) and to all the pro-inflammatory cytokines analyzed. Once again, the plasmatic level of C at baseline was significantly as well as negatively associated to the WIP-1 mRNA expression, and positively associated to IL-1β, IL-18, and IL-6. The cytokines were strongly and positively associated with each other. None of the variables considered, correlated significantly with the CRP.

The correlation values obtained at T0 were not confirmed at T1 for Age and the MMSE score, Age and C, and Age as well as immune markers evaluated. The same significant loss also occurred when the correlation were analyzed for the EG at T1.

Table 4 presents the multiple linear regression analysis results. The MMSE score was considered as a dependent variable. The variables which show significant correlation vs. MMSE score were considered as potential independent variables for the analysis. In order to assess multi-collinearity, the diagnostic factors, Tolerance and VIF were evaluated. As a consequence, none of the variables were excluded. The analysis was carried out using Age, C, WIP-1, IL-1β, IL-18, and IL-6 as independent variables that were added stepwise. The model including Age, C, and amount of the WIP-1 mRNA in *ex vivo* PBMCs resulted as the most significant, accounting for 13.4% of the MMSE variance [$F_{(1,60)} = 4.105$; $p = 0.010$] (Supplementary Table 1).

Neuropsychological Test

Cognitive functioning was directly assessed using a neuropsychological test battery. In 62 apparent older adults, the global cognitive function was measured by the MMSE at T0, and after 6 months of the MT program (T1). At the same time, verbal memory was assessed to measure the ability to retain (Short

TABLE 3 | Pearson (r) correlation between Age, Body Mass Index (BMI), Mini Mental State Examination (MMSE) score, Cortisol (C) and immune markers at baseline in apparently healthy older adults ($n = 62$).

	BMI	MMSE	C	WIP1	IL-1β	IL-18	IL-6	CRP
Age	−0.235	−0.303*	0.345#	−0.368#	0.320*	0.371#	0.327*	0.013
BMI	_	0.032	0.043	0.147	0.190	0.034	0.254*	−0.136
MMSE		_	−0.340#	0.322#	−0.298*	−0.324#	−0.296*	−0.024
C			_	−0.299*	0.272*	0.363#	0.252*	0.161
WIP-1				_	−0.426#	−0.660#	−0.505#	0.158
IL-1β					_	0.463#	0.426#	−0.188
IL-18						_	0.340#	−0.189
IL-6							_	−0.206

*WIP-1, Wild type p53-induced phosphatase 1; IL, Interleukin; CRP, C-reactive protein. *$p < 0.05$; #$p < 0.01$.*

TABLE 4 | Regression analysis summary for biological variables predicting cognitive function.

Dependent variable	Independent variables	R^2	R^2 adjusted	F	ANOVA (p)
MMSE score	Age	0.085	0.069	5.478	0.023
MMSE score	Age, C	0.147	0.117	4.986	0.010
MMSE score	Age, C, WIP1	0.178	0.134	4.105	0.010
MMSE score	Age, C, WIP1, IL-1β	0.193	0.135	3.342	0.016
MMSE score	Age, C, WIP1, IL-1β, IL-18	0.194	0.121	2.655	0.032
MMSE score	Age, C, WIP1, IL-1β, IL-18, IL-6	0.207	0.119	2.347	0.044

The MMSE score was regressed on Age, Cortisol (C), Wild type p53-induced phosphatase 1 (WIP-1), IL-1β, IL-18, IL-6 in an stepwise regression analysis (n = 62). All variables were considered at baseline. IL, Interleukin.

Term Memory, STM) and recall (Long Term Memory, LTM) verbal information using the RAVL Test. Visual memory was evaluated through the ROCF Test. Attention was assessed by the TMT A, the TMT B and the test of Attentive Matrices.

The results reported in **Table 4** show that the MMSE score significantly increased at T1 in EG when compared to their baseline score (28.3 ± 1.6 vs. 26.1 ± 1.7, $p < 0.01$). We also observed that the MMSE score of EG at T1 was significantly higher than those of CG (28.3 ± 1.6 vs. 26.4 ± 1.9, $p < 0.01$).

With close attention, we observed significant differences pre- and post- the MT program for attentive test targets. The medium number of targets attempted was augmented in the EG over time (48.8 ± 6.2 vs. 55.7 ± 5.1, $p < 0.05$), and between the EG and the CG at T1 (55.7 ± 5.1 vs. 50.2 ± 5.0, $p < 0.05$). The two groups did not differ significantly in their TMT A ($p = 0.611$) or TMT B ($p = 0.684$) performance concerning completion time at T0. However, the completion time was different between the two groups at the T1, in which it was lower in the EG both for the TMT A (35.1 ± 8.1 vs. 42.2 ± 11.5, $p > 0.05$), that the TMT B (66.2 ± 20.7 vs. 78.9 ± 27.7, $p > 0.05$), but it did not reach the significance. The EG did not show significant variation of scores when compared to themselves at T0 for the TMT A (35.1 ± 8.1 vs. 41.8 ± 10.5, $p > 0.05$), and B (66.2 ± 20.7 vs. 79.3 ± 28.4, $p > 0.05$). We again found that the RAVLT score followed the same trend over time observed in the EG for global cognitive assessment. In detail, the STM score significantly increased from 2.5 ± 1.2 to 3.4 ± 1.0 ($p < 0.01$), and the LTM significantly improved from 2.3 ± 1.2 to 3.3 ± 1.2 ($p < 0.01$). We did not observe any variation over time in the CG. Nonetheless, the EG showed a significantly higher RAVLT score for the STM at T1 when compared to CG, whereas the groups did not differ significantly for STM and for any neuropsychological measure at the baseline (**Table 5**).

The data obtained for Visual memory through the ROCF Test, suggested that the MT program also significantly improved this function in subjects of the EG over time. We did not observe any difference between groups at the T1 (**Table 5**).

Age Dependent WIP-1 mRNA and Protein Expression

The expression of WIP-1 has been shown to decrease during aging in murine model (Wong et al., 2009). In order to confirm on human the differentiated expression of the WIP-1 during age, qPCR, and WB experiments were performed to detect the mRNA and protein level, respectively, on freshly isolated PBMCs from healthy young and older adults. The results showed in **Figure 2**, suggest that older adults were characterized by significantly lower levels of the WIP-1 expression.

Memory Training Modulation on WIP-1 mRNA and Protein Expression

We determined the expression of the WIP-1 mRNA transcripts in freshly isolated PBMCs from selected healthy older adults by the qPCR at the baseline and after 6 months of the MT program in the CG and in the EG. **Figure 3A** shows the results from subjects of both groups. Compared with the CG, the PBMCs obtained from the EG were found to contain much higher levels of the mRNA for the WIP-1 at T1, determined as its expression

TABLE 5 | Neuropsychological assessment of global cognitive function, attention, verbal and visual memory from a Control Group (CG) and from an Experimental Group (EG) at baseline (T0) and also after 6 months of a Memory Training Program (T1).

Neuropsychological tests	CG-T0 ($n = 28$) (mean ± SD)	EG-T0 ($n = 34$) (mean ± SD)	CG-T1 ($n = 28$) (mean ± SD)	EG-T1 ($n = 34$) (mean ± SD)
Dementia (MMSE)	26.7 ± 1.7	26.1 ± 1.7	26.4 ± 1.9	28.3 ± 1.6[a,b]
TMT				
Part A (s)	41.3 ± 12.5	41.8 ± 10.5	42.2 ± 11.5	35.1 ± 8.1
Part B (s)	78.1 ± 29.0	79.3 ± 28.4	78.9 ± 27.7	66.2 ± 20.7
AM (targets)	48.2 ± 5.4	48.8 ± 6.2	50.2 ± 5.0	55.7 ± 5.1[a,b]
RAVLT				
STM	2.6 ± 1.5	2.5 ± 1.2	2.7 ± 1.3	3.4 ± 1.0[a,b]
LTM	2.8 ± 1.1	2.3 ± 1.2	2.6 ± 1.3	3.3 ± 1.2[a,b]
ROCF				
Copy	26.8 ± 7.1	27.2 ± 8.2	27.5 ± 9.3	32.4 ± 5.8[a,b]
Delayed recall	12.4 ± 5.1	11.8 ± 5.0	13.2 ± 7.8	14.4 ± 3.8[a,b]

The MMSE was used to quantify global cognitive functioning and cognitive change over time. The Attentive Matrices Test (AM) was used to measure selective and sustained attention (max targets = 60). The Trail Making Test (TMT) (Part A and Part B) provides information on visual search, the speed of processing, mental flexibility, selective visual attention and the ability of shifting. The score is represented by the time spent on test completion. The ability to retain (Short Term Memory, STM) and recall (Long Term Memory, LTM) verbal information was measured using the Rey Auditory Verbal Learning Test (RAVLT). The Rey-Osterrieth Complex Figure (ROCF) Test was used to assess visuo-spatial constructional functions, visuo-graphic memory, and a few planning aspects.

