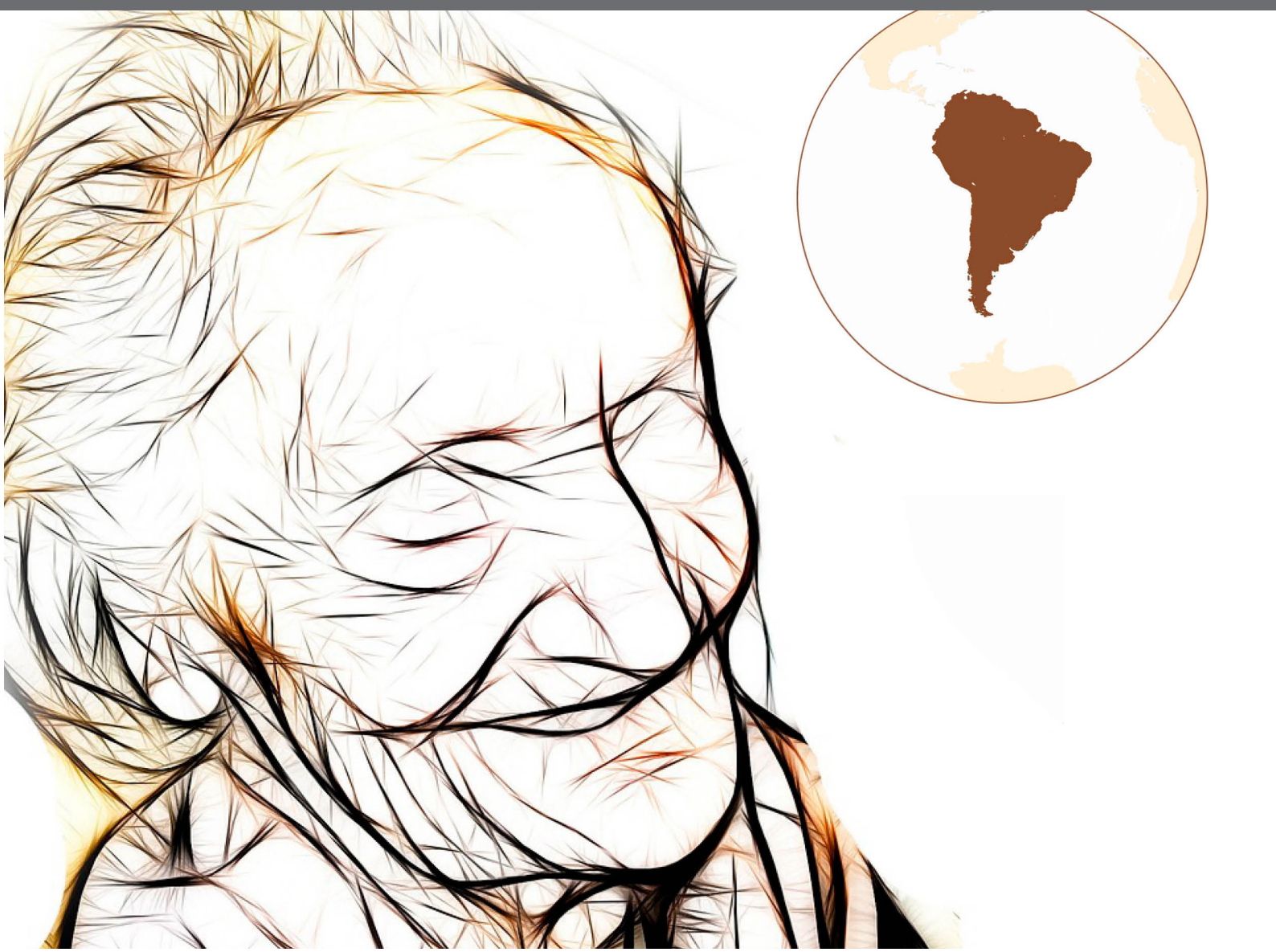
HUMAN AND ANIMAL MODELS FOR TRANSLATIONAL RESEARCH ON NEURODEGENERATION: CHALLENGES AND OPPORTUNITIES FROM SOUTH AMERICA

EDITED BY: Agustín Ibáñez, Lucas Sedeño, Adolfo M. García, Robert M.J. Deacon and Patricia Cogram

PUBLISHED IN: Frontiers in Aging Neuroscience



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88945-494-5
DOI 10.3389/978-2-88945-494-5

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

HUMAN AND ANIMAL MODELS FOR TRANSLATIONAL RESEARCH ON NEURODEGENERATION: CHALLENGES AND OPPORTUNITIES FROM SOUTH AMERICA

Topic Editors:
Agustín Ibáñez, Institute of Cognitive and Translational Neuroscience (INCYT), INECO Foundation, Favaloro University, Buenos Aires, Argentina; National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina; Universidad Autónoma del Caribe, Barranquilla, Colombia; Center for Social and Cognitive Neuroscience (CSCN), School of Psychology, Universidad Adolfo Ibáñez, Santiago, Chile, and Centre of Excellence in Cognition and its Disorders, Australian Research Council (ACR), Sydney, Australia
Lucas Sedeño, Institute of Cognitive and Translational Neuroscience (INCYT), INECO Foundation, Favaloro University, Buenos Aires, Argentina, and National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina
Adolfo M. García, Institute of Cognitive and Translational Neuroscience (INCYT), INECO Foundation, Favaloro University, Buenos Aires, Argentina; National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina, and Faculty of Education, National University of Cuyo (UNCuyo), Mendoza, Argentina
Robert M.J. Deacon, Institute of Cognitive and Translational Neuroscience (INCYT), INECO Foundation, Favaloro University, Buenos Aires, Argentina, and Institute of Ecology and Biodiversity, Faculty of Science, University of Chile, Santiago, Chile
Patricia Cogram, Institute of Cognitive and Translational Neuroscience (INCYT), INECO Foundation, Favaloro University, Buenos Aires, Argentina, and Institute of Ecology and Biodiversity, Faculty of Science, University of Chile, Santiago, Chile

In this cover artwork, our aim is to show the huge burden of dementia in South American countries. Figure is a modified version reproduced with authorization (Creative Commons Zero - CC0) from: https://goo.gl/cesmaa. The authors thank S. Abrevaya for helping with the figure edition.

Neurodegenerative diseases are the most frequent cause of dementia, representing a burden for public health systems (especially in middle and middle-high income countries). Although most research on this issue is concentrated in first-world centers, growing efforts in South America are affording important breakthroughs. This emerging agenda poses new challenges for the region but also new opportunities for the field.

This book aims to integrate the community of experts across the globe and the region, and to establish new challenges and developments for future investigation. We present research focused on neurodegenerative research in South America. We introduce studies assessing the interplay among genetic, neural, and behavioral dimensions of these diseases, as well as articles on vulnerability factors, comparisons of findings from various countries, and works promoting multicenter and collaborative networking. More generally, our book covers a broad scope of human-research approaches (behavioral assessment, neuroimaging, electromagnetic techniques, brain connectivity, peripheral measures), animal methodologies (genetics, epigenetics, proteomics, metabolomics, other molecular biology tools), species (all human and non-human animals, sporadic, and genetic versions), and article types (original research, review, and opinion papers). Through this wide-ranging proposal, we hope to introduce a fresh approach to the challenges and opportunities of research on neurodegeneration in South America.

Citation: Ibáñez, A., Sedeño, L., García, A. M., Deacon, R. M.J., Cogram, P., eds. (2018). Human and Animal Models for Translational Research on Neurodegeneration: Challenges and Opportunities From South America. Lausanne: Frontiers Media.
doi: 10.3389/978-2-88945-494-5

Table of Contents

3.2. PATHOPHYSIOLOGICAL MODELS OF DIFFERENT NEURODEGENERATIVE CONDITIONS

3.3. PROTEOSTASIS NETWORK: FROM ANIMAL TO HUMAN APPROACHES

3.4. COGNITIVE INTERVENTIONS AS AN EARLY NON-PHARMACOLOGICAL STRATEGY: FROM ANIMAL RESEARCH TO HUMAN TRANSLATION

frontiers in Aging Neuroscience

EDITORIAL
published: 06 April 2018
doi: 10.3389/fnagi.2018.00095

Editorial: Human and Animal Models for Translational Research on Neurodegeneration: Challenges and Opportunities From South America

Agustín Ibáñez [1,2,3,4,5], Lucas Sedeño [1,2], Adolfo M. García [1,2,6], Robert M. J. Deacon [1,7] and Patricia Cogram [1,7]*

[1] Laboratory of Experimental Psychology and Neuroscience (LPEN), Institute of Cognitive and Translational Neuroscience (INCYT), INECO Foundation, Favaloro University, Buenos Aires, Argentina, [2] National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina, [3] Universidad Autónoma del Caribe, Barranquilla, Colombia, [4] Center for Social and Cognitive Neuroscience (CSCN), School of Psychology, Universidad Adolfo Ibáñez, Santiago, Chile, [5] Centre of Excellence in Cognition and its Disorders, Australian Research Council (ACR), Sydney, NSW, Australia, [6] Faculty of Education, National University of Cuyo (UNCuyo), Mendoza, Argentina, [7] Faculty of Science, Institute of Ecology and Biodiversity, University of Chile, Santiago, Chile

Keywords: neurodegenerative diseases, South America, research, clinical protocols, neurosciences, public health, animals, human experimentation

Editorial on the Research Topic

Human and animal models for translational research on neurodegeneration: challenges and opportunities from South America

OPEN ACCESS

Edited by:
David Ronald Brown, University of Bath, United Kingdom

Reviewed by:
Boon-Seng Wong, Singapore Institute of Technology, Singapore

****Correspondence:***
Agustín Ibáñez
aibanez@ineco.org.ar

Received: *01 December 2017*
Accepted: *20 March 2018*
Published: *06 April 2018*

Citation:
Ibáñez A, Sedeño L, García AM, Deacon RMJ and Cogram P (2018) Editorial: Human and Animal Models for Translational Research on Neurodegeneration: Challenges and Opportunities From South America. Front. Aging Neurosci. 10:95. doi: 10.3389/fnagi.2018.00095

NEURODEGENERATION: A VOICE FROM THE SOUTH

Facing the alarming growth of dementia and neurodegenerative conditions has become a critical priority across the globe (Alzheimer's Disease International, 2009; Lancet, 2015; Shah et al., 2016; Parra et al., 2018). Neurodegenerative diseases are the most frequent cause of dementia, representing a burden for public health systems (especially in middle and middle-high income countries). Although most research on this subject is concentrated in first-world centers, growing efforts in South American countries (SACs) are affording important breakthroughs. This emerging agenda poses not only new challenges for the region, but also new opportunities for the field at large. SACs have witnessed a promising development of relevant research in humans and animals, giving rise to new regional challenges. As highlighted in a recent experts' consensus paper Latin-American countries (LAC), and SACs in particular (Parra et al., 2018), face a critical situation. Higher demographic rates and the predicted prevalence of dementia have reached and even exceeded those of developing countries. In SACs, low- and middle-income countries (e.g., Bolivia, Paraguay), the prevalence of dementia will double that of high-income countries, while upper-middle-income countries in the region (e.g., Argentina, Brazil, Chile, Colombia, Peru, Uruguay, and Venezuela) will experience the greatest impact of dementia. The WHO estimated that the standardized prevalence of dementia in Latin America was 8.5%, but multiple SACs have been underrepresented or underestimated in such a calculation (Parra et al., 2018). Moreover, raw prevalence rates across studies are characterized by high variability within and between countries (e.g., Argentina: 8.3; Brazil: 7.1-2.0; Chile: 4.4-7.0; Colombia: 6.0; Peru: 6.72-9.3; Uruguay: 3.1; Venezuela: 5.7-13,7) (Parra et al., 2018). In addition, most of these studies are undermined by various limitations and methodological problems. Even considering these data, SACs possess the

highest global prevalence of dementia after North Africa/Middle East in people above the age of 60 (Parra et al., 2018). Moreover, the harmonization of global strategies against dementia in these contexts is hindered not only by reduced epidemiological data, but also by the lack of standardized clinical practice, insufficient training of physicians, limited resources, and poor governmental support, let alone poverty and more general cultural barriers and stigmas. All of these factors have impacted the type and amount of research conducted in SACs. A regional network, based on multi-institutional actors from research, governmental, and private sectors is fundamental to overcome these challenges (Parra et al., 2018).