[a]*$p < 0.05$ vs. CG at the same time (Unpaired Student t-test);*

[b]*$p < 0.05$ vs. themselves at T0 (Paired t-test).*

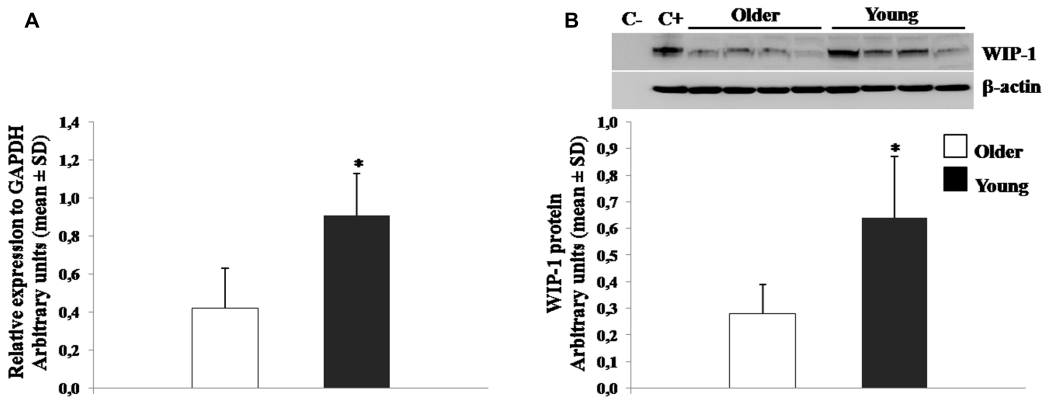


FIGURE 2 | Wild type p53-induced phosphatase 1 (WIP-1) expression levels in freshly isolated Peripheral Blood Mononuclear Cells (PBMCs) from Young and Older adults at baseline. **(A)** mRNA was quantified using qPCR in Young adults ($n = 31$) and Older adults ($n = 62$). Sequence-specific primers to the WIP-1 gene were used to quantify the expression of WIP-1 promoter. Values are expressed as relative expression to GAPDH as the reference gene. Values are means ± SD of three experiments performed in triplicate. **(B)** WIP-1 protein expression in PBMCs from Young adults as compared to Older adults at T0. Top, representative Western blot image of WIP-1 and β-actin. Bottom, each immunoreactive band was analyzed by densitometry and normalized to β-actin levels. Each value is a mean ± SD of three different experiments performed in triplicate. *$p < 0.05$ vs. older adults.

relative to the reference gene GAPDH obtained from all subjects at each time. We did not observe any difference between groups at T0. Nevertheless, the WIP-1 mRNA expression was significantly higher in the PBMCs of the EG at T1 when compared to their value at the baseline. In detail, the detected amount of the WIP-1 mRNA in PBMCs from subjects of the EG at T1, is increased by more than 40% when compared to the EG at T0, and the CG at T1.

The levels of the WIP-1 protein in PBMCs were measured by the WB. In accordance with the mRNA levels, the PBMCs from the CG had significantly lower levels of the WIP-1 protein versus the EG after the MT program. The expression of the WIP-1 protein was also significantly increased within the EG over time. According to those reported for the mRNA, the protein level did not significantly differ between groups at T0 (**Figure 3B**).

Cortisol, CRP, and Cytokines Production

In order to highlight the effects of the MT on the C level and the low-grade of inflammation which characterizes healthy older adults, the ELISA test was used to detect the plasmatic level of C as well as determining the non-specific marker for inflammation CRP in both groups at baseline and after the MT. Significant variation for CRP was not observed. As reported in **Table 6**, our data suggest that the MT significantly modulates the C plasma level. That level had significantly decreased in the EG at T1 when compared vs. CG at T1 and vs. EG at T0. In order to investigate

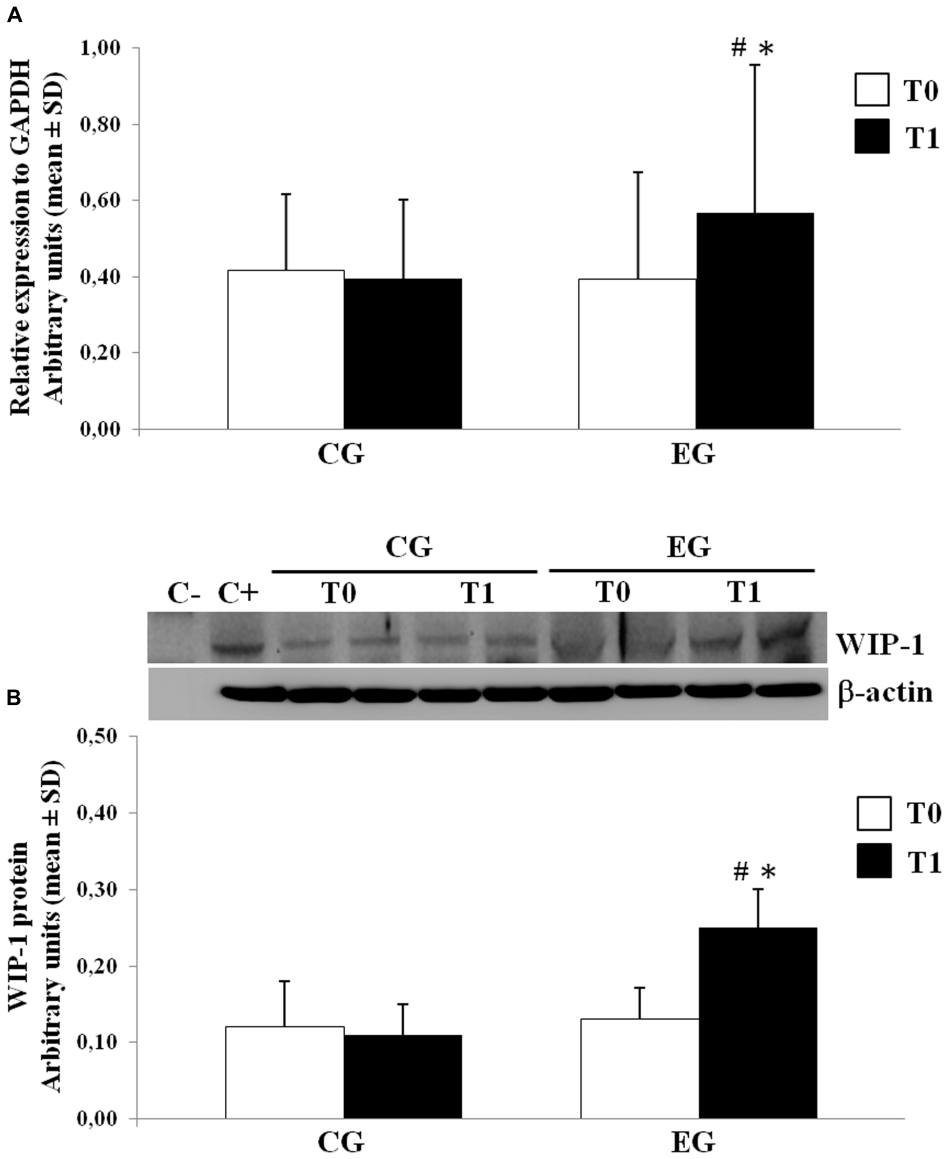


FIGURE 3 | Wild type p53-induced phosphatase 1 expression levels in freshly isolated PBMCs from Control Group (CG) and Experimental Group (EG) at baseline and after 6 months of memory training program. **(A)** mRNA was quantified using qPCR in CG (n = 28) and EG (n = 34). Sequence-specific primers to the WIP-1 gene were used to quantify the expression of WIP-1 promoter. Values are expressed as relative expression to GAPDH as the reference gene. **(B)** WIP-1 protein expression in PBMCs from EG at T1 as compared to CG and EG at baseline (T0). Top, representative Western blot image of WIP-1 and β-actin. Bottom, each immunoreactive band was analyzed by densitometry and normalized to β-actin levels. Each value is a mean ± SD of three different experiments performed in triplicate. $^{*}p < 0.05$ vs. T0; $^{\#}p < 0.05$ vs. CG at the same time.

any involvement of the MT on cytokine production in healthy older adults, the plasma levels of the IL-1β, the IL-18, and the IL-6 were evaluated. **Table 5** also shows the concentrations of cytokines detected in plasma of both groups at T0 and T1. The levels of the IL-1β, the IL-18 and the IL-6 detected at T0 did not significantly differ between the CG and the EG. On the other hand, the submission to the MT program significantly decreased the plasmatic level of all cytokines analyzed at T1 in the EG. Consequently, the subjects of this group showed a significant decrease over time.

The Role of WIP-1 in Regulating NF-kB Signaling in PBMCs from Healthy Older Adults

The role of the NF-κB in the expression of pro-inflammatory cytokines has been broadly reviewed (Chung et al., 2011). The post-translational modification of the NF-κB (p65) affects its transcriptional activity; in particular, several reports have documented negative regulation of the NF-κB by the WIP-1, also through the direct dephosphorylation of Ser-536 of p65 (Chew

TABLE 6 | Time-dependent effect of memory training on Cortisol and the plasmatic pro-inflammatory mediators.

	CG-T0 (mean ± SD)	EG-T0 (mean ± SD)	CG-T1 (mean ± SD)	EG-T1 (mean ± SD)
Cortisol (pg/mL)	136.2 ± 56.4	134.9 ± 58.2	131.5 ± 67.7	109.3 ± 48.1[a,b]
IL-1β (pg/mL)	26.1 ± 8.3	25.9 ± 8.7	25.9 ± 7.9	22.8 ± 8.4[a,b]
IL-18 (pg/mL)	239.2 ± 48.0	241.8 ± 45.1	238.0 ± 41.0	212.1 ± 43.1[a,b]
IL-6 (pg/mL)	1.41 ± 0.26	1.46 ± 0.26	1.40 ± 0.27	1.23 ± 0.16[a,b]

Cortisol, IL-1β, IL-18, and IL-6 plasma level are expressed as means ± SD (IL-1β, IL-18, and IL-6 CG at T0 and T1, n = 28; IL-1β, IL-18, and IL-6 EG at T0, n = 34; IL-1β and IL-18 EG at T1, n = 34; IL-6 EG, n = 31). T0, baseline time; T1, 6 months from the baseline time; CG, Control Group; EG, Experimental Group; IL, Interleukin. [a]$p < 0.05$ *vs. T0;* [b]$p < 0.05$ *vs. CG at the same time.*

et al., 2009). We next further performed WB studies to determine the phosphorylation of p65 at Ser-536, using specific phospho-p65 antibody on nuclear protein extracts. In the EG, we observed a down-regulation of nuclear translocation of the p-p65^{Ser536} as compared to the CG group at T1. As a result, the phosphorylated p65 in the EG showed low levels at T1 when compared to their own levels at T0 (**Figure 4A**).

In order to better investigate the role of this phosphatase in regulating the NF-κB nuclear translocation, the PBMCs from the CG and the EG at T0 and T1 were cultured and treated with a selective inhibitor of the WIP-1 (GSK 2830371) and/or LPS + INFγ, while nuclear protein extracts were detected by an antibody specific for the p-p65. As shown in **Figure 4B**, treatment with the LPS + INFγ increased the p-p65^{Ser536} expression in nuclei. This response was significantly elevated in the PBMCs from the CG as compared to the EG at T1. It should also be noted that the WIP-1 inhibition resulted in a significant increase in the constitutive and the LPS + INFγ-induced p65 phosphorylation and nuclear translocation in both groups. These findings suggested that the WIP-1 expression is significantly involved in the negative regulation of the NF-κB activity.

Furthermore, the inhibition of the activity of the WIP-1 resulted in a significantly higher constitutive and induced the release of the IL-1β, of the IL-18 and of the IL-6 in the medium of cultured cells (**Table 7**). We did not observe any significant differences between cells treated with an inhibitor and a medium alone, either in the p-p65^{Ser536} nuclear levels (**Figure 4B**) or in cytokines release (**Table 7**).

Altogether, these findings suggest a role for the WIP-1 activity in mediating the NF-κB nuclear levels and pro-inflammatory cytokines released in older adults. Furthermore, the submission to 6 months of the MT significantly restored the WIP-1 inhibitory function.

DISCUSSION

In the current study, we assume that the MT through mnemonic strategies could be considered as treatment that similarly to other stimuli of the EE, could enhance the cognitive and physical well-being of older adults. We studied a sample of apparently healthy older adults and used a longitudinal approach to test the hypothesis that the MT program could counteract the higher level of plasmatic C and sterile inflammation that characterizes adulthood as well as a decline in the cognitive function. We also investigated whether the phosphatase WIP-1 in *ex vivo* PBMCs could be considered a "master" molecule involved in modulating the level of plasmatic pro-inflammatory cytokines IL-6, IL-1β, and IL-18.

Concerning our correlation's data, the associations recorded between cytokines, Age, MMSE score and mRNA of WIP-1 suggested a role of this phosphatase in the inhibition of the release of the IL-6, the IL-1β, and the IL-18. We first purposed a significant connection between global cognitive functioning and the IL-18 in healthy subjects, and highlighted a previously unrecognized relationship between plasmatic C levels and immune markers analyzed. Our results again reinforced the positive correlation between Age and C, as well as the negative association between the global cognitive function and C (Carvalhaes-Neto et al., 2003; Otte et al., 2005). Worth noting is that in a linear regression analysis, Age, C, and the WIP-1 mRNA expression resulted as the best predictors of variance in global cognitive performance and explained approximately the 13% variance in the MMSE score. We were in accordance with previous findings suggesting positive association between the cytokines measured and Age (Dinarello, 2006; Miles et al., 2008), and the IL-6 and BMI (Mohamed-Ali et al., 1997). We also agree with other authors in observing any significant association between the CRP plasmatic level and global cognitive functioning (Elderkin-Thompson et al., 2012).

As far as MT is concerned, accordingly with other studies, our results highlight better performance relating to verbal and visuo-spatial memory after 6 months of laboratory participation. Interestingly, as suggested by a significant decrease in the completion time of attentive matrices, MT contributes to attention performance enhancement. In regards to this, we are in line with the assumption that working memory used effectively is necessary to continuously select what is being represented by paying major attention to the way in which the working memory functions, and to update what is represented according to the relevance of the task (Palladino et al., 2001).

Cognitive improvement was paralleled over time by the lowering of the plasmatic level of the glucocorticoid hormone C, and by a significant reduction in the plasmatic levels of the pro-inflammatory cytokines evaluated. These findings suggest a previously unrecognized beneficial effect of the MT program participation, as increased C levels have been associated to several age-related pathologies that include muscle atrophy (Dohi et al., 2005), osteoporosis/hypercalcemia (Hetyey et al., 2007), hyperglycemia/hyperlipidemia, atherosclerosis, type II diabetes