Nevertheless, until now most research groups still work in isolation or in sporadic collaboration, without developing large-scale multicenter studies or active cooperation networks. The field could grow exponentially by combining the strengths of regional research with higher visibility, a translational philosophy, and enhanced global networking. Importantly, collaborative developments may promote the establishment of translational centers studying neurodegeneration. This Research Topic engages researchers from the world over, helping to integrate the international community of experts and to establish new challenges and developments for future investigation. We present original research in SACs, including studies assessing the interplay among genetic, neural, and behavioral dimensions of these diseases, as well as articles on vulnerability factors, comparisons of findings from various countries, and works promoting multicenter and collaborative networking. More generally, our Research Topic covers a broad scope of human research approaches (behavioral assessment, neuroimaging, electromagnetic techniques, brain connectivity, peripheral measures), animal methodologies (genetics, epigenetics, proteomics, metabolomics, other molecular biology tools), target species (human and non-human animals, sporadic, and genetic versions), and article types (mainly original research articles, but also case reports, data reports, commentaries, opinions, and reviews), all based on work conducted in SACs. Thus, in capturing the breakthroughs, possibilities, and limitations of such a promising niche, the present Research Topic (titled *Human and animal models for translational research on neurodegeneration: challenges and opportunities from South America*) represents a valuable forum to initialize a constructive dialogue and reflect on the present and future of neurodegenerative research in the region. Here, we summarize the main contributions included in the volume. Through this wide-ranging proposal, we hope to introduce a fresh approach to the challenges and opportunities of research on neurodegeneration across these countries, focusing on two overarching levels of evidence (human and animal research), as summarized below.

STUDIES ON HUMANS

Concerning human research, SACs offer invaluable possibilities to pursue neurogenetic studies and clinical trials. This region possesses the world's largest population of familial Alzheimer's and Huntington's disease (AD and HD, respectively), among others, alongside multiple novel and rare functional genomic variants of other disorders. Moreover, poor socioeconomic conditions in several communities provide a natural scenario to study the role of vulnerability, resilience, and genetic-cultural interaction on disease progression. These opportunities are already being exploited by consolidated research groups in Argentina, Chile, Colombia, Peru, and Brazil, among others, via cutting-edge approaches which include connectomics, omics-biomarkers, and neuropsychological assessment. Moreover, the emerging cognitive neuroscience of neurodegeneration in the region (Parra et al., 2010, 2015; Ibanez and Manes, 2012; Baez et al., 2013, 2014a,b, 2015, 2016a,b, 2017; García-Cordero et al., 2015, 2016; Melloni et al., 2015, 2016; Pietto et al., 2016; Santamaría-García et al., 2016, 2017; Sedeño et al., 2016, 2017; Abrevaya et al., 2017; Birba et al., 2017; Calvo et al., 2017; Dottori et al., 2017; Garcia et al., 2017a; García et al., 2017b,c; Ibáñez et al., 2017; García and Ibáñez, 2018; Kumfor et al., 2018) has provided manifold pathways of synergy with multimethodological approaches to genetic, clinic, neuropsychological, and neuroscientific data.

The research on human neurodegeneration presented in this collection includes works on: (a) global challenges to dementia, from diagnosis to public health; (b) different dimensions of assessment (low socio-educational levels, cultural and competence-related variability, and robust evaluations to face heterogeneous contexts); (c) neurodegeneration discrimination and disease progression (through combinations of behavioral measures, neuroscientific approaches, and biomarkers for improving differential diagnosis between dementia subtypes), the characterization of specific initial alterations in each disease, and the identification of factors that manifest in early aging; and (d) the impact of non-pharmacological interventions for dementia using non-invasive brain stimulation.

Global Challenges and Public Health

A first group of studies assesses diverse aspects of the global challenges related to dementia, from diagnosis to public health at a regional level. After reviewing critical sociodemographic and epidemiological data form LACs, Baez and Ibanez propose to evaluate the plausibility of international expert recommendations regarding dementias in these countries. Key issues of this evaluation include diagnosis, demographic specificities of LACs, lack of social awareness of these diseases, deficiencies in the health system, the need for standardizing diagnostic practices, and the existing barriers in terms of resources and cultural factors. Similarly, Custodio et al. outline a challenging picture of epidemiological data in LACs, evidencing the major impact of unprecedented demographic changes and projections of dementia for people between 65 and 69 years old. The situation is worst for low-income people whose families cover the majority of the cost related to the disease. This is even worse for illiterate people, where the majority of the costs are covered by families. Accordingly, the authors propose a critical assessment of regional differences and similarities for the implementation of long-term care policies and plans. For their own part, Cardona-Gómez and Lopera assess the intertwine of novel animal and human translational research on molecular targets and pre/clinical

studies. Then they discuss on cases of pure and mixed dementias in the region, and, finally, they recommend the implementation of a protocol clarification policy for developing clinical trials and local intervention strategies.

Multiple and Multimodal Assessment

Another set of articles focuses on various dimensions of assessment, including the effects of low socioeconomic status and educational level, the role of clinical competence, and the relevance of trans-culturally valid tasks. The low detection of dementia is a major problem in SACs, accentuated by the lack of validated and standardized tools. In a cross-sectional study, Custodio et al. evaluate the robustness of the memory alteration test (MAT) in low-educated patients with mild cognitive impairment (MCI) and AD by using validity measures (sensitivity, specificity, and correctly classified percentage), internal consistency (Cronbach's alpha coefficient), and concurrent validity (Pearson's ratio coefficient between the MAT and Clinical Dementia Rating scores). All measures, they conclude, provide robust and adequate classification scores.

Some instruments, like the working memory binding (WMB) task (Parra et al., 2009, 2010), seem to have high sensitivity and specificity to detect AD in early stages. It has been suggested that cultural aspects such as education, age, and memory abilities may impact in relatively culture-free tasks (Parra et al., 2018). Building on this line of research, Hoefeijzers et al. provide evidence of a new WMB task composed by everyday items that is not affected by education. This result suggests that the WMB test may be culturally and educationally unbiased for the screening of abnormal aging trajectories.

Neurodegenerative Diseases: Progression and Differentiation

A third set of works integrates behavioral and neuroscientific insights (including biomarker research) to examine differential diagnosis across dementia subtypes, the characterization of specific initial alterations for each disease, and the identification of factors that impact early aging.

Russo et al. investigate whether memory recognition and deferred recall measures of episodic memory, in combination with cerebrospinal fluid (CSF) biomarkers, can predict the conversion from MCI (397 amnestic-MCI patients of the Alzheimer's disease Neuroimaging Initiative, ADNI) to AD (at 24 months of follow-up). Predictive models of memory, together with risk factors (age, sex, education, APOE genotype and CSF biomarkers), evidence that memory measures alongside amyloid biomarkers can predict the conversion of MCI to AD in the ADNI cohort, especially when combined with amyloid biomarkers. This highlights the relevance of a multimodal approach to anticipate the MCI progression to dementia.

Guevara et al. introduce an ante-mortem method to differentiate progressive supranuclear palsy (PSP) from idiopathic Parkinson's disease (IPD) at early stages. To this end, the authors combine normalized measures of brain atrophy with clinical metrics (Unified Parkinson's Disease Rating Scale Part III, Hoehn and Yahr, Clinical Global Impression for Disease Severity Scale, and the Frontal Assessment Battery). Their results show that whole-brain and gray matter volumes distinguished PSP from IPD, and that clinical-imaging correlations were indicative of clinical presentation and differentiation.

Campêlo et al. investigate the relationship among single nucleotide polymorphisms of alpha-synuclein gene (SNCA) and risk for PD in a Brazilian sample, considering potential interactions with environmental factors and specific clinical outcomes (cognitive, motor, and mood impairments). Their findings confirm the association between SNCA and PD risk (and early onset PD). Specific SNCA alleles were significantly more frequent in PD patients with cognitive impairment, and negative association with protective factors (cognitive activity and smoking habits). This study constitutes the first description of SNCA polymorphism and PD in a South-American sample.

The relation among familial antecedents of late-onset AD (LOAD), cognitive impairment, and sleep patterns in asymptomatic subjects was investigated by Abulafia et al. Middle-aged children of patients with LOAD (O-LOAD), in comparison with controls, displayed deficits in episodic memory and language. Moreover, the former group showed a phase-delayed rhythm of body temperature. Also, cognitive performance in these subjects was associated with cardiac autonomic sleep-wake variables (greater sympathetic activity at night was related to worse cognitive performance).

Long-lasting neurofunctional influences during childhood can impact pathological aging. Iron deficiency anemia (IDA) is a marker of iron micronutrient deficit affecting myelination, dopamine neurotransmission, and neuronal metabolism. Algarin et al. explore the connection between IDA in infancy and altered connectivity patterns of the default-mode network (DMN, an aging-sensitive resting-state network) in young adults. Compared to controls, participants with IDA evidenced atypical DMN connectivity. These preliminary findings suggest that a common nutritional problem among human infants may be important for understanding aberrant aging mechanisms.

Senescence has been associated with metabolic changes including mitochondrial fission and fusion events. Stab et al. assess the cell senescence and structural remodeling of mesenchymal stromal/stem cells (from human mitochondrial tissue) isolated from adipose tissue *in vitro*. Cell morphology aging was associated with an increase in β-galactosidase activity. Old cells showed increased mitochondrial mass, augmented superoxide production, and decreased mitochondrial membrane potential. Morphological changes were related to increases in mitochondrial fusion proteins, Mitofusion 1, and Dynamin-related GTPase. Thus, aged, adipose tissue-derived mitochondrial stem cells developed a senescent phenotype.

Non-invasive Brain Stimulation as Non-pharmacological Interventions

A fourth set of studies evaluate the impact of non-pharmacological interventions for dementia. Non-invasive brain stimulation (NIBS) methods can induce plastic changes in the brain and modulate cognitive functions in humans. Thus, their potential use for early stages of dementia has become a promissory strategy.

Birba et al. conducted a systematic review of the effects of NIBS on MCI and subjective cognitive impairment (SCI) in preventing or delaying the development of AD. In particular, they discuss the impact of NIBS on specific target functions, including recognition of verbal and non-verbal stimuli, attention, psychomotor speed, and everyday memory. Moreover, they identify a number of methodological issues (differences among tasks, designs, and samples size) that arguably underlie the mixed results obtained so far. They also outline further methodological approaches to boost the efficacy and specificity of NIBS in MCI and SCI. These issues are critical for developing robust treatments for both conditions.

Finally, another report by Birba et al. provides the first evidence that direct electrical brain stimulation can enhance performance in the working memory binding (WMB) task, a sensitive tool for early AD. WMB deficits constitute a robust clinical and preclinical marker of AD, associated with early atrophy of posterior brain regions. Profiting from a unique approach, the authors show that direct intracranial electrical stimulation of the parietal cortex can induce a selective improvement in WMB performance. These preliminary but promising results promote new opportunities to improve binding functions in preclinical AD through brain stimulation.

STUDIES ON ANIMALS

The region also constitutes a rich platform for developments via animal research. Preclinical testing of new therapeutic concepts has been difficult due to the lack of naturally occurring disease models. Although availability of genetically engineered mouse models has partly addressed this challenge, neurodegenerative diseases rarely occur in non-human animals (Jucker, 2010); and the causes of non-familial dementia are multifactorial and age-related (Hurley et al., 2018). In consequence, non-genetic and natural models of dementia are still required. Advances could be made by studying species such as the *Octodon degus*, an endemic rodent from Chile that spontaneously develops an analog of dementia at behavioral and neurobiological levels (Ardiles et al., 2012; Hurley et al., 2018). In addition, the *O. degus* can also naturally develop several other conditions like diabetes mellitus type 2, macular and retinal degeneration and atherosclerosis, conditions that are often associated with aging and comorbid disorders with dementia (Hurley et al., 2018). Consequently, the *O. degus* is a suitable novel experimental model that can be utilized for the development of disease-modifying treatments for dementia. In addition, scientific efforts in the region have been enhanced through new models from promising groups (e.g., single and double immunohistochemistry, inmunoblot, RT-PCR, behavioral phenotyping, CNS lesioning techniques, neuronal tracers for connectivity studies, small craniotomy for drug delivery). These approaches are now fueled by the engagement of international centers and the development of multicenter alliances.

The section on animal research provides timely works on regionally relevant topics. These include (a) natural models of AD; (b) pathophysiological models of different neurodegenerative conditions, such as frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), and AD; (c) the role of the proteostasis network in basic organisms; and (d) cognitive interventions as early non-pharmacological therapeutics.

Natural Rodent Models of Neurodegeneration

In a *Data Report Article*, Altimiras et al. offer the first characterization of the *O. degus*'s brain transcriptome (in order to support its use as a natural model of AD), together with a comparison between the transcriptomes of AD-like and healthy specimens. Of note, this work includes an unprecedented report of whole transcriptome sequencing (RNA-seq) of the *Octogon degus* brain. Results reveal differences in novel and previously reported genes for AD and related disorders (CHRNA6, AMD1, WISP1, COX8A, APOC-I, among others). In addition, the comparison of human and *O. degus* AD-like brain transcriptomes evidences multiple common genes in both species. These findings highlight the relevance of this rodent species to foster progress in AD research.