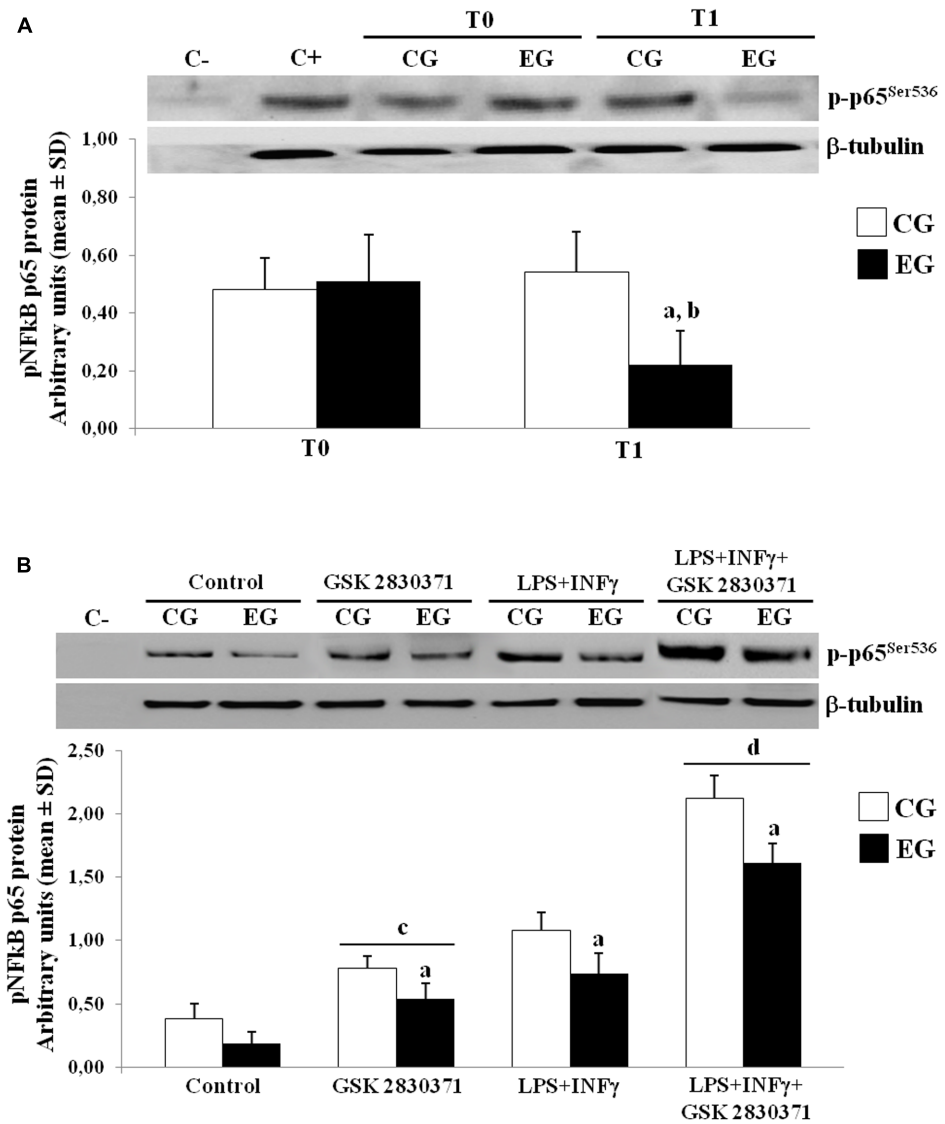


FIGURE 4 | Phosphorylated levels of p65 in nuclei of PBMCs of older adults. **(A)** p-p65^{Ser536} protein expression in nuclei of freshly isolated PBMCs from CG and EG at T1 as compared to baseline values (T0). Top, representative Western blot image of p-p65^{Ser536}. **(B)** PBMCs from CG and EG were cultured and then treated with WIP-1 inhibitor (GSK 2830371, 10 μM), LPS (10 μg/ml) + INFγ (20 ng/ml) or WIP-1 inhibitor and LPS (10 μg/ml) + INFγ (20 ng/ml), as described in "Materials and Methods" Section. Western immunoblotting was performed on nuclear proteins with antibody anti-p-p65^{Ser536} and anti-β-tubulin as the control. The image represents six different gels performed twice. Values are expressed relative to control (mean ± SD). $^{a}p < 0.05$ vs. CG; $^{b}p < 0.05$ vs. EG at T0; $^{c}p < 0.05$ vs. control PBMCs; $^{d}p < 0.05$ vs. PBMCs treated with LPS and INFγ.

and major depression (Bindu et al., 2005; Leggio et al., 2005; Bauer, 2008). The peripheral level of the IL-6 contributes to age-related neural atrophy (Willette et al., 2010), it is considered a marker of increased risk of frailty (Ershler and Keller, 2000), and is associated with reduced muscle strength (Barbieri et al., 2003) during adulthood. No less important, the circulating levels of the IL-1β and of the IL-18 which are considered detrimental for aging successfully were involved in the mediation of several downstream effects of the NLRP3 inflammasome activation (Martinon et al., 2009; Youm et al., 2013). In particular, the IL-1β is thought to play a predominant role in the development of several age-related degenerative diseases including type 2 diabetes and Alzheimer's Disease (AD) (Youm et al., 2011; Heneka et al., 2013). Both cytokines have been suggested to regulate obesity-induced inflammation, insulin resistance, and plaque instability and atherogenesis (Stienstra et al., 2011; Vandanmagsar et al., 2011; Wen et al., 2011; Shi et al., 2015).

At the same time, the MT could contribute to generate a positive loop in which memory performance improvement was further sustained by the lowering of the plasmatic level of C and of pro-inflammatory cytokines. In this respect, the higher level of C was related to the parameters of cognitive

TABLE 7 | Comparison of the IL-1β, of the IL-18, and of the IL-6 release in conditioned medium by non-stimulated and the LPS + INFγ and/or WIP-1 inhibitor (GSK2830371, 10 μM) treated Peripheral Blood Mononuclear Cells (PBMCs) (pg/ml/10^6 cells) from 28 subjects of Control Group (CG) and 34 of the Experimental Group (EG) after 6 months of being on a Memory Training Program (T1).

	Control (pg/mL, mean ± SD)		WIP-1 inhibitor (pg/mL, mean ± SD)		LPS/INFγ (pg/mL, mean ± SD)		LPS/INFγ + WIP-1 inhibitor (pg/mL, mean ± SD)	
	CG	EG	CG	EG	CG	EG	CG	EG
IL1β	9.7 ± 4.2	5.6 ± 3.1[a]	35.9 ± 4.4	22.4 ± 6.2[a,b]	398.2 ± 37.4	321.4 ± 25.4[a]	512.0 ± 58.7	380.6 ± 54.8[a,b]
IL-18	36.9 ± 5.4	26.2 ± 6.1[a]	76.1 ± 6.8	51.7 ± 14.4[a,b]	282.6 ± 18.7	212.6 ± 16.1[a]	371.2 ± 22.2	324.1 ± 31.5[a,b]
IL-6	128.7 ± 25.4	94.1 ± 18.9[a]	156.2 ± 26.6	135.8 ± 19.2[a,b]	213.8 ± 22.7	178 ± 28.1[b]	244.6 ± 32.3	212.8 ± 0.3[a,b]

Supernatants were collected, stored at −80°C until analysis, and then measured for IL-1β, IL-18, and IL-6 concentration using a commercial enzyme-linked immunosorbent assay (ELISA). All samples for a given assay were analyzed in duplicate at the same time. The ELISA values have an error range lower than 10%. The variation coefficient both inter-assay and intra-assay was <5%.

Values are presented as means ± SD. [a]$p < 0.05$ vs. respective CG; [b]$p < 0.05$ vs. respective WIP-1 inhibitor no treated cells.

frailty (Carvalhaes-Neto et al., 2003), and more recently has been directly associated to cognitive performance in a large sample of non-demented subjects aged at least 65 years (Ouanes et al., 2017). Nevertheless, the IL-6 has been associated with poorer performance on tests of encoding and the recall of information and working memory (Elderkin-Thompson et al., 2012). The concentrations of the IL-1β were extensively related to the negative effects on memory in animal and human studies (Yirmiya and Goshen, 2011). To the best of our knowledge, there are no studies that have been previously associated with the IL-18 to some domain of cognition in healthy subjects. However, Zhang et al. (2013) suggests a role of a higher plasmatic level of this cytokine in determining the Visuo-spatial/Constructional deficit that characterizes naïve schizophrenic patients and has resulted in its circulatory level being significantly and positively associated with cognitive impairment in AD patients (Bossù et al., 2008).

The higher plasma levels of the IL-6, the IL-1β, and the IL-18 contribute to explain the more elevated NF-κB activation observed in freshly isolated PBMCs at the baseline vs. the EG after the MT. Our data prompted the fact that the MT inhibition of pro-inflammatory cytokines release from the PBMCs could be influenced by the restoration of the WIP-1 expression.