Braidy et al. use the *O. degus* model to evaluate the biometal imaging and role of metal uptake transporters in AD pathogenesis and aging. Their work hinges on the hypothesis that *O. degus* may develop neuropathological abnormalities in the distribution of redox active biometals, due to alterations in the expression of lysosomal protein, major Fe/Cu transporters, and selected Zn transporters (ZnTs and ZIPs). Using laser ablation inductively coupled plasma mass spectrometry, they find elevated quantitative images of biometals (Fe, Ca, Zn, Cu, and Al) in the aged *O. degus*, which in turn showed an age-dependent rise. Some of these metals were specifically enriched in the cortex and hippocampus. Whole-brain extracts evidenced age-related deregulation of metal trafficking pathways (impaired lysosomal function, demonstrated by increased cathepsin D protein expression). An age-related reduction in the expression of subunit B2 of V-ATPase was also identified, alongside significant increases in amyloid beta peptide 42 (Aβ42), and metal transporter ATP13a2. Finally, enhanced expression of transporter of divalent metal species, 5′-aminolevulinate synthase 2 (ALAS2), and the proto-oncogene, FOS was associated with aging. Thus, these results suggest that transition metals in the brain may be enriched with age in the *O. degus*, and that metal dyshomeostasis in specific brain regions may be related to age.

Pathophysiological Models of Different Neurodegenerative Conditions

Two additional studies provide pathophysiological models of FTD, ALS, and AD. FTD and ALS are both associated with TAR DNA-binding protein 43 (TDP-43). To investigate the behavioral phenotype associated with this proteinopathy, Alfieri et al. implemented a transgenic (Tg) mouse model that conditionally overexpresses human wild-type TDP 43 protein (hTDP-43-WT) in forebrain neurons. They analyzed the motor, social, and cognitive performance of this species. The young hTDP-43-WT Tg mice presented a mild degree of spasticity. Analysis of social and cognitive behavior showed a rapid installment of deficits in

social interaction, working memory, and recognition memory. After long-term (up to 12 months) transgene induction, a motor phenotype (previously absent in younger mice) was identified. Thus, this work points to the time-dependent emergence of a motor phenotype, a clinical presentation of FTD with involving motor deficits, and a complementary animal model for studying TDP-43 proteinopathies.

The effect of chronic treatment with reserpine (an inhibitor of vesicular monoamine transporter-2), which induces dyskinesia in PD, has been proposed to be attenuated in spontaneously hypertensive rats (SHRs). Leão et al. evaluated whether SHRs (in comparison with Wistar rats) present differential susceptibility to repeated reserpine-induced deficits in a progressive model of PD. Only reserpine-treated Wistar rats presented increased motor signs. After a withdrawal period, both strains recovered from motor impairment, but SHRs were slower to reach control levels. Immunohistochemistry for tyrosine hydroxylase (TH) and α-synuclein (α-syn) after the last injection or 15 days after withdrawal showed a reduction in TH and an increase in α-syn immunoreactivity in the substantia nigra and dorsal striatum (recovered after 15 days of withdrawal). The SHRs were resistant to reserpine-induced TH decrement in the substantia nigra, and presented reduced immunoreactivity to α-syn in the dorsal striatum relative to Wistar rats, irrespective of treatment. In brief, SHRs may be resilient to motor and neurochemical impairments induced by the repeated low-dose reserpine.

Accumulation of β in AD begins many years before clinical onset. Yet, given that massive accumulation of Aβ appears in 30% of healthy aged individuals, compensatory mechanisms and/or additional neurotoxic or protective factors need to be discovered. Belfiori-Carrasco et al. provide a novel genetic screen in the drosophila brain that identifies modifiers of age-dependent amyloid β toxicity. One hundred and ninety-nine deficiency lines accounting for ∼6,300 genes were analyzed. Six lines significantly modified Aβ42 neurotoxicity, including the CG11796 and CG17249 (orthologs to human HPD and PRCC, respectively) as candidates. These modifiers of Aβ42 neurotoxicity in Drosophila open avenues for new validation studies into their possible role in sporadic AD.

Proteostasis Network: From Animal to Human Approaches

Abnormal protein aggregation is a transversal pathological mechanism in neurodegeneration. Thus, the capacity of neurons to handle alterations in the proteome seems to be specifically altered in aging. Martínez et al. critically asses the proteostasis network in basic organisms, highlighting the challenges for moving toward human research in this domain. Although several reports are pointing to this network as a relevant adjustor of organismal aging in several species, its relevance to human aging remains unknown. The authors discuss multiples challenges (regarding buffering capacity, neural control of organismal proteostasis, connections among stress and aging in protein misfolding disorders, control the cell-nonautonomous UPR as a therapeutic strategy) in the light of new drug-based avenues to intervene in brain aging.

Cognitive Interventions as an Early Non-pharmacological Strategy: From Animal Research to Human Translation

To conclude, Gehres et al. set forth a challenging opinion on cognitive interventions as an early non-pharmacological strategy in AD, considering a translational perspective from animal research to human behavior. The critical concepts of cognitive reserve, cognitive interventions, and early-life exposure to environmental enrichment (EE) are reviewed from the vantage point of animal research, with new vistas for neurodegenerative human conditions. After an informative revision, the authors conclude that study designs that aim to unravel EE-specific mechanisms are crucial and could guide the generation of non-pharmacological strategies. Moreover, the combination of EE with better models of sporadic AD, in conjunction with CSF/PET biomarkers, could promote novel insights on novel therapeutic targets for AD.

CONCLUSIONS

Amid a multiple collection of theories, experimental approaches, and models, all these studies highlight both the rise of world-class research in SACs, as well as the specificity of problems and opportunities in the region. Moreover, they provide relevant evidence for the harmonization of multilevel approaches to neurodegenerative research. Overall, this integrated and pluralistic approach to neurodegeneration in SACs can provide the basic building blocks for a future translational network based on (but not limited to) experimental research, focusing on policy changes and the development of international collaborations (Parra et al., 2018). In sum, this book demonstrates that SACs are highly active in generating first-class translational and multicenter research on neurodegeration, thus amplifying the powerful voice of the South in this worldwide program.

AUTHOR CONTRIBUTIONS

AI: designed the proposal. AI, LS, and AG: wrote the first draft, discussed contributions from all co-authors, and approved the final version. All authors (AI, LS, AG, RD, and PC) searched the literature, participated in discussing the contents of the paper, contributed to editing and approved the final version of the article.

ACKNOWLEDGMENTS

This work was supported by CONICET, CONICYT/FONDECYT (Regular 1170010), FONDAP (15150012), the INECO Foundation, and the Interamerican Development Bank (IDB).

REFERENCES

Abrevaya, S., Sedeño, L., Fitipaldi, S., Pineda, D., Lopera, F., Buritica, O., et al. (2017). The road less traveled: alternative pathways for action-verb processing in Parkinson's disease. *J. Alzheimers Dis.* 55, 1429–1435. doi: 10.3233/JAD-160737

Alzheimer's Disease International (2009). *World Alzheimer Report: Executive Summary*. World Alzheimer Report: Executive Summary. Alzheimer's Disease International.

Ardiles, A. O., Tapia-Rojas, C. C., Mandal, M., Alexandre, F., Kirkwood, A., Inestrosa, N. C., et al. (2012). Postsynaptic dysfunction is associated with spatial and object recognition memory loss in a natural model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 109, 13835–13840. doi: 10.1073/pnas.1201209109

Baez, S., Couto, B., Herrera, E., Bocanegra, Y., Trujillo-Orrego, N., Madrigal-Zapata, L., et al. (2013). Tracking the cognitive, social, and neuroanatomical profile in early neurodegeneration: type III cockayne syndrome. *Front. Aging Neurosci.* 5:80. doi: 10.3389/fnagi.2013.00080

Baez, S., Couto, B., Torralva, T., Sposato, L. A., Huepe, D., Montañes, P., et al. (2014a). Comparing moral judgments of patients with frontotemporal dementia and frontal stroke. *JAMA Neurol.* 71, 1172–1176. doi: 10.1001/jamaneurol.2014.347

Baez, S., Herrera, E., Gershanik, O., Garcia, A. M., Bocanegra, Y., Kargieman, L., et al. (2015). Impairments in negative emotion recognition and empathy for pain in Huntington's disease families. *Neuropsychologia* 68, 158–167. doi: 10.1016/j.neuropsychologia.2015.01.012

Baez, S., Kanske, P., Matallana, D., Montañes, P., Reyes, P., Slachevsky, A., et al. (2016a). Integration of Intention and Outcome for Moral Judgment in Frontotemporal Dementia: Brain Structural Signatures. *Neurodegener. Dis.* 16, 206–217. doi: 10.1159/000441918

Baez, S., Manes, F., Huepe, D., Torralva, T., Fiorentino, N., Richter, F., et al. (2014b). Primary empathy deficits in frontotemporal dementia. *Front. Aging Neurosci.* 6:262. doi: 10.3389/fnagi.2014.00262

Baez, S., Morales, J. P., Slachevsky, A., Torralva, T., Matus, C., Manes, F., et al. (2016b). Orbitofrontal and limbic signatures of empathic concern and intentional harm in the behavioral variant frontotemporal dementia. *Cortex* 75, 20–32. doi: 10.1016/j.cortex.2015.11.007

Baez, S., Pino, M., Berrío, M., Santamaría-García, H., Sedeño, L., García, A. M., et al. (2017). Corticostriatal signatures of schadenfreude: evidence from Huntington's disease. *J. Neurol. Neurosurg. Psychiatry* 89, 112–116. doi: 10.1136/jnnp-2017-316055

Birba, A., García-Cordero, I., Kozono, G., Legaz, A., Ibáñez, A., Sedeño, L., et al. (2017). Losing ground: frontostriatal atrophy disrupts language embodiment in Parkinson's and Huntington's disease. *Neurosci. Biobehav. Rev.* 80, 673–687. doi: 10.1016/j.neubiorev.2017.07.011

Calvo, N., Ibáñez, A., Muñoz, E., and García, A. M. (2017). A core avenue for transcultural research on dementia: on the cross-linguistic generalization of language-related effects in Alzheimer's disease and Parkinson's disease. *Int. J. Geriatr. Psychiatry*. doi: 10.1002/gps.4712. [Epub ahead of print].

Dottori, M., Sedeño, L., Martorell Caro, M., Alifano, F., Hesse, E., Mikulan, E., et al. (2017). Towards affordable biomarkers of frontotemporal dementia: a classification study via network's information sharing. *Sci. Rep.* 7:3822. doi: 10.1038/s41598-017-04204-8

Garcia, A. M., Abrevaya, S., Kozono, G., Cordero, I. G., Cordoba, M., Kauffman, M. A., et al. (2017a). The cerebellum and embodied semantics: evidence from a case of genetic ataxia due to STUB1 mutations. *J. Med. Genet.* 54, 114–124. doi: 10.1136/jmedgenet-2016-104148

García, A. M., Bocanegra, Y., Herrera, E., Moreno, L., Carmona, J., Baena, A., et al. (2017b). Parkinson's disease compromises the appraisal of action meanings evoked by naturalistic texts. *Cortex* 100, 111–126. doi: 10.1016/j.cortex.2017.07.003

García, A. M., and Ibáñez, A. (2018). When embodiment breaks down: language deficits as novel avenues into movement disorders. *Cortex* 100, 1–7. doi: 10.1016/j.cortex.2017.12.022

García, A. M., Sedeño, L., Trujillo, N., Bocanegra, Y., Gomez, D., Pineda, D., et al. (2017c). Language deficits as a preclinical window into Parkinson's disease: evidence from asymptomatic parkin and dardarin mutation carriers. *J. Int. Neuropsychol. Soc.* 23, 150–158. doi: 10.1017/S1355617716000710

García-Cordero, I., Sedeño, L., De La Fuente, L., Slachevsky, A., Forno, G., Klein, F., et al. (2016). Feeling, learning from, and being aware of inner states: interoceptive dimensions in neurodegeneration and stroke. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 371:20160006. doi: 10.1098/rstb.2016.0006

García-Cordero, I., Sedeño, L., Fraiman, D., Craiem, D., De La Fuente, L. A., Salamone, P., et al. (2015). Stroke and neurodegeneration induce different connectivity aberrations in the insula. *Stroke* 46, 2673–2677. doi: 10.1161/STROKEAHA.115.009598

Hurley, M. J., Deacon, R. M. J., Beyer, K., Ioannou, E., Ibáñez, A., Teeling, J. L., et al. (2018). The long-lived *Octodon degus* as a rodent drug discovery model for Alzheimer's and other age-related diseases. *Pharmacol. Ther.* doi: 10.1016/j.pharmthera.2018.03.001. [Epub ahead of print].