Our observations on human are consistent with the previous finding on rodents, in which the gene expression of WIP-1 resulted lower during aging (Wong et al., 2009). The PBMCs from older adults at the baseline, in which the WIP-1 expression was lower, were characterized by the higher $p65^{Ser536}$ nuclear translocation when compared to the PBMCs from older adults treated with the MT in which the WIP-1 expression and activity was enhanced.

The analyses of cultured *ex vivo* PBMCs have shown that the WIP-1 could suppress the release of targets of the transcription factor NF-κB (i.e., cytokines medium release), with or without the LPS/INFγ stimulation. Our data also suggests that cultured PBMCs from older adults after the MT participation, were less sensitive toward the LPS/INFγ stimulation.

We are in accordance with the previous study which suggested an inhibiting role of the WIP-1 to the NF-κB signaling pathway. As showed by Chew et al. (2009), the over-expression of the WIP-1 or silencing it reduced or increased the NF-κB activation downstream treatment with the IL-1β. Once again, it was purposed that the enhancement of the NF-κB activity by the WIP-1 knockdown was strictly due to the levels of phosphorylation of the p65 on the Ser536. We can conclude that the WIP-1 could reduce the release of these cytokines by the inhibition of the NF-κB through direct de-phosphorylation of the p65 sub-unit on the Ser536.

Summarizing, our data confirms the validity of the MT, through mnemonic strategies in counteracting the aging associated decline of verbal, visual memory and of attention. In addition, we first suggested that this method of cognitive training contributes also to improve the other features of the phenotype of older adults by the decreasing level of the circulating C and pro-inflammatory cytokines and inflammation. Nevertheless, we suggest that the effect exerted on cytokines by MT could be due to the restoration of a higher level of the WIP-1 expression and activity.

The main limitation of the study is regards "How" the MT can contribute in restoring the WIP-1 expression and decreasing the pro-inflammatory cytokines level. Considering the associations observed between C, MMSE score, cytokines, and WIP-1 expression, we hypothesized a mechanism in which the lowering of this hormone could represent a potential link between cognitive stimulation and the observed decrease of low-grade inflammation that characterizes older adults.

The MT is a practice based on working memory tasks, which involves the hippocampus and its connections (Chengyang et al., 2016). At the present, we speculate that the MT could contribute to restore the inhibiting role on the HPA axis activity (Herman et al., 2003). It is indeed known that the hippocampal type I receptors were strictly expressed in the hippocampus and resulted diminished in aging. These receptors mediated the feedback control of the HPA axis, through the activation of tonic inhibitory projections mediated by GABAergic neurons proceeded to the Para Ventricular Nucleus of the hypothalamus (Swanson, 1991; Herman et al., 1996). To follow, the effect of MT on the diminishing C medium level, could determine the modulations on pro-inflammatory cytokines secretion recorded by analysis of *ex vivo* PBMCs. To this end, recent findings suggested that *in vivo* administration of C could enhance the migratory response of human monocytes and decrease the mRNA level of the IkBα protein, leading to higher nuclear levels of the p65 sub-unit of NF-kB (Yeager et al., 2016). Other studies

support the existence of a positive feedback between GCs secretion and the activation of the Toll Like Receptor pro-inflammatory signaling pathway that was mediated by the synergistic action of GCs and pro-inflammatory stimuli (Bornstein et al., 2004; Hermoso et al., 2004). GCs have also been shown to induce the expression of NLRP3 inflammasome, in both cultured and primary macrophages, leading to higher secretion of IL1β, and IL-6 (Busillo et al., 2011). These results provide a potential explanation for some of the negative effects of higher level of C in older adults (Cruz-Topete and Cidlowski, 2015).

With regards to the WIP-1 expression, further analysis as to whether its expression in *ex vivo* PBMCs could be modulated by *in vivo* administration of C in young and older adults with or without synergy with pro-inflammatory stimulation will be done in the future. Furthermore, it will be of interest to investigate the age-related promoter status methylation of the PPM1D gene to test the hypothesis that an epigenetic mechanism (e.g., hypermethylation of PPM1D promoter κB site) could contribute to the WIP-1 repression, and could explain the progressive long life reduction of the WIP-1 expression previously observed in several cell types by other studies (Wong et al., 2009; Zhu et al., 2009).

CONCLUSION

Our work provides insight some new properties of the MT in counteracting aging and a purposely planned WIP-1 deficiency as a part of its pathophysiology, bearing in mind that this phosphatase exerts an important role within the mechanisms that generally act in order to counteract and reduce the negative effects of environmental agents and those which were impaired during adulthood.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: MP, MF, and AG. Performed the experiments: RT, ILF, AR, AF, SF, CT, and GC. Analyzed the data: MP, RT, MCV, MDL, and LS. Wrote the manuscript: MP and RT.

FUNDING

The Italian Ministry of Education and Research.

ACKNOWLEDGMENTS

Dr. Filomena Natarelli for her support in the editing of the paper. The Italian Ministry of Education and Research for its financial support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnmol.2017.00233/full#supplementary-material

REFERENCES

Ablij, H. C., and Meinders, A. E. (2002). C-reactive protein: history and revival. *Eur. J. Intern. Med.* 13, 412–422. doi: 10.1016/S0953-6205(02)00132-2

Baltes, P. B., and Baltes, M. M. (1990). "Psychological perspectives on successful aging: the model of selective optimization with compensation," in *Successful Aging*, eds P. B. Baltes and M. M. Baltes (Cambridge: University Press), 1–34. doi: 10.1017/CBO9780511665684

Barbieri, M., Ferrucci, L., Ragno, E., Corsi, A., Bandinelli, S., Bonafè, M., et al. (2003). Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. *Am. J. Physiol. Endocrinol. Metab.* 284, E481–E487. doi: 10.1152/ajpendo.00319.2002

Bauer, M. E. (2008). Chronic stress and immunosenescence: a review. *Neuroimmunomodulation* 15, 241–250. doi: 10.1159/000156467

Baumans, V. (2005). Environmental enrichment for laboratory rodents and rabbits: requirements of rodents, rabbits, and research. *Ilar. J.* 46, 162–170. doi: 10.1093/ilar.46.2.162

Biasucci, L. M. (2004). CDC/AHA workshop on markers of inflammation and cardiovascular disease: application to clinical and public health practice. Clinical use of inflammatory markers in patients with cardiovascular diseases: a background paper. *Circulation* 110, 560–567. doi: 10.1161/01.CIR.0000148983.88334.80

Bindu, B., Rekha, J., and Kutty, B. M. (2005). Post insult enriched housing improves the 8-arm radial maze performance but not the Morris water maze task in ventral subicular lesioned rats. *Brain. Res.* 1063, 121–131. doi: 10.1016/j.brainres.2005.09.044

Bornstein, S. R., Zacharowski, P., Schumann, R. R., Barthel, A., Tran, N., Papewalis, C., et al. (2004). Impaired adrenal stress response in Toll-like receptor 2-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 101, 16695–16700. doi: 10.1073/pnas.0407550101

Bossù, P., Ciaramella, A., Salani, F., Bizzoni, F., Varsi, E., Di Iulio, F., et al. (2008). Interleukin-18 produced by peripheral blood cells is increased in Alzheimer's disease and correlates with cognitive impairment. *Brain Behav. Immun.* 22, 487–492. doi: 10.1016/j.bbi.2007.10.001

Bowers, S. L., Bilbo, S. D., Dhabhar, F. S., and Nelson, R. J. (2008). Stressor-specific alterations in corticosterone and immune responses in mice. *Brain Behav. Immun.* 22, 105–113. doi: 10.1016/j.bbi.2007.07.012

Busillo, J. M., Azzam, K. M., and Cidlowski, J. A. (2011). Glucocorticoids sensitize the innate immune system through regulation of the NLRP3 inflammasome. *J. Biol. Chem.* 286, 38703–38713. doi: 10.1074/jbc.M111.275370

Carvalhaes-Neto, N., Huayllas, M. K., Ramos, L. R., Cendoroglo, M. S., and Kater, C. E. (2003). Cortisol, DHEAS and aging: resistance to cortisol suppression in frail institutionalized elderly. *J. Endocrinol. Invest.* 26, 17–22. doi: 10.1007/BF03345117

Caswell, M. (1993). Effect of patient age on tests of the acute-phase response. *Arch. Pathol. Lab. Med.* 117, 906–909.