Ibáñez, A., Billeke, P., De La Fuente, L., Salamone, P., Garcia, A. M., and Melloni, M. (2017). Reply: towards a neurocomputational account of social dysfunction in neurodegenerative disease. *Brain* 140:e15. doi: 10.1093/brain/aww316

Ibanez, A., and Manes, F. (2012). Contextual social cognition and the behavioral variant of frontotemporal dementia. *Neurology* 78, 1354–1362. doi: 10.1212/WNL.0b013e3182518375

Jucker, M. (2010). The benefits and limitations of animal models for translational research in neurodegenerative diseases. *Nat. Med.* 16, 1210–1214. doi: 10.1038/nm.2224

Kumfor, F., Ibañez, A., Hutchings, R., Hazelton, J. L., Hodges, J. R., and Piguet, O. (2018). Beyond the face: how context modulates emotion processing in frontotemporal dementia subtypes. *Brain* 141, 1172–1185. doi: 10.1093/brain/awy002

Lancet, N. (2015). A neurology revival in Latin America. *Lancet Neurol.* 14:1143. doi: 10.1016/S1474-4422(15)00307-5

Melloni, M., Billeke, P., Baez, S., Hesse, E., De La Fuente, L., Forno, G., et al. (2016). Your perspective and my benefit: multiple lesion models of self-other integration strategies during social bargaining. *Brain* 139, 3022–3040. doi: 10.1093/brain/aww231

Melloni, M., Sedeño , L., Hesse, E., García-Cordero, I., Mikulan, E., Plastino, A., et al. (2015). Cortical dynamics and subcortical signatures of motor-language coupling in Parkinson's disease. *Sci. Rep.* 5:11899. doi: 10.1038/srep11899

Parra, M. A., Abrahams, S., Fabi, K., Logie, R., Luzzi, S., and Della Sala, S. (2009). Short-term memory binding deficits in Alzheimer's disease. *Brain* 132, 1057–1066. doi: 10.1093/brain/awp036

Parra, M. A., Abrahams, S., Logie, R. H., Méndez, L. G., Lopera, F., and Della Sala, S. (2010). Visual short-term memory binding deficits in familial Alzheimer's disease. *Brain* 133, 2702–2713. doi: 10.1093/brain/awq148

Parra, M. A., Baez, S., Allegri, R., Nitrini, R., Lopera, F., Slachevsky, A., et al. (2018). Dementia in Latin America: assessing the present and envisioning the future. *Neurology* 90, 222–231. doi: 10.1212/WNL.0000000000004897

Parra, M. A., Saarimäki, H., Bastin, M. E., Londoño, A. C., Pettit, L., Lopera, F., et al. (2015). Memory binding and white matter integrity in familial Alzheimer's disease. *Brain* 138, 1355–1369. doi: 10.1093/brain/awv048

Pietto, M., Parra, M. A., Trujillo, N., Flores, F., García, A. M., Bustin, J., et al. (2016). Behavioral and electrophysiological correlates of memory binding deficits in patients at different risk levels for Alzheimer's disease. *J. Alzheimers Dis.* 53, 1325–1340. doi: 10.3233/JAD-160056

Santamaría-García, H., Baez, S., Reyes, P., Santamaria-Garcia, J. A., Santacruz-Escudero, J. M., Matallana, D., et al. (2017). A lesion model of envy and Schadenfreude: legal, deservingness and moral dimensions as revealed by neurodegeneration. *Brain* 140, 3357–3377. doi: 10.1093/brain/awx269

Santamaría-García, H., Reyes, P., Garcia, A., Baez, S., Martinez, A., Santacruz, J. M., et al. (2016). First symptoms and neurocognitive correlates of behavioral variant frontotemporal dementia. *J. Alzheimers Dis.* 54, 957–970. doi: 10.3233/JAD-160501

Sedeño, L., Couto, B., García-Cordero, I., Melloni, M., Baez, S., Morales Sepúlveda, J. P., et al. (2016). Brain network organization and social executive performance in frontotemporal dementia. *J. Int. Neuropsychol. Soc.* 22, 250–262. doi: 10.1017/S1355617715000703

Sedeño, L., Piguet, O., Abrevaya, S., Desmaras, H., Garcia-Cordero, I., Baez, S., et al. (2017). Tackling variability: a multicenter study to provide a gold-standard network approach for frontotemporal dementia. *Hum. Brain Mapp.* 38, 3804–3822. doi: 10.1002/hbm.23627

Shah, H., Albanese, E., Duggan, C., Rudan, I., Langa, K. M., Carrillo, M. C., et al. (2016). Research priorities to reduce the global burden of dementia by 2025. *Lancet Neurol.* 15, 1285–1294. doi: 10.1016/S1474-4422(16)30235-6

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

OPINION
published: 28 October 2016
doi: 10.3389/fnagi.2016.00253

Dementia in Latin America: An Emergent Silent Tsunami

Sandra Baez[1, 2] *and Agustín Ibáñez*[1, 3, 4, 5]*

[1] *Laboratory of Experimental Psychology and Neuroscience, Institute of Cognitive and Translational Neuroscience, INECO Foundation, Favaloro University, Buenos Aires, Argentina,* [2] *Departamento de Psicología, Universidad de los Andes, Bogotá, Colombia,* [3] *Departamento de Psicología, Universidad Autónoma del Caribe, Barranquilla, Colombia,* [4] *Center for Social and Cognitive Neuroscience, School of Psychology, Universidad Adolfo Ibáñez, Santiago de Chile, Chile,* [5] *Australian Research Council Centre of Excellence in Cognition and its Disorders, Sydney, NSW, Australia*

Keywords: Latin America, dementia, priority, barriers, diagnosis

Recently the Lancet Neurology Commission (Winblad et al., 2016) has provided expert recommendations and highlighted that European Union (EU) is well positioned to take the work lead to prevent and cure the Alzheimer's disease and other dementias, and to provide models for care. This panorama strongly contrasts with the one of Latin America. Although there is an evident growing interest in dementia among Latin American countries (LAC) (Lancet, 2015), important barriers in this region involves big challenges to join the fight against dementia. In this article, we identify some key issues regarding dementia diagnosis that could trigger immediate actions in LAC, contrasting them with the EU scenario (Winblad et al., 2016).

Demographic characteristics of LAC have substantially changed over the past 25 years, with an extensive decline of mortality and life expectancy increasing (Barreto et al., 2012). Demographical transitions have contributed to a large and rapid growth in the number of people suffering from dementia (Sousa et al., 2010). Predictions suggest that by 2050, the number of people aged 60 years will increase by 1.25 billion, with 79% living in the world's less developed regions (Prince et al., 2013). In spite of the huge economic and social impact that dementia is causing in LAC (Manes, 2016), loss of awareness and deficiencies in health system are more accentuated in LAC than in the EU. Some of these obstacles are addressed in this article, including the limited access to health facilities, the need for standardizing diagnostic practices, and the existing barriers regarding resources and culture.

In LAC, the diagnosis is usually made by specialists (i.e., neurologists, psychiatrists, or gerontologists) and sporadically by a general practitioner (GP). However, only private health insurances cover such specialized services. In contrast, in many European countries most of patients with dementia are diagnosed by the GP and some patients are referred to neurologists or psychiatrists in private practice (Winblad et al., 2016). Both in LAC and in the EU only a very small proportion of patients are diagnosed in specialized centers such as memory clinics. Unlike EU [where the public health system tends to dominate (Winblad et al., 2016)], in most LAC the division of private and public health systems determines the quality and promptness of the diagnosis, as well as the proportion of people that can access health care facilities. At the public level, there are no centers of excellence providing multidisciplinary and individualized assessments. This, added to socioeconomic inequalities, emphasizes the importance of delineating actions toward these outstanding needs in LAC (Maestre, 2012).

In addition, basic recommendations and guidelines for dementia diagnosis are only available in some LAC (e.g., Chile, Argentina, and Brazil; Fuentes et al., 2008; Allegri, 2011; Caramelli et al., 2011; Chavez et al., 2011). In contrast, most of the EU countries have National Plans or guidelines for dementia diagnosis, the care for patients, and the recommended treatment (Winblad et al., 2016). Although some LAC has reached awareness regarding the importance of harmonizing diagnostic actions, this is not true for the regional level. The acceptance by scientific and academic

OPEN ACCESS

Edited by:
Aurel Popa-Wagner, University of Rostock, Germany

Reviewed by:
Raluca Sandu Vintilescu, University of Medicine and Pharmacy of Craiova, Romania

***Correspondence:**
Agustín Ibáñez
aibanez@ineco.org.ar

Received: *11 July 2016*
Accepted: *14 October 2016*
Published: *28 October 2016*

Citation:
Baez S and Ibáñez A (2016) Dementia in Latin America: An Emergent Silent Tsunami. Front. Aging Neurosci. 8:253. doi: 10.3389/fnagi.2016.00253

communities about international guidelines on dementia is increasing, but with no adequate support from Latin American governments.

Regarding the diagnostic procedures, in most LAC, diagnosis of dementia is primarily clinical, and detailed cognitive assessments are offered mainly in private institutions. Diagnosis relies on the history, interview with the patient and the family, cognitive screening tests, and laboratory tests. Imaging and biomarkers are very restricted to a few private centers. In EU countries, the instruments employed for dementia diagnosis include comprehensive and detailed cognitive batteries, scales of functional impairment, informant-based questionnaires about basic and instrumental activities of daily living, and assessments of neuropsychiatric symptoms, quality of life, and disease burden. Structural neuroimaging is well established in the clinical diagnosis and the use of biomarkers is becoming part of the clinical routine in memory clinics (Winblad et al., 2016). Currently, dementia biomarkers are not sufficiently standardized for the use in everyday clinical practice, but standardization initiatives are ongoing in the EU countries. This kind of initiatives are lacking in LAC.

Finally, several cultural issues affect dementia diagnosis in LAC. For instance, low education and illiteracy are key problems affecting most LAC (Prince et al., 2003). The illiteracy rate in the older population is approximately 10% (Nitrini et al., 2009). This problem is highly relevant since the prevalence of dementia in illiterates is two times higher than that in literates (Nitrini et al., 2009). In addition, LAC are not homogenous in terms of language (e.g., aboriginal groups). However, neuropsychological tests used as part of the diagnosis process have been adapted and translated from those designed to assess populations with a different cultural background (Nitrini et al., 2004; Parra, 2014). The basis of cultural effects is poorly understood, but population-based studies (Sosa et al., 2009) suggest that normative data should not be generalized across populations with different sociocultural contexts. The extent to which these cultural factors are influencing the dementia prevalence in LAC needs to be investigated with upmost priority.

The key issues presented here highlight the importance of developing harmonized global strategies in LAC in order to overcome the existing barriers preventing an accurate and early dementia diagnosis. Some research groups in Latin America are already addressing relevant clinical issues regarding Alzheimer's disease (Parra et al., 2009, 2010, 2015; Pietto et al., 2016) and other dementias (Cardona et al., 2013; Ibáñez et al., 2013; Baez et al., 2014a,b, 2015, 2016a,b,c; Kargieman et al., 2014; Garcia-Cordero et al., 2015, 2016; Melloni et al., 2015, 2016; Santamaria-Garcia et al., 2016; Sedeno et al., 2016). The urgent need now is to develop and implement health-care strategies and national plans that meet the needs of individuals with dementia and their families. Though some LAC are developing national plans, important challenges remain to improve the quality of dementia diagnosis (Manes, 2016). Latin American governments should strengthen health services, improve training for health professionals to diagnose/treat dementia and promote the creation of public memory clinics. In facing the fight against dementia, LAC should capitalize on the experience of EU countries.

AUTHOR CONTRIBUTIONS

Both authors developed the study concept, drafted the manuscript and approved its final version.

FUNDING

This work was partially supported by grants from CONICET, CONICYT/FONDECYT Regular (1130920 and 1140114), FONCyT-PICT 2012-0412, FONCyT-PICT 2012-1309, FONDAP 15150012, and the INECO Foundation.

REFERENCES

Allegri, R. (2011). Clinical practice guideline. *Neurol. Argent.* 3, 120–137. doi: 10.1016/S1853-0028(11)70026-X

Baez, S., Couto, B., Torralva, T., Sposato, L. A., Huepe, D., Montañes, P., et al. (2014a). Comparing moral judgments of patients with frontotemporal dementia and frontal stroke. *JAMA Neurol.* 71, 1172–1176. doi: 10.1001/jamaneurol.2014.347

Baez, S., Herrera, E., Gershanik, O., Garcia, A. M., Bocanegra, Y., Kargieman, L., et al. (2015). Impairments in negative emotion recognition and empathy for pain in Huntington's disease families. *Neuropsychologia* 68, 158–167. doi: 10.1016/j.neuropsychologia.2015.01.012

Baez, S., Kanske, P., Matallana, D., Montañes, P., Reyes, P., Slachevsky, A., et al. (2016a). Integration of intention and outcome for moral judgment in frontotemporal dementia: brain structural signatures. *Neurodegener. Dis.* 16, 206–217. doi: 10.1159/000441918

Baez, S., Manes, F., Huepe, D., Torralva, T., Fiorentino, N., Richter, F., et al. (2014b). Primary empathy deficits in frontotemporal dementia. *Front. Aging Neurosci.* 6:262. doi: 10.3389/fnagi.2014.00262

Baez, S., Morales, J. P., Slachevsky, A., Torralva, T., Matus, C., Manes, F., et al. (2016b). Orbitofrontal and limbic signatures of empathic concern and intentional harm in the behavioral variant frontotemporal dementia. *Cortex* 75, 20–32. doi: 10.1016/j.cortex.2015.11.007

Baez, S., Santamaría-García, H., Orozco, J., Fittipaldi, S., García, A. M., Pino, M., et al. (2016c). Your misery is no longer my pleasure: Reduced schadenfreude in Huntington's disease families. *Cortex* 83, 78–85. doi: 10.1016/j.cortex.2016.07.009

Barreto, S. M., Miranda, J. J., Figueroa, J. P., Schmidt, M. I., Munoz, S., Kuri-Morales, P. P., et al. (2012). Epidemiology in Latin America and the Caribbean: current situation and challenges. *Int. J. Epidemiol.* 41, 557–571. doi: 10.1093/ije/dys017

Caramelli, P., Texeira, A., Buchpiguel, C., Lee, H., Livramento, J., Fernandez, L., et al. (2011). Group recommendations in alzheimer's disease and vascular dementia of the brazilian academy of neurology. diagnosis of alzheimer's disease in Brazil: supplementary exams. *Dement. Neuropsychol.* 5, 167–177. Available online at: http://www.scielo.br/pdf/dn/v5n3/1980-5764-dn-5-03-00167.pdf

Cardona, J. F., Gershanik, O., Gelormini-Lezama, C., Houck, A. L., Cardona, S., Kargieman, L., et al. (2013). Action-verb processing in Parkinson's disease: new pathways for motor-language coupling. *Brain Struct. Funct.* 218, 1355–1373. doi: 10.1007/s00429-013-0510-1

Chavez, M., Godinho, C., Porto, C. S., Mansur, L., Carthery-Goulart, M., Yassuda, M. S., et al. (2011). Group recommendations in Alzheimer's disease and vascular dementia of the Brazilian academy of neurology. cognitive, functional and behavioral assessment: Alzheimer's disease. *Dement. Neuropsychol.* 5, 153–166. Available online at: http://148.215.2.10/articulo.oa?id=339529029003

Fuentes, P., Donoso, A., Slachevsky, A., Villalobos, A., and Flores, L. (2008). *Guía Clínica Trastornos Cognitivos y Demencia en el Adulto Mayor*. Chile: MINSAL.

Garcia-Cordero, I., Sedeno, L., de la Fuente, L. A., Slachevsky, A., Forno, G., Klein, F., et al. (2016). The functional neuroanatomical organization of interoceptive dimensions: multimodal evidence from neurodegeneration and stroke. *Philos. Trans. R. Soc.* 371:20160006. doi: 10.1098/rstb.2016-0006

García-Cordero, I., Sedeño, L., Fraiman, D., Craiem, D., de la Fuente, L. A., Salamone, P., et al. (2015). Stroke and neurodegeneration induce different connectivity aberrations in the Insula. *Stroke* 46, 2673–2677. doi: 10.1161/STROKEAHA.115.009598

Ibáñez, A., Cardona, J. F., Dos Santos, Y. V., Blenkmann, A., Aravena, P., Roca, M., et al. (2013). Motor-language coupling: direct evidence from early Parkinson's disease and intracranial cortical recordings. *Cortex* 49, 968–984. doi: 10.1016/j.cortex.2012.02.014

Kargieman, L., Herrera, E., Baez, S., García, A. M., Dottori, M., Gelormini, C., et al. (2014). Motor-language coupling in Huntington's disease families. *Front. Aging Neurosci.* 6:122. doi: 10.3389/fnagi.2014.00122

Lancet, N. (2015). A neurology revival in Latin America. *Lancet Neurol.* 14, 1143. doi: 10.1016/S1474-4422(15)00307-5

Maestre, G. E. (2012). Assessing dementia in resource-poor regions. *Curr. Neurol. Neurosci. Rep.* 12, 511–519. doi: 10.1007/s11910-012-0300-9

Manes, F. (2016). The huge burden of dementia in Latin America. *Lancet Neurol.* 15, 29. doi: 10.1016/S1474-4422(15)00360-9

Melloni, M., Billeke, P., Baez, S., Hesse, E., de la Fuente, L., Forno, G., et al. (2016). Your perspective and my benefit: multiple lesion models of self-other integration strategies during social bargaining. *Brain J. Neurol.* doi: 10.1093/brain/aww231. [Epub ahead of print].

Melloni, M., Sedeño, L., Hesse, E., García-Cordero, I., Mikulan, E., Plastino, A., et al. (2015). Cortical dynamics and subcortical signatures of motor-language coupling in Parkinson's disease. *Sci. Rep.* 5:11899. doi: 10.1038/srep11899

Nitrini, R., Bottino, C. M., Albala, C., Custodio Capuñay, N. S., Ketzoian, C., Llibre Rodriguez, J. J., et al. (2009). Prevalence of dementia in Latin America: a collaborative study of population-based cohorts. *Int. Psychogeriatr.* 21, 622–630. doi: 10.1017/S1041610209009430

Nitrini, R., Caramelli, P., Herrera Júnior, E., Porto, C. S., Charchat-Fichman, H., Carthery, M. T., et al. (2004). Performance of illiterate and literate nondemented elderly subjects in two tests of long-term memory. *J. Int. Neuropsychol. Soc.* 10, 634–638. doi: 10.1017/S1355617704104062

Parra, M. (2014). Overcoming barriers in cognitive assessment of Alzheimer's disease. *Dement. Neuropsychol.* 8, 95–98. doi: 10.1590/S1980-57642014DN82000002

Parra, M. A., Abrahams, S., Fabi, K., Logie, R., Luzzi, S., and Della Sala, S. (2009). Short-term memory binding deficits in Alzheimer's disease. *Brain J. Neurol.* 132, 1057–1066. doi: 10.1093/brain/awp036

Parra, M. A., Abrahams, S., Logie, R. H., Méndez, L. G., Lopera, F., and Della Sala, S. (2010). Visual short-term memory binding deficits in familial Alzheimer's disease. *Brain J. Neurol.* 133, 2702–2713. doi: 10.1093/brain/awq148

Parra, M. A., Saarimäki, H., Bastin, M. E., Londoño, A. C., Pettit, L., Lopera, F., et al. (2015). Memory binding and white matter integrity in familial Alzheimer's disease. *Brain J. Neurol.* 138, 1355–1369. doi: 10.1093/brain/awv048

Pietto, M., Parra, M. A., Trujillo, N., Flores, F., García, A. M., Bustin, J., et al. (2016). Behavioral and electrophysiological correlates of memory binding deficits in patients at different risk levels for Alzheimer's Disease. *J. Alzheimers Dis.* 53, 1325–1340. doi: 10.3233/JAD-160056

Prince, M., Acosta, D., Chiu, H., Scazufca, M., and Varghese, M., (2003). Dementia diagnosis in developing countries: a cross-cultural validation study. *Lancet* 361, 909–917. doi: 10.1016/S0140-6736(03)12772-9

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., and Ferri, C. P. (2013). The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement.* 9, 63.e2–75.e2. doi: 10.1016/j.jalz.2012.11.007

Santamaria-García, H., Reyes, P., Garcia, A., Baéz, S., Martinez, A., Santacruz, J. M., et al. (2016). First symptoms and neurocognitive correlates of behavioral variant frontotemporal dementia. *J. Alzheimers Dis.* 54, 957–970. doi: 10.3233/JAD-160501

Sedeño, L., Couto, B., García-Cordero, I., Melloni, M., Baez, S., Morales Sepúlveda, J. P., et al. (2016). Brain network organization and social executive performance in frontotemporal dementia. *J. Int. Neuropsychol. Soc.* 22, 250–262. doi: 10.1017/S1355617715000703

Sosa, A. L., Albanese, E., Prince, M., Acosta, D., Ferri, C. P., Guerra, M., et al. (2009). Population normative data for the 10/66 dementia research group cognitive test battery from Latin America, India and China: a cross-sectional survey. *BMC Neurol.* 9:48. doi: 10.1186/1471-2377-9-48

Sousa, R. M., Ferri, C. P., Acosta, D., Guerra, M., Huang, Y., Jacob, K., et al. (2010). The contribution of chronic diseases to the prevalence of dependence among older people in Latin America, China and India: a 10/66 Dementia Research Group population-based survey. *BMC Geriatr.* 10:53. doi: 10.1186/1471-2318-10-53

Winblad, B., Amouyel, P., Andrieu, S., Ballard, C., Brayne, C., Brodaty, H., et al. (2016). Defeating Alzheimer's disease and other dementias: a priority for European science and society. *Lancet Neurol.* 15, 455–532. doi: 10.1016/S1474-4422(16)00062-4

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: 13 July 2017
doi: 10.3389/fnagi.2017.00221

Dementia in Latin America: Epidemiological Evidence and Implications for Public Policy

Nilton Custodio[1], Ana Wheelock[2,3], Daniela Thumala[2,4] and Andrea Slachevsky[2,5,6,7,8]**

[1]Unidad de diagnóstico de deterioro cognitivo y prevención de demencia, Departamento de Neurología, Instituto Peruano de Neurociencias, Lima, Peru, [2]Gerosciences Center for Brain Health and Metabolism (GERO), Santiago, Chile, [3]Department of Surgery and Cancer, National Institute for Health Research Imperial Patient Safety Translational Research Centre, Imperial College London, London, United Kingdom, [4]Psychology Department, Faculty of Social Sciences, Universidad de Chile, Santiago, Chile, [5]Physiopathology Department, ICBM, and East Neuroscience Department, Faculty of Medicine, Universidad de Chile, Santiago, Chile, [6]Cognitive Neurology and Dementia Unit, Neurology Department, Hospital del Salvador, Santiago, Chile, [7]Center for Advanced Research in Education (CIAE), Universidad de Chile, Santiago, Chile, [8]Servicio de Neurología, Departamento de Medicina, Clínica Alemana-Universidad del Desarrollo, Santiago, Chile

Population aging is among the most important global transformations. Today, 12% of the world population is of age 60 and over and by the middle of this century this segment will represent 21.5%. The increase in population of those aged 80 and over, also referred to as the "oldest old" or the "very elderly", will be even more pronounced, going from 1.7% of the population to 4.5% within the same period. Compared to European and North American countries, Latin America (LA) is experiencing this unprecedented demographic change at a significantly faster rate. Due to demographic and health transitions, the number of people with dementia will rise from 7.8 million in 2013 to over 27 million by 2050. Nowadays, the global prevalence of dementia in LA has reached 7.1%, with Alzheimer's Disease (AD) being the most frequent type. This level is similar to those found in developed countries; however, the dementia rate is twice as high as that of the 65–69 years age group in developed countries. In addition, the prevalence and incidence of dementia is higher among illiterate people. Mortality rates due to dementia have risen considerably. The burden and costs of the disease are high and must be covered by patients' families. The prevention of dementia and the development of long-term care policies and plans for people with dementia in LA, which take into account regional differences and similarities, should be urgent priorities.

Keywords: Latin America, dementia, epidemiology, public policy, Alzheimer, dementia plan, caregiver burden, cost of dementia

OPEN ACCESS

Edited by:
Patricia Cogram, Fraunhofer Research, Chile

Reviewed by:
Paulo Caramelli, Universidade Federal de Minas Gerais, Brazil
Gladys Elena Maestre, University of Texas Rio Grande Valley, United States

****Correspondence:***
Nilton Custodio
ncustodio@ipn.pe
Andrea Slachevsky
andrea.slachevsky@uchile.cl

Received: *23 November 2016*
Accepted: *23 June 2017*
Published: *13 July 2017*

Citation:
Custodio N, Wheelock A, Thumala D and Slachevsky A (2017) Dementia in Latin America: Epidemiological Evidence and Implications for Public Policy. Front. Aging Neurosci. 9:221. doi: 10.3389/fnagi.2017.00221

INTRODUCTION

Population aging is among the most important global transformations. Today, 12% of the world population is of age 60 and over and by the middle of this century this segment will represent 21.5%. The increase in population of aged 80 and over, also referred as the "oldest old" or the "very elderly", will be even more pronounced, rising from 1.7% of the population to 4.5% within the same period (United Nations, 2015). Compared to European and North American countries, Latin America (LA) is experiencing this unprecedented demographic change at a significantly faster rate (Bongaarts, 2009). Due to demographic and health transitions, the number of people with dementia in LA will rise from 7.8 million in 2013 to over 27 million by 2050 (Bupa and Alzheimer's Disease International, 2013; Baez and Ibáñez, 2016). The aim of this review is to provide an overview of the epidemiological state of affairs and a critical appraisal of the

public policies that are being designed/implemented vs. those that are needed to address the current dementia-related challenges.