Cavallini, E., Pagnin, A., and Vecchi, T. (2003). Aging and everyday memory: the beneficial effect of memory training. *Arch. Gerontol. Geriatr.* 37, 241–257. doi: 10.1016/S0167-4943(03)00063-3

Chengyang, L., Daqing, H., Jianlin, Q., Haisheng, C., Qingqing, M., Jin, W., et al. (2016). Short-term memory deficits correlate with hippocampal-thalamic functional connectivity alterations following acute sleep restriction. *Brain Imaging Behav.* doi: 10.1007/s11682-016-9570-1 [Epub ahead of print].

Chesnokova, V., Pechnick, R. N., and Wawrowsky, K. (2016). Chronic peripheral inflammation, hippocampal neurogenesis, and behavior. *Brain. Behav. Immun.* 58, 1–8. doi: 10.1016/j.bbi.2016.01.017

Chew, J., Biswas, S., Shreeram, S., Humaidi, M., Wong, E. T., Dhillion, M. K., et al. (2009). WIP1 phosphatase is a negative regulator of NF-kappaB signalling. *Nat. Cell. Biol.* 11, 659–666. doi: 10.1038/ncb1873

Chung, S., Sundar, I. K., Hwang, J. W., Yull, F. E., Blackwell, T. S., Kinnula, V. L., et al. (2011). NF-κB inducing kinase, NIK mediates cigarette smoke/TNFα-induced histone acetylation and inflammation through differential activation of IKKs. *PLoS ONE* 6:e23488. doi: 10.1371/journal.pone.0023488

Cruz-Topete, D., and Cidlowski, J. A. (2015). One hormone, two actions: anti- and pro-inflammatory effects of glucocorticoids. *Neuroimmunomodulation* 22, 20–32. doi: 10.1159/000362724

Curlik, D. M. II, and Shors, T. J. (2013). Training your brain: do mental and physical (MAP) training enhance cognition through the process of neurogenesis in the hippocampus? *Neuropharmacology* 64, 506–514. doi: 10.1016/j.neuropharm.2012.07.027

De Beni, R. (2009). *Psicologia dell'Invecchiamento*. Bologna: Il Mulino.

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., and Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocr. Rev.* 19, 269–301. doi: 10.1210/er.19.3.269

Deuschle, M., Gotthardt, U., Schweiger, U., Weber, B., Körner, A., Schmider, J., et al. (1997). With aging in humans the activity of the hypothalamus-pituitary-adrenal system increases and its diurnal amplitude flattens. *Life Sci.* 61, 2239–2246. doi: 10.1016/S0024-3205(97)00926-0

Dinarello, C. A. (2006). Interleukin 1 and interleukin 18 as mediators of inflammation and the aging process. *Am. J. Clin. Nutr.* 83, 447S–455S.

Ding, Y., Gao, Z. G., Jacobson, K. A., and Suffredini, A. F. (2010). Dexamethasone enhances ATP-induced inflammatory responses in endothelial cells. *J. Pharmacol. Exp. Ther.* 335, 693–702. doi: 10.1124/jpet.110.171975

Dohi, K., Satoh, K., Ohtaki, H., Shioda, S., Miyake, Y., Shindo, M., et al. (2005). Elevated plasma levels of bilirubin in patients with neurotrauma reflect its pathophysiological role in free radical scavenging. *In Vivo* 19, 855–860.

Elderkin-Thompson, V., Irwin, M. R., Hellemann, G., and Kumar, A. (2012). Interleukin-6 and memory functions of encoding and recall in healthy and depressed elderly adults. *Am. J. Geriatr. Psychiatry* 20, 753–763. doi: 10.1097/JGP.0b013e31825d08d6

Ershler, W. B., and Keller, E. T. (2000). Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu. Rev. Med.* 51, 245–270. doi: 10.1146/annurev.med.51.1.245

Felaco, M., Di Maio, F. D., De Fazio, P., D'Arcangelo, C., De Lutiis, M. A., Varvara, G., et al. (2000). Localization of the e-NOS enzyme in endothelial cells and odontoblasts of healthy human dental pulp. *Life Sci.* 68, 297–306. doi: 10.1016/S0024-3205(00)00935-8

Ferrari, E., and Magri, F. (2008). Role of neuroendocrine pathways in cognitive decline during aging. *Ageing Res. Rev.* 7, 225–233. doi: 10.1016/j.arr.2008.07.001

Fiscella, M., Zhang, H., Fan, S., Sakaguchi, K., Shen, S., Mercer, W. E., et al. (1997). Wip1, a novel human protein phosphatase that is induced in response to ionizing radiation in a p53-dependent manner. *Proc. Natl. Acad. Sci. U.S.A.* 94, 6048–6053. doi: 10.1073/pnas.94.12.6048

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Franceschelli, S., Pesce, M., Vinciguerra, I., Ferrone, A., Riccioni, G., Patruno, A., et al. (2011). Licocalchone-C extracted from Glycyrrhiza glabrainhibits lipopolysaccharide-interferon-γ inflammation by improving antioxidant conditions and regulating inducible nitric oxide synthase expression. *Molecules* 16, 5720–5734. doi: 10.3390/molecules16075720

Franceschi, C. (2007). Inflammaging as a major characteristic of old people: can it be prevented or cured? *Nutr. Rev.* 65(12 Pt 2), S173–S176. doi: 10.1111/j.1753-4887.2007.tb00358.x

Franceschi, C., and Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J. Gerontol. A Biol. Sci. Med. Sci.* 69(Suppl. 1), S4–S9. doi: 10.1093/gerona/glu057

Gabay, C., and Kashner, I. (1999). Acute phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* 340, 448–454. doi: 10.1056/NEJM199902113400607

Geerlings, M. I., Sigurdsson, S., Eiriksdottir, G., Garcia, M. E., Harris, T. B., Gudnason, V., et al. (2015). Salivary cortisol, brain volumes, and cognition in community-dwelling elderly without dementia. *Neurology* 85, 976–983.

Giordano, R., Bo, M., Pellegrino, M., Vezzari, M., Baldi, M., Picu, A., et al. (2005). Hypothalamus-pituitary-adrenal hyperactivity in human aging is partially refractory to stimulation by mineralocorticoid receptor blockade. *J. Clin. Endocrinol. Metab.* 90, 5656–5662. doi: 10.1210/jc.2005-0105

Gorelick, P. B., Scuteri, A., Black, S. E., Decarli, C., Greenberg, S. M., Iadecola, C., et al. (2014). American heart association stroke council, council on epidemiology and prevention, council on cardiovascular nursing, council on cardiovascular radiology and intervention, and council on cardiovascular Hannan AJ. Environmental enrichment and brain repair: harnessing the therapeutic effects of cognitive stimulation and physical activity to enhance experience-dependent plasticity. *Neuropathol. Appl. Neurobiol.* 40, 13–25. doi: 10.1111/nan.12102

Green, D. R., Galluzzi, L., and Kroemer, G. (2011). Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* 333, 1109–1112. doi: 10.1126/science.1201940

Hannan, A. J. (2014). Environmental enrichment and brain repair: harnessing the therapeutic effects of cognitive stimulation and physical activity to enhance experience-dependent plasticity. *Neuropathol. Appl. Neurobiol.* 1, 13–25. doi: 10.1111/nan.12102

Heneka, M. T., Kummer, M. P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., et al. (2013). NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* 493, 674–678. doi: 10.1038/nature11729

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., et al. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front. Neuroendocrinol.* 24:151–180. doi: 10.1016/j.yfrne.2003.07.001

Herman, J. P., Prewitt, C. M., and Cullinan, W. E. (1996). Neuronal circuit regulation of the hypothalamo-pituitary-adrenocortical stress axis. *Crit. Rev. Neurobiol.* 10, 371–394. doi: 10.1615/CritRevNeurobiol.v10.i3-4.50

Hermoso, M. A., Matsuguchi, T., Smoak, K., and Cidlowski, J. A. (2004). Glucocorticoids and tumor necrosis factor alpha cooperatively regulate toll-like receptor 2 gene expression. *Mol. Cell. Biol.* 24, 4743–4756. doi: 10.1128/MCB.24.11.4743-4756.2004