THE PREVALENCE OF DEMENTIA IN LATIN AMERICA

As a result of the demographic transition in LA, the total number of individuals over 60 years of age will increase, reaching approximately 57 million by 2025 (CEPAL, 2014). On the other hand, low socioeconomic and educational levels in the region are additional elements that account for the major increase in the prevalence of dementia. For these reasons, dementias are beginning to be regarded as a public health priority. Systematic reviews of studies on the prevalence of dementia conducted in developed countries have revealed a slightly downward trend for the USA and Europe, but an upwards trend for Asia (Winblad et al., 2016), The analysis of eight population studies conducted in Brazil, Cuba, Chile, Peru and Venezuela showed that the global prevalence is 7.1% (confidence interval, CI 95%: 6.8–7.4; Nitrini et al., 2009). The studies reveal that the prevalence of dementia increases with age, doubling every 5 years from 65 years of age onwards. It rises from 2.40% (CI 95%: 2.11–2.72) in the 60–64 group to 33.07% (CI 95%: 29.98–36.20) in the 90–94 group (Nitrini et al., 2009). However, there is a significant variation in the estimate of prevalence of dementia, ranging from 2% in a Brazilian study (Ramos-Cerqueira et al., 2005) to 13% in a Venezuelan study (Maestre et al., 2002). These studies suggest prevalence similar to the ones reported for developed regions, whose rates range from 4.2% in Canada to 14.5% in Spain (Winblad et al., 2016). We must stress the fact that at least three studies, those conducted in Catanduva-Sao Paulo (Herrera et al., 2002; Nitrini et al., 2004) and Lima (Custodio et al., 2008), used the same screening tests for diagnosing dementia, while Llibre's study in Cuba (Llibre et al., 1999) used a uniform structured interview (**Table 1**).

With respect to the types of dementia found in the Brazilian city of Catanduva, 25% of the participants were assessed in their urban homes at ≥65 years of age. Specialists examined those with suspected dementia. A 7.1% rate of dementia was found, with 55.1% of Alzheimer's Disease (AD) cases (Herrera et al., 2002). A similar protocol was used in Lima with a sample of 1532 individuals. 105 cases of dementia were found, with AD being the most frequent diagnosis (56.2%). This prevalence increased with age and was mostly observed in female participants (Custodio et al., 2008). AD is the most frequent cause of dementia in LA, representing 56.3% of cases, followed by AD with cerebrovascular disease (CVD), which reaches 15.5%, and vascular dementia (VD), which accounts for 8.7%. These results are consistent with those reported in previous research (Herrera et al., 2002; Nitrini et al., 2004; Ramos-Cerqueira et al., 2005). However, the fact that magnetic resonance imaging was not used may have distorted the true proportion of individuals with AD+CVD and VD (Grinberg et al., 2007; França-Resende et al., 2016).

Few studies conducted in our region have focused on other degenerative causes of dementia, such as frontotemporal dementia (FTD). Community-based studies conducted in LA with individuals aged ≥55 years have found that the prevalence of FTD ranges from 12 to 18 cases per 1000 people, and that it is higher among Brazilians (2.6%–2.8%; Herrera et al., 2002; Lopes, 2006) than among Peruvians (1.90%; Custodio et al., 2008) and Venezuelans (1.53%; Maestre et al., 2002; **Table 2**). The values reported are intermediate with respect to global studies (Custodio et al., 2013). In general, the exact prevalence of FTD is unknown. The prevalence estimated in studies with outpatients and in European memory centers ranges from 0.002% to 0.031%; from 0.078% to 1.56%, and from 0.054% to 0.135% between 45 years and 64 years, 65 years and 74 years, and at ≥75 years respectively (Rosso et al., 2003; Gilberti et al., 2012). However, recent community-based studies suggest that FTD may be more common than previously estimated (Bernardi et al., 2012). Borroni's (Borroni et al., 2010) in Brescia, Italy, reported a higher prevalence than the literature, reaching 17.6 cases per 100,000 people. This study encourages us to pay attention to FTD in people over 75 years old, in whom the prevalence reached 54/100,000 inhabitants. The research examined suggests that the prevalence of FTD in LA may be lower than that reported for developed countries.

TABLE 1 | Prevalence of dementia: community-based studies 1994–2000 (Lopes et al., 2007).

Age	Number of studies	Prevalence of dementia (%) (CI** 95%)	Average increase in prevalence
65–69	17	1.2 (0.8–1.5)	—
70–74	19	3.7 (2.6–4.7)	3.0
75–79	21	7.9 (6.2–9.5)	2.1
80–84	20	16.4 (13.8–18.9)	2.0
85–89	16	24.6 (20.5–28.6)	1.5
90–94	6	39.9 (34.4–45.3)	1.6
>95	6	54.8 (45.6–63.9)	1.3

**CI, confidence interval.*

THE INCIDENCE OF DEMENTIA IN LA

In the study conducted in Catanduva, Sao Paulo (Nitrini et al., 2004), 1119 individuals aged ≥65 years were reassessed on average 3.25 years after their first assessment, a process that revealed 50 cases of dementia (28 AD cases). The incidence rate of dementia was 13.8 per 1000 people/year for individuals ≥65 years, while that of AD was 7.7. This is comparable to the levels reported by researchers working in Europe, North America and Asia (Satizabal et al., 2016). The incidence rate of dementia doubled every 5 years; however, it was found to be lower in the last 5-year period studied (**Table 3**). This may be due to the number of individuals in the age group over 90 years ($n = 16$), where only two new cases of dementia were found. This phenomenon has also been observed in other studies (Satizabal et al., 2016). In contrast with prior research, the study conducted in Catanduva (Nitrini et al., 2004) did not demonstrate any significant differences linked to gender; however, female participants displayed a high incidence of dementia, especially AD, in very elderly groups (over 85 years of age). The incidence

TABLE 2 | Prevalence of frontotemporal dementia in four studies conducted in Latin America (LA; adapted from Custodio et al., 2013).

Country	Location	Age group	Prevalence			Causes of dementia	
			Global dementia (95% CI[a])	FTD[b]	DUE[c]	FTD (%)	DUE (%)
Brazil (Herrera et al., 2002; Nitrini et al., 2004)	Catanduva, Sao Paulo	≥65	7.1% (6.0–8.5)	0.18%	0.90%	2.60	12.70
	Ribeirao Preto, Sao Paulo	≥60	6.0% (4.6–7.3)	0.7%	0.43%	2.80	7.20
		≥65	7.2% (5.7–8.6)	–	–	–	–
Venezuela (Maestre et al., 2002)	Santa Lucia, Maracaibo	≥55	8.04% (7.01–9.19)	0.12%	0.45%	1.53	5.61
		≥65	13.27%[†]	–	–	–	–
Peru (Custodio et al., 2008)	Cercado de Lima, Lima	≥65	6.85% (5.53–8.08)[‡]	0.13%	0.87%	1.90	12.70

[a]*CI, confidence interval;* [b]*FTD, fronto-temporal dementia;* [c]*DUE, dementia of unknow etiology;* [†]*data calculated by author;* [‡]*confidence interval data provided by author. Only raw, non-adjusted data are presented.*

of dementia was higher in males than in females in the group ≤85 years, although this difference was not statistically significant. The study carried out by the 10/66 Dementia Research Group with individuals ≥60 years old living in urban areas of Cuba, the Dominican Republic and Venezuela and in urban and rural areas of Peru, Mexico and China (Prince et al., 2013) reported a higher incidence rate of dementia in women than in men and revealed that it increased exponentially with age. After standardizing the EURODEM incident cohort for age (analysis of four prospective studies conducted in Denmark, France, Netherlands and United Kingdom), the incidence rate of dementia according to 10/66 criteria ranged from 20 to 30 per 1000 people/year, which is slightly higher than the 18.4 per 1000 people/year calculated according to the DSM-III-R criteria reported by the EURODEM. On the other hand, the incidence rates of dementia according to 10/66 criteria were approximately 1.5–2.5 times higher than those obtained with the DSM-IV dementia criteria.

RISK FACTORS ASSOCIATED WITH DEMENTIA

The Influence of Age and Gender on the Prevalence of Dementia in LA

According to studies conducted in LA, the prevalence of dementia increases with age: from 2.40% (95% CI: 2.11–2.72) in the 65–69 group to 20.20% (95% CI: 18.62–21.78) in the 85–89 group and 33.07% (95% CI: 29.98–36.20) among participants aged 90–94 years (Nitrini et al., 2004). These results confirm that the prevalence rates of dementia increase exponentially with age (Prince et al., 2016; Winblad et al., 2016).

TABLE 3 | Rate of incidence of dementia by age group and sex per 1000 inhabitants/year (adapted from Nitrini et al., 2004).

Age	*N*	Women (CI** 95%)	Men (CI 95%)	Total (CI 95%)
65–69	2	2.4 (0.52–0.725)	3.9 (0.845–11.60)	3.0 (0.4–10.7)
70–74	9	4.0 (1.74–7.77)	9.3 (5.72–15.05)	6.4 (3.0–12.1)
75–79	14	13.7 (9.36–23.87)	20.5 (14.04–32.92)	16.4 (9.1–27.1)
80–84	10	19.2 (3.87–32.05)	35.8 (21.12–58.66)	25.0 (12.2–44.4)
85–89	13	68.4 (5.13–98.60)	24.3 (11.64–45.83)	48.2 (26.5–77.8)
≥90***	2	44.0 (9.46–113.46)	34.2 (7.38–91.74)	38.5 (4.8–118.0)

***CI, confidence interval,* ****Only one patient (97) was over 94 years old.*

In terms of gender, studies conducted in LA show higher rates for both men and women in the 65–69 years group, and for women in the 70–74 years group, compared with the results reported by European studies (Lobo et al., 2000; Nitrini et al., 2004; Winblad et al., 2016; **Table 4**). The studies carried out in LA reported slightly higher rates for female participants in all age groups (Nitrini et al., 2004). Similar rates were reported in studies conducted in Europe (Lobo et al., 2000; Winblad et al., 2016), LA, India and China (Prince et al., 2013). It is interesting to note that in LA, the prevalence in the 65–69 group is higher than in developed countries (**Table 4**). A number of reasons may be contributing to the greater prevalence observed in relatively young individuals in developing countries, especially their limited access to primary care and their low educational level. The lack of primary health care can predispose these individuals to suffering from dementia caused by controllable or curable diseases, such as hypertension or syphilis. Low educational levels have been consistently linked to high dementia rates; in this regard, it can be argued that low educational levels are linked to early manifestations of cognitive decline, whereas individuals with higher educational levels tend to have a cognitive reserve that delays the emergence of clinical signs of dementia (Fratiglioni and Wang, 2007; Manly et al., 2007).

The Influence of Education on Dementia

An inverse relationship has been demonstrated to exist between educational level and dementia. The prevalence of dementia in Lima reached 3.7% in individuals with more than 8 years of education, but it was much higher (15.2%) in illiterate participants (Custodio et al., 2008). Through a univariate analysis, the study conducted in Catanduva also revealed a greater incidence of dementia among illiterate participants; however, a multivariate analysis showed that the relationship was more significant when controlling for age and female sex (Nitrini et al., 2004). In Chile, two studies reported higher prevalence of cognitive impairment and dementia in rural contexts and in people with low educational levels: cognitive impairment was 5.6 times higher among adults with low educational levels (17.2%) compared to those with high educational levels

TABLE 4 | Comparison of dementia prevalence associated with gender considering data provided by seven LA studies and European studies (adapted from Nitrini et al., 2009).

	Latin American studies						European studies	
	Women			Men			Women	Men
Age	Dementia *N*	Participants *N*	Mean (%) (95% CI**)	Dementia *N*	Participants *N*	Mean (%) (95% CI)	Mean (%)	Mean (%)
65–69	149	5620	2.65 (2.25–3.10)	79	3479	2.27 (1.80–2.81)	1.0	1.6
70–74	196	4781	4.10 (3.55–4.69)	65	2317	2.81 (2.17–3.57)	3.1	2.9
75–79	293	3802	7.71 (6.89–8.59)	112	1888	5.93 (4.90–7.09)	6.0	5.6
80–84*	291	2326	12.51 (11.17–13.94)	162	1489	10.88 (9.34–2.55)	12.6	11.0
85–89	281	1244	22.59 (20.30–24.97)	182	960	18.96 (16.49–21.55)	20.2	12.8
90+	189	500	37.80 (33.56–42.28)	105	390	26.92 (22.54–31.67)	30.8	22.1

**CI, confidence interval.*

(3%; González et al., 2009; ENS, 2010; Fuentes and Albala, 2014).

The systematic review published by Sharp and Gatz (2011), which involved 71 studies conducted in the last 25 years, suggests that the education-dementia link may be more complex. This may be due to the fact that differences in terms of the methodologies and samples used make it difficult to compare studies; however, the analysis of the educational factor could have a differential impact in different cultures and cohorts. The mechanisms that have been advanced to explain the education-dementia link include cerebral reserve and cognitive reserve (Stern, 2009), which appear to be robust. Likewise, there is consensus among researchers that education is a protective factor against dementia (Caamaño-Isorna et al., 2006; Baumgart et al., 2015). Within the context of the cognitive reserve hypothesis, if two individuals with the same brain volume suffer from dementia, the one with the largest cognitive reserve is believed to be better equipped to tolerate the cerebral pathological burden for longer, thus delaying the first clinical manifestations of the disease (Qiu et al., 2001). In addition, the survival rates of patients with dementia and high education levels are low compared with those of patients with low education levels (Qiu et al., 2001). Stern's theoretical explanation (Stern, 2012) is that, when individuals with high cognitive reserves reach the point where cognitive function is affected, they have a cerebral pathological burden nearing their total capacity, and at that point clinical manifestations are florid and evolve quickly, which resembles rapidly progressive dementia. On the other hand, it is interesting to trace the influence of non-modifiable risk factors such as allele ε4 of apolipoprotein E (APOE) and its effect considering education level. In this regard, there are no doubts of the role of APOE ε4 as a risk factor for AD; however, education may be relevant for balancing the effect of APOE ε on the clinical manifestations of dementia. Only a few studies have been conducted on the latter issue. An analysis of the data yielded by three studies conducted in Northern Europe (Sweden and Finland) with 3436 participants over 65 years old suggests that genetic factors (APOE ε4) and environmental factors (education) may act independently as risk factors for dementia; in addition, they may interact: high education levels could balance the negative effects of APOE ε4 on the occurrence of dementia (Wang et al., 2012). There is no information about the influence of education and Apo-E on AD.

DEMENTIA-RELATED MORTALITY

People living with dementia have a risk of death 2–4 times higher than that of similarly aged people without dementia (Ientile et al., 2013). In middle-income countries, the mortality risk is 1.56–5.69 times higher than in individuals without dementia (Nitrini et al., 2005). Survival after a dementia diagnosis ranges from 3 years to 12 years, depending on diagnostic criteria, age, severity at the time of diagnosis, and place of diagnosis (Brodaty et al., 2012; Kua et al., 2014). Fitzpatrick et al. (2005) reported an average survival rate of 7.1 years (95% CI: 6.7–7.5 years) for AD and 3.9 years (3.5–4.2 years) for VD. Helzner et al. (2008), using a multi-ethnic cohort of 323 individuals in the USA, described that AD reduced life expectancy by 3 years in subjects diagnosed between 70 years and 75 years of age, and by 1–2 years when diagnosis was made at a later age. In advanced dementia cases, average survival reached 1.3 years, with a mortality rate of 25% at 6 months. Similarly, Mitchell et al. (2009), with a cohort of 323 nursing home patients with advanced dementia, report an average survival time of 1 year and 3 months and a mortality risk of 24.7% at 6 months. At 18 months of evolution, more than 54.8% of patients had died. Severe dementia often causes complications such as immobility, swallowing disorders and malnutrition, thus increasing the risk of developing intercurrent diseases that may cause death. It has been reported that in the last 3 months of survival, 37.3% of patients suffered from pneumonia, 32.2% had a fever, and 90.4% displayed swallowing disorders. Pneumonia has been identified as the complication most frequently leading to death

(Mitchell et al., 2009; for a review, see Slachevsky et al., 2016).

A more than threefold increase in dementia-related mortality was observed between 1990 and 2010; thus, the number of deaths went up from 141,200 (CI 95% 110,800–208,500) to 485,700 (CI 95% 307,800–590,500), which represents a 244% increase. The age-adjusted mortality rate rose from 3.6 (CI 95% 2.8–5.4) to 7.1 (CI 95% 4.5–8.6) per 100,000 inhabitants, which constitutes a 95.4% increase. Globally, AD and other dementias are the 50th cause of death (Prince et al., 2016). In the Andean part of LA, they are the 68th cause and the 53th in South America, with major intra-regional disparities (Lozano et al., 2012; Prince et al., 2016). In Chile, in 2012, with 3852 deaths (3.89% of the total number of deaths), dementias constituted the sixth specific cause of death (Lozano et al., 2012). A global study on disease burden conducted in 2010 showed that the number of deaths attributed to dementias rose by 526%, which means that they are the cause of death with the largest increase in percentage (Lozano et al., 2012). In Chile, regional variability has been reported with respect to dementia-related mortality. Two regions display higher rates: the Metropolitan Region, in the center of the country, and Antofagasta, in the north (Russ et al., 2016). It has been suggested that this difference may be due to the high urbanization rates of these regions and/or to pollution (Russ et al., 2016). Nevertheless, dementia-related mortality data should be interpreted with caution. This is because many people living with dementia are not formally diagnosed (Lang et al., 2017) and few physicians have received dementia training, thus they may underreport dementia as a cause of death (O'Neill et al., 2013; Olavarría et al., 2016; Russ et al., 2016). Since a formal diagnosis is required for it to be recorded on a death certificate, it is possible that dementia underdiagnosis is affecting the accuracy of data on dementia-related mortality (Russ et al., 2016).

THE IMPACT OF DEMENTIAS

Disease Burden

The DALY (disability-adjusted life years) index is used to measure and compare disease burdens in the population. It represents the sum of the years lost due to premature death and the years lost as a result of disability (Alzheimer's Association, 2014). The World Health Organization has estimated that dementias contribute with 11.2% of the years of life with disability in people over 60 years old, which is higher than the time added by, cardiovascular diseases and cancer (Ballard et al., 2011). A global study of disease burden conducted in 2010 showed that the DALYs due to AD and other dementias rose from 5,695,000 (4,516,000–6,982,000) in 1990 to 11,349,000 (9,147,000–1,3741,000) in 2010, representing a 99.3% increase. The DALYs per 100,000 people increased from 107 (85–132) in 1990 to 165 (133–199) in 2010, representing a 53.3% increase. Globally, dementias are the 49th disease in terms of DALYs. In Central America, they occupy the 50th spot, the 62nd in Andean LA and the 26th in South America (Murray et al., 2012). In Chile, they are the fastest-growing diseases in terms of DALY causation: 200% between 1990 and 2010 (Murray et al., 2012). Likewise, dementias are the fastest-growing diseases in terms of premature death causes, rising from the 49th place in 1990 to the 17th in 2010 (Lozano et al., 2012).

Burden of Caring

A high proportion of people with dementia need care ranging from the provision of instrumental daily living activities to full personal care and round-the-clock supervision (Sorensen et al., 2006). Therefore, dementia affects not only the patient, but also the person who supports them, which effectively doubles the number of people concerned (Ferrario et al., 2003; Georges et al., 2008). Caregivers are either formal, i.e., people who are paid to care for a patient with dementia, such as healthcare professionals, or informal, i.e., individuals who provide care and/or support to a family member, friend or neighbor who is chronically ill, frail, or who has a physical or mental disability (Slachevsky et al., 2013). In Latin American societies, patients with dementia are mainly cared for by families, and to a lesser extent by public institutions. That is, care and attention are provided by people who are physically and emotionally close to them and who have the largest responsibility in the ill person's care, despite having no prior training and receiving no payment. Several studies have examined informal caregivers, two of which included samples with more than 200 subjects. In Chile, the CUIDEME study involved 292 family caregivers who lived mainly in Santiago, the capital city. There were more female (80%) than male caregivers. Most of them were daughters and spouses of the patients. Severe burden was reported in 63% of the caregivers, and 47% exhibited psychiatric morbidity. Burden was associated with caregiver psychiatric distress, family dysfunction, severity of neuropsychiatric symptoms, and functional disability, but neither the patient's age, gender, nor socioeconomic status (SES) impacted burden (Slachevsky et al., 2013). In Cuba, in a descriptive study with 237 caregivers, a survey was administered to informal caregivers of elderly people with ictus and dementia, who resided in the Abel Santamaría neighborhood of Santiago de Cuba. The analysis demonstrated that 71.7% of caregivers were female and 28.3% male. Their average age was 49.83 years, and the average time devoted to the task was 42.86 months. 49.4% of the caregivers were children or partners of the people with dementia studied. 53.2% were full-time caregivers, while 46.7% worked part-time (Turtós Carbonell et al., 2016). In Bogotá, Colombia, in a descriptive study conducted with 52 informal caregivers of patients with Alzheimer's, 82.7% of whom were female, 57.7% of the population was not aware of any support networks. 36.5% of the caregivers were 51–60 years old and 25.0% were between 41 years and 50 years old. 23.1% were under 40 years old, 9.6% were 61–70 years old, and only 5.8% were over 70. 55.8% of the caregivers were children of the patients and 30.8% were their spouses. 55.8% of the caregivers reported caring for a person with AD for 36 months and 34.6% did so between 13 months and 36 months. 36.5% of the caregivers were housewives, 26.9% were pensioners, 25% were in work, and 5.8% were unemployed. Caregiver depression, assessed with

the State-Trait Depression Inventory, occurs due to taking care of a person who is ill (Cerquera Córdova et al., 2012). In Peru, in three research centers located in Lima, 92 informal caregivers were interviewed to analyze their caregiver burden with the Zarit Burden Interview (ZBI) and the Beck Depression Inventory (BDI-II). In this sample, 75% of the participants were over 55.5 years old. Most were female (81.5%) and spouses of the patient (60.87%). In addition, over 75% of them had been a caregiver for at least 1 year, 90.2% considered that their leisure time had been reduced, and 83.7% felt that their health had deteriorated. This study demonstrated that caregivers display high burden levels. Also, a multivariate analysis revealed that only the BDI-II was a solid predictor of ZBI (Custodio et al., 2014). In Brazil, at the Center for AD of the Federal University of Rio de Janeiro, 145 caregivers were interviewed to analyze three dimensions of caregiver burden: emotional exhaustion (EE), depersonalization (DP) and reduced personal accomplishment (RPA). In addition, the researchers studied the demographic characteristics of the patients' caregivers and clinics. High levels of EE were present in 42.1% of the sample, while DP was found in 22.8%. RPA was present in 38.6% of the participating caregivers. Caregiver depression and patient delusions were the most significant predictors of EE (Truzzi et al., 2012). Similarly, in a sample of people living in Sao Paulo, 165 caregivers of patients with dementia (61 with AD, 25 with some cognitive impairment but not dementia, and 79 healthy controls) were interviewed to assess caregiver burden according to the ZBI and establish correlations with the results of the patients' Neuropsychiatric Inventory (NPI). This study demonstrated that neuropsychiatric symptoms are significantly associated with caregiver stress (Cunha Folquitto et al., 2013).

Economic Cost of Dementia

In 2010, the total estimated worldwide cost of dementia was US$ 817.9 billion, roughly one percent of the global gross domestic product (GDP). This cost has three components: (i) direct costs that include medical expenses (visits, tests, medication); (ii) social costs associated with paid formal caregiving by health professionals or institutionalization; and (iii) indirect costs associated with informal caregivers—family members, friends, or neighbors—who are unpaid but forgo paid jobs and thereby suffer a productivity loss. The level and composition of the cost of dementia varies widely across countries. In high-income countries, the cost is 1.2 percent of GDP and is mostly formal. In contrast, in low-income countries it is just 0.24 percent of GDP and most costs are informal (World Health Organization and Alzheimer's Disease International, 2012; WHO, 2015). In contrast with high-income countries, informal care costs predominate in low and mid-low income countries. The costs of community care by paid social caregivers and home caregivers are practically nonexistent (Wimo et al., 2017). Only a handful of studies have focused on the cost and heterogeneity of the instruments used to assess the costs involved. Allegri et al. (2007), using a sample of 80 community-dwelling patients with dementia and 25 institutionalized patients, reported that direct costs increased according to the degree of cognitive deterioration (US$ 3420.40 in mild cases and US$ 9657.60 in severe cases) and institutionalization (US$ 3189.20 for outpatients vs. US$ 14,447.68 for institutionalized patients). Costs also differ depending on the type of dementia (US$ 5112 for VD, US$ 4625 for AD and US$ 4,924 for FTD). In Peru, Custodio et al. (2015), using a sample of 136 outpatients receiving care at a private clinic, reported an average cost of US$ 1500 per trimester for AD, US$ 1860 for FTD and US$ 1291 for VD. These costs are significantly greater than those of patients without dementia (US$ 230). Families are reported to spend a large part of their income on the care of people with dementia. In Brazil, Veras et al. (2008) reported that direct costs represent approximately 66% of family income, ranging from 75% in mild stages to 62% in severe stages, a figure that reaches 81% in the case of comorbidities such as high blood pressure and diabetes. Liu (2013) (unpublished data cited in Prince et al., 2015) reported a higher cost of public care (US$ 6750) compared to private care (US$ 1887), with both values being calculated using international dollars. In Chile, Hojman et al. (2017), using a sample of 330 informal primary caregivers, reported an average monthly cost per patient of US$ 943. Direct medical costs account for 21%, direct social costs represent 5%, and indirect costs constitute 74% of the total figure. The mean monthly cost is inversely related to SES. The monthly cost for high SES is US$ 690 and US$ 1023 for low SES. In this study, between one third and one half of the variation explained by SES is not due to the severity gradient, suggesting that SES is a key determinant in the cost of dementia, regardless of severity.

In contrast with high income countries, LA is characterized by the predominance of informal care costs (Wimo et al., 2010). Medical costs represent a relatively small percentage of the total costs of dementias. Costs associated with community care provided by paid social caregivers and home caregivers are practically nonexistent, which may be due to the lack of developed health services that can meet the needs of patients with chronic, non-transmissible diseases such as dementia (Hojman et al., 2017).

It is important to stress that the studies mentioned above have major limitations, including the fact that they employed convenience sampling and only enrolled patients diagnosed with dementias and who were being medically monitored. For this reason, these results must be extrapolated cautiously, considering that most people with dementias do not receive medical care.

NATIONAL DEMENTIA POLICIES

In LA, outdated health systems have failed to provide the complex and multidisciplinary actions required by people with chronic diseases such as dementia (Bossert and Leisewitz, 2016). Following recommendations by the WHO, the Pan American Health Organization and NGOs such as AD International (World Health Organization and Alzheimer's Disease International, 2012), several LA countries have started to develop national strategies to address the dementia crisis. Yet, much work remains to be done.

One of the countries spearheading these efforts in the region is Costa Rica, who launched in 2014 a National Plan for AD and

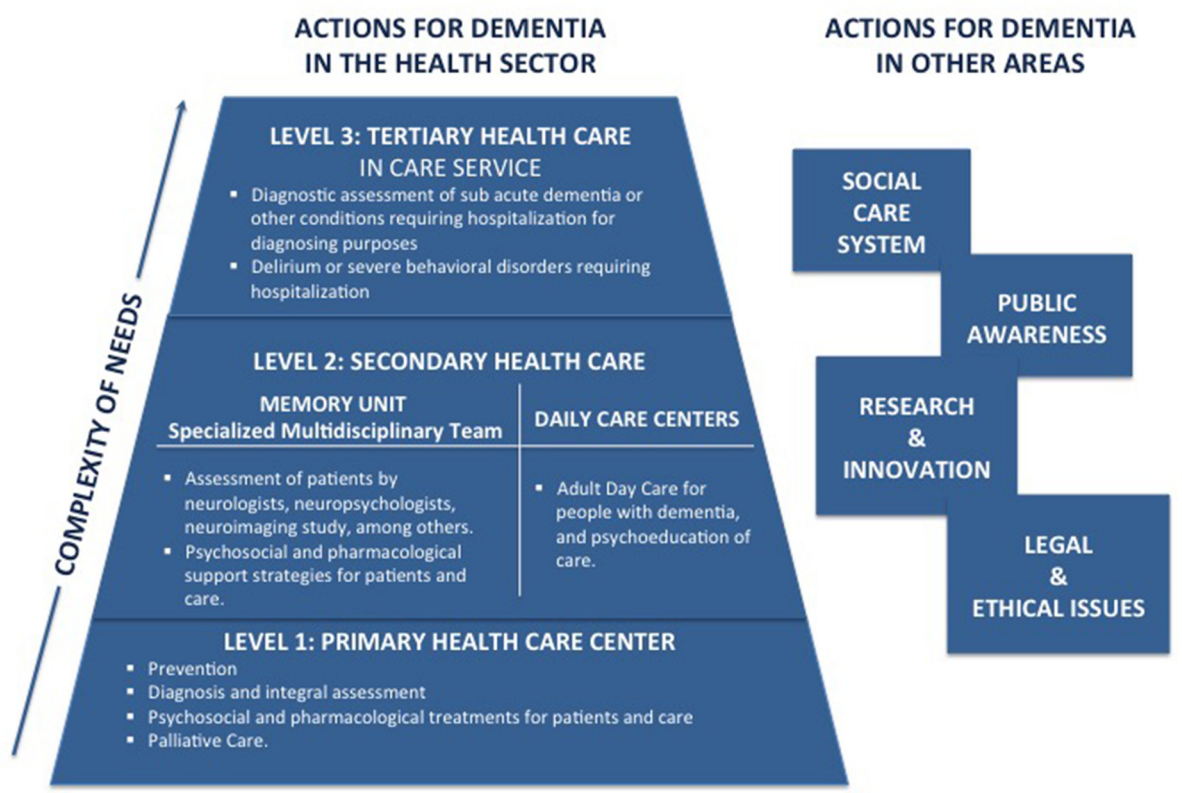


FIGURE 1 | Biopsychosocial model of dementia care in the Chilean's National Dementia Plan (adapted from Ministerio de Salud, 2015) (prepared by AS).

Related Diseases (Conapam, 2014). The plan's original objectives were transformed into seven cross-cutting principles which are guiding its implementation: (1) human rights of people with dementia; (2) empowerment and participation of people with dementia and their caregivers; (3) evidence-based practices for risk reduction and dementia care combined with research in the public and private sectors; (4) multisectoral collaboration on the response of public health to dementia; (5) health, social and community coverage for dementia; (6) equality in the response of public health in relation to dementia; and (7) care, prevention, promotion and rehabilitation treatment and development of dementia care. In 3 years, Costa Rica has implemented a number of initiatives, including community and clinical memory centers, programs, facilities and training for caregivers, acquisition of neuroimaging equipment, access to medication approved for Alzheimer's through the social security system, among others.

In 2016, Argentina launched the National Strategic Plan for a Healthy Brain, AD and other Dementias (PAMI, 2016). The plan encompasses five key areas: improving awareness, training professionals, caregivers and family members, improving access to diagnosis and treatment, reducing risk and encouraging research. Its implementation is underway.

In 2015, the Chilean government implemented an intersectoral work group—comprised of experts in neurology, geriatrics, mental health, public policy and the civil society—to propose a National Plan for Dementia (Ministerio de Salud, 2015). The plan was submitted to public consultation and has yet to be officially launched (Gajardo and Abuseleme, 2016). Nevertheless, the plan is currently being implemented and funding has been granted for Memory Clinics in secondary health care facilities, for a pilot program for dementia in primary care facilities and for dementia care training for health professionals (**Figure 1**).

In addition, since 2013, the government of Chile has funded daily care facilities for dementia patients, which are an important component of the dementia plan.

Other countries, however, are lagging behind. Bolivia, for example, has approved specific laws for people with dementia, but no financial commitments have been made. In 2013, Mexico's National Institutes of Geriatrics, Neurology and Psychiatry developed a proposal for a National Dementia Plan. Its primary aim was to promote the well-being of people affected by AD and theirs caregivers and families through the strengthening of the Mexican healthcare system and the support of other responsible institutions (Gutiérrez-Robledo and Arrieta-Cruz, 2015). However, this plan has yet to be approved by the government.

Given the challenges posed by the increasing number of people living with dementia, LA countries' current efforts to

tackle the looming dementia crisis are simply insufficient. Governments must urgently work toward closing the gap between the need for prevention, treatment and care of dementia and the actual provision of these services (World Health Organization and Alzheimer's Disease International, 2012). National dementia policies also need to consider how key contextual factors such as poverty, inequality and limited resources, impact the health of the population and access to health services (Marmot, 2005; Baez and Ibáñez, 2016; Bossert and Leisewitz, 2016; Hojman et al., 2017).

With regard to prevention, policies must address the challenges posed by a higher prevalence of dementia in groups with less education, by the increased incidence of cardiovascular diseases, which in turn, are associated with a higher risk of dementia, and by economic and geographical barriers to access to health services (Glassman et al., 2010; Garcia-Subirats et al., 2014). Dementia prevention efforts should also be shared by other government departments. For instance, recent evidence suggests that improvement in living conditions and higher levels of formal education account for a reduced risk of dementia in later life (Wu et al., 2017). Thus, increasing access to formal education could contribute to reduce the prevalence of dementia in LA (Meng and D'arcy, 2012).

A key policy challenge is that access to appropriate support services for people with dementia is hindered by the low level of detection of dementia in LA (Lang et al., 2017). This is largely due to the social stigma associated with dementia and lack of dementia awareness both among the general public and healthcare professionals. Therefore, efforts should also focus on addressing these barriers (Romero and Ge, 2007; Maestre, 2012; Olavarría et al., 2016).

A related obstacle to dementia diagnosis is the lack of validated and standardized instruments to assess functionality and cognition in illiterate and low-educated populations and populations with diverse cultural background, such as indigenous population vs. city population (Maestre, 2012; Parra, 2014).

An important question is when in the course of the dementia syndrome it is advisable to make a diagnosis. There is currently an intense debate about the benefits and drawbacks of early diagnosis vs. timely diagnosis—the latter being defined as the access to accurate diagnosis at a time in the disease process when it can be of most benefit for the patient (Le Couteur et al., 2013). From a public health perspective, the available evidence suggests that there is no benefit in screening for cognitive impairment at the population level, and that both screening and early diagnosis could actually cause harm (Boustani et al., 2003; Le Couteur et al., 2013). In the absence of a cure for AD and other neurodegenerative dementias, timely diagnosis is, therefore, recommended (Brooker et al., 2014). Nevertheless, the concept of timely diagnosis is ill-defined in the field of dementia. For some scholars, it corresponds to a diagnosis in the presence of neuropathology, early cognitive changes and possible disability and subjective impairment, i.e., at a stage that overlaps with early diagnosis (Dubois et al., 2016). For others, timely diagnosis corresponds to either a diagnosis established at the onset of cognitive decline and disability, responding to patient and caregivers' concerns, or to a diagnosis established in the presence of significant evidence of cognitive decline and disability (Prince et al., 2011; Brooker et al., 2014). In LA, the benefits and disadvantages associated with the diagnosis of dementia at different stages should be evaluated and a clear definition of timely diagnosis within the LA context should be sought. We argue that given the high rate of undetected dementia, the prevailing misconceptions on aging, the low awareness of dementia in the general public and health professionals, inadequate training in dementia among health professionals, and little access to specialist service, the benefit of case detection for "at risk" groups needs to be discussed (Baez and Ibáñez, 2016; Manes, 2016; Lang et al., 2017).

Concerning treatment and care, most LA countries have outdated health systems which are not currently able to offer the resource-intensive and multidisciplinary programs that people with dementia and their caregivers require (Bossert and Leisewitz, 2016). The capacity to develop integrated health and social care services for patients with dementia continues to challenge both developed and developing economies and will certainly be paramount to the success of public policies attempting to address the rising tide of dementia. Meanwhile, raising public awareness about dementia and ensuring that those living with these illnesses are treated with respect and dignity should be a priority (Gajardo and Abuseleme, 2016; Slachevsky and Gajardo, in press).

CONCLUSIONS

The increase in the prevalence of dementia and the resulting economic and societal impact, are growing concerns in LA. Yet, only a handful of epidemiological studies examining the prevalence of risk and dementia types, and protective factors, have been conducted in the region. Moreover, there is scarce information regarding the socio-sanitary conditions of people with dementia, their environment and associated costs. Among other issues, there is no data evaluating the impact of diagnosis and access to socio-sanitary support services on the evolution of the disease. From a public health perspective, the vast and rapid increase in the number of people with dementia due to demographic and health changes, warrants the prioritization of both dementia prevention policies long-term strategies to provide care for people living with dementia (Sousa et al., 2010).

Finally, it should be noted that although LA can be considered as a whole, its levels of development are heterogeneous. Risk factors associated with dementia, as well as its social impact varies between countries and within each country. Therefore, research and public policies in this field require consideration of the similarities that characterize the LA region, as well as its differences and particularities.

AUTHOR CONTRIBUTIONS

All the authors developed the study concept, drafted the manuscript and approved its final version.

ACKNOWLEDGMENTS

AS is supported by Comisión Nacional de Investigación Científica y Tecnológica (CONICYT)/FONDECYT/11404223, CONICYT/FONDAP/15150012 and Associative Research Program of CONICYT under Basal Funds Grant for Centers of Excellence FB 0003. AW and DT are supported by CONICYT/FONDAP/15150012. The authors would like to thank Asociación Costarricense de Alzheimer y otras Demencias Asociadas (ASCADA), for kindly providing up-to-date information about the progress of Costa Rica's National Plan for Alzheimer's Disease and Related Diseases.

REFERENCES

Allegri, R. F., Butman, J., Arizaga, R. L., Machnicki, G., Serrano, C., Taragano, F. E., et al. (2007). Economic impact of dementia in developing countries: an evaluation of costs of Alzheimer-type dementia in Argentina. *Int. Psychogeriatr.* 19, 705–718. doi: 10.1017/s1041610206003784