Hetyey, C. S., Manczur, F., Dudás-Györki, Z., Reiczigel, J., Ribiczey, P., Vajdovich, P., et al. (2007). Plasma antioxidant capacity in dogs with naturally occurring heart diseases. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* 54, 36–39. doi: 10.1111/j.1439-0442.2007.00911.x

Jankowsky, J. L., Melnikova, T., Fadale, D. J., Xu, G. M., Slunt, H. H., Gonzales, V., et al. (2005). Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J. Neurosci.* 25, 5217–5224. doi: 10.1523/JNEUROSCI.5080-04.2005

Jin, R. O., Mason, S., Mellon, S. H., Epel, E. S., Reus, V. I., and Mahan, L. (2016). Cortisol/DHEA ratio and hippocampal volume: a pilot study in major depression and healthy controls. *Psychoneuroendocrinology* 72, 139–146. doi: 10.1016/j.psyneuen.2016.06.017

Kempermann, G., Gast, D., and Gage, F. H. (2002). Neuroplasticity in old age: sustained five fold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann. Neurol.* 52, 135–143. doi: 10.1002/ana.10262

Langlais, D., Couture, C., Balsalobre, A., and Drouin, J. (2008). Regulatory network analyses reveal genome-wide potentiation of LIF signaling by glucocorticoids and define an innate cell defense response. *PLoS Genet.* 4:e1000224. doi: 10.1371/journal.pgen.1000224

Lee, B. K., Glass, T. A., Wand, G. S., McAtee, M. J., Bandeen-Roche, K., Bolla, K. I., et al. (2008). Apolipoprotein e genotype, cortisol, and cognitive function in community-dwelling older adults. *Am. J. Psychiatry* 165, 1456–1464. doi: 10.1176/appi.ajp.2008.07091532

Leggio, M. G., Mandolesi, L., Frederico, F., Spirito, F., Ricci, B., Gelfo, F., et al. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behav. Brain Res.* 163, 78–90. doi: 10.1016/j.bbr.2005.04.009

Lezak, M. D. (1995). *Neuropsychological Assessment*. New York, NY: Oxford University Press.

Ligthart, G. J., Corberand, J. X., Fournier, C., Galanaud, P., Hijmans, W., Kennes, B., et al. (1984). Admission criteria for immunogerontological studies in man: the SENIEUR protocol. *Mech. Ageing Dev.* 28, 47–55. doi: 10.1016/0047-6374(84)90152-0

Lupien, S. J., de Leon, M., de Santi, S., Convit, A., Tarshish, C., Nair, N. P., et al. (1998). Cortisol levels during human aging

predict hippocampal atrophy and memory deficits. *Nat. Neurosci.* 1, 69–73.

Lupien, S. J., Evans, A., Lord, C., Miles, J., Pruessner, M., Pike, B., et al. (2007). Hippocampal volume is as variable in young as in older adults: implications for the notion of hippocampal atrophy in humans. *Neuroimage* 34, 479–485. doi: 10.1016/j.neuroimage.2006.09.041

Maggio, M., Basaria, S., Ceda, G. P., Ble, A., Ling, S. M., Bandinelli, S., et al. (2005). The relationship between testosterone and molecular markers of inflammation in older men. *J. Endocrinol. Invest.* 28(11 Suppl. Proceedings), 116–119.

Mangnus, L., Nieuwenhuis, W. P., van Steenbergen, H. W., Huizinga, T. W., Reijnierse, M., and van der Helm-van Mil, A. H. (2016). Body mass index and extent of MRI-detected inflammation: opposite effects in rheumatoid arthritis versus other arthritides and asymptomatic persons. *Arthritis. Res. Ther.* 18, 245. doi: 10.1186/s13075-016-1146-3

Martinon, F., Mayor, A., and Tschopp, J. (2009). The inflammasomes: guardians of the body. *Annu. Rev. Immunol.* 27, 229–265. doi: 10.1146/annurev.immunol.021908.132715

Mendelson, J. H., Goletiani, N., Sholar, M. B., Siegel, A. J., and Mello, N. K. (2008). Effects of smoking successive low- and high-nicotine cigarettes on hypothalamic-pituitary-adrenal axis hormones and mood in men. *Neuropsychopharmacology* 33, 749–760. doi: 10.1038/sj.npp.1301455

Messner, B., and Bernhard, D. (2014). Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. *Arterioscler. Thromb. Vasc. Biol.* 34, 509–515. doi: 10.1161/ATVBAHA.113.300156

Metti, A. L., Aizenstein, H., Yaffe, K., Boudreau, R. M., Newman, A., Launer, L., et al. (2015). Trajectories of peripheral interleukin-6, structure of the hippocampus, and cognitive impairment over 14 years in older adults. *Neurobiol. Aging* 36, 3038–3044. doi: 10.1016/j.neurobiolaging.2015.07.025

Miles, E. A., Rees, D., Banerjee, T., Cazzola, R., Lewis, S., Wood, R., et al. (2008). Age-related increases in circulating inflammatory markers in men are independent of BMI, blood pressure and blood lipid concentrations. *Atherosclerosis* 196, 298–305. doi: 10.1016/j.atherosclerosis.2006.11.002

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., et al. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J. Clin. Endocrinol. Metab.* 82, 4196–4200.

Morris, N. L., Ippolito, J. A., Curtis, B. J., Chen, M. M., Friedman, S. L., Hines, I. N., et al. (2015). Alcohol and inflammatory responses: summary of the 2013 Alcohol and Immunology Research Interest Group (AIRIG) meeting. *Alcohol* 49, 1–6. doi: 10.1016/j.alcohol.2014.07.018

O'Hara, R., Schröder, C. M., Mahadevan, R., Schatzberg, A. F., Lindley, S., Fox, S., et al. (2007). Serotonin transporter polymorphism, memory and hippocampal volume in the elderly: association and interaction with cortisol. *Mol. Psychiatry* 12, 544–555. doi: 10.1038/sj.mp.4001978

Otte, C., Hart, S., Neylan, T. C., Marmar, C. R., Yaffe, K., and Mohr, D. C. (2005). A meta-analysis of cortisol response to challenge in human aging: importance of gender. *Psychoneuroendocrinology* 30, 80–91. doi: 10.1016/j.psyneuen.2004.06.002

Ouanes, S., Castelao, E., Gebreab, S., von Gunten, A., Preisig, M., and Popp, J. (2017). Life events, salivary cortisol, and cognitive performance in nondemented subjects: a population-based study. *Neurobiol. Aging* 51, 1–8. doi: 10.1016/j.neurobiolaging.2016.11.014

Palladino, P., Cornoldi, C., De Beni, R., and Pazzaglia, F. (2001). Working memory and updating processes in reading comprehension. *Mem. Cognit.* 29, 344–354. doi: 10.3758/BF03194929

Patruno, A., Franceschelli, S., Pesce, M., Maccallini, C., Fantacuzzi, M., Speranza, L., et al. (2012). Novel aminobenzyl-acetamidine derivative modulate the differential regulation of NOSs in LPS induced inflammatory response: role of PI3K/Akt pathway. *Biochim. Biophys. Acta* 1820, 2095–2104. doi: 10.1016/j.bbagen.2012.08.014

Pesce, M., Ferrone, A., Rizzuto, A., Tatangelo, R., Iezzi, I., Ladu, S., et al. (2014). The SHP-1 expression is associated with cytokines and psychopathological status in unmedicated first episode schizophrenia patients. *Brain Behav. Immun.* 41, 251–260. doi: 10.1016/j.bbi.2014.04.008

Pesce, M., Speranza, L., Franceschelli, S., Ialenti, V., Iezzi, I., Patruno, A., et al. (2013). Positive correlation between serum interleukin-1β and state anger in rugby athletes. *Aggress. Behav.* 39, 141–148. doi: 10.1002/ab.21457

Reedy, S. D., Boone, K. B., Cottingham, M. E., Glaser, D. F., Lu, P. H., Victor, T. L., et al. (2013). Cross validation of the Lu and colleagues (2003) rey-osterrieth complex figure test effort equation in a large known-group sample. *Arch. Clin. Neuropsychol.* 28, 30–37. doi: 10.1093/arclin/acs106

Reitan, R. M. (1958). Validity of the trail making test as an indicator of organic brain damage. *Percept. Mot. Skills* 8, 271–276. doi: 10.2466/pms.1958.8.3.271

Rosano, C., Marsland, A. L., and Gianaros, P. J. (2012). Maintaining brain health by monitoring inflammatory processes: a mechanism to promote successful aging. *Aging Dis.* 3, 16–33.

Salminen, A., and Kaarniranta, K. (2011). Control of p53 and NF-κB signaling by WIP1 and MIF: role in cellular senescence and organismal aging. *Cell. Signal.* 23, 747–752. doi: 10.1016/j.cellsig.2010.10.012

Sher, K. J., Bartholow, B. D., Peuser, K., Erickson, D. J., and Wood, M. D. (2007). Stressresponse- dampening effects of alcohol: attention as a mediator and moderator. *J. Abnorm. Psychol.* 116, 362–377. doi: 10.1037/0021-843X.116.2.362

Shi, X., Sun, H., Zhou, D., Xi, H., and Shan, L. (2015). Arctigenin attenuates lipopolysaccharide-induced acute lung injury in rats. *Inflammation* 38, 623–631. doi: 10.1007/s10753-014-9969-z

Simpson, J., and Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats–behavioural and neurochemical aspects. *Behav. Brain Res.* 222, 246–264. doi: 10.1016/j.bbr.2011.04.002

Singhal, G., Jaehne, E. J., Corrigan, F., and Baune, B. T. (2014). Cellular and molecular mechanisms of immunomodulation in the brain through environmental enrichment. *Front. Cell. Neurosci.* 8:97. doi: 10.3389/fncel.2014.00097

Sox, H., and Liang, M. (1986). The erythrocyte sedimentation rate. *Ann. Intern. Med.* 104, 515–523. doi: 10.7326/0003-4819-104-4-515

Speranza, L., Franceschelli, S., Pesce, M., Menghini, L., Patruno, A., Vinciguerra, I., et al. (2009). Anti-inflammatory properties of the plant *Verbascum mallophorum*. *J. Biol. Regul. Homeost. Agents* 23, 189–195.

Speranza, L., Pesce, M., Franceschelli, S., Bucciarelli, T., Gallina, S., Riccioni, G., et al. (2013). The biological evaluation of ADMA/SDMA and eNOS in patients with ACHF. *Front. Biosci.* 5:551–557. doi: 10.2741/e637

Spinnler, H., and Tognoni, G. (1987). Italian group on the neuropsychological study of ageing: Italian standardization and classification of neuropsychological tests. *Ital. J. Neurol. Sci.* 6, 1–120.

Stienstra, R., van Diepen, J. A., Tack, C. J., Zaki, M. H., van de Veerdonk, F. L., Perera, D., et al. (2011). Inflammasome is a central player in the induction of obesity and insulin resistance. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15324–15329. doi: 10.1073/pnas.1100255108

Sudheimer, K. D., O'Hara, R., Spiegel, D., Powers, B., Kraemer, H. C., Neri, E., et al. (2014). Cortisol, cytokines, and hippocampal volume interactions in the elderly. *Front. Aging Neurosci.* 6:153. doi: 10.3389/fnagi.2014.00153

Sun, B., Hu, X., Liu, G., Ma, B., Xu, Y., Yang, T., et al. (2014). Phosphatase Wip1 negatively regulates neutrophil migration and inflammation. *J. Immunol.* 192, 1184–1195. doi: 10.4049/jimmunol.1300656

Sutliffe, J. T., Wilson, L. D., de Heer, H. D., Foster, R. L., and Carnot, M. J. (2015). C-reactive protein response to a vegan lifestyle intervention. *Complement. Ther. Med.* 23, 32–37. doi: 10.1016/j.ctim.2014.11.001

Swanson, L. W. (1991). Biochemical switching in hypothalamic circuits mediating responses to stress. *Prog. Brain Res.* 87, 181–200. doi: 10.1016/S0079-6123(08)63052-6

Tan, X., Zhang, J., Jin, W., Li, L., Xu, W., Zheng, H., et al. (2013). Wip1 phosphatase involved in lipopolysaccharide-induced neuroinflammation. *J. Mol. Neurosci.* 51, 959–966. doi: 10.1007/s12031-013-0080-y

Tatomir, A., Micu, C., and Crivii, C. (2014). The impact of stress and glucocorticoids on memory. *Clujul. Med.* 87, 3–6. doi: 10.15386/cjm.2014.8872.871.at1cm2

Travison, T. G., O'Donnell, A. B., Araujo, A. B., Matsumoto, A. M., and McKinlay, J. B. (2007). Cortisol levels and measures of body composition in middle-aged and older men. *Clin. Endocrinol. (Oxf)* 67, 71–77. doi: 10.1111/j.1365-2265.2007.02837.x

Vandanmagsar, B., Youm, Y. H., Ravussin, A., Galgani, J. E., Stadler, K., Mynatt, R. L., et al. (2011). The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat. Med.* 17, 179–188. doi: 10.1038/nm.2279

Vogel, S., Fernández, G., Joëls, M., and Schwabe, L. (2016). Cognitive adaptation under stress: a case for the mineralocorticoid receptor. *Trends Cogn. Sci.* 20, 192–203. doi: 10.1016/j.tics.2015.12.003

Wen, H., Gris, D., Lei, Y., Jha, S., Zhang, L., Huang, M. T., et al. (2011). Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat. Immunol.* 12, 408–415. doi: 10.1038/ni.2022

Willette, A. A., Bendlin, B. B., McLaren, D. G., Canu, E., Kastman, E. K., Kosmatka, K. J., et al. (2010). Age-related changes in neural volume and microstructure associated with interleukin-6 are ameliorated by a calorie-restricted diet in old rhesus monkeys. *Neuroimage* 51, 987–994. doi: 10.1016/j.neuroimage.2010.03.015

Wong, E. S., Le Guezennec, X., Demidov, O. N., Marshall, N. T., Wang, S. T., Krishnamurthy, J., et al. (2009). p38MAPK controls expression of multiple cell cycle inhibitors and islet proliferation with advancing age. *Dev. Cell* 17, 142–149. doi: 10.1016/j.devcel.2009.05.009

Yeager, M. P., Pioli, P. A., Collins, J., Barr, F., Metzler, S., Sites, B. D., et al. (2016). Glucocorticoids enhance the in vivo migratory response of human monocytes. *Brain Behav. Immun.* 54, 86–94. doi: 10.1016/j.bbi.2016.01.004

Yesavage, J. A., Brink, T. L., Rose, T. L., Lum, O., Huang, V., Adey, M., et al. (1982). Development and validation of a geriatric depression screening scale: a preliminary report. *J. Psychiatr. Res.* 17, 37–49. doi: 10.1016/0022-3956(82)90033-4

Yirmiya, R., and Goshen, I. (2011). Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav. Immun.* 25, 181–213. doi: 10.1016/j.bbi.2010.10.015

Youm, Y. H., Adijiang, A., Vandanmagsar, B., Burk, D., Ravussin, A., and Dixit, V. D. (2011). Elimination of the NLRP3-ASC inflammasome protects against chronic obesity-induced pancreatic damage. *Endocrinology* 152, 4039–4045. doi: 10.1210/en.2011-1326

Youm, Y. H., Grant, R. W., McCabe, L. R., Albarado, D. C., Nguyen, K. Y., Ravussin, A., et al. (2013). Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell. Metab.* 18, 519–532. doi: 10.1016/j.cmet.2013.09.010

Zhang, X. Y., Tang, W., Xiu, M. H., De Yang, F., Tan, Y. L., Wang, Z. R., et al. (2013). Interleukin 18 and cognitive impairment in first episode and drug naïve schizophrenia versus healthy controls. *Brain Behav. Immun.* 32, 105–111. doi: 10.1016/j.bbi.2013.03.001

Zhu, Y. H., Zhang, C. W., Lu, L., Demidov, O. N., Sun, L., Yang, L., et al. (2009). Wip1 regulates the generation of new neural cells in the adult olfactory bulb through p53-dependent cell cycle control. *Stem Cells* 27, 1433–1442. doi: 10.1002/stem.65

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer VB and handling Editor declared their shared affiliation, and the handling Editor states that the process nevertheless met the standards of a fair and objective review.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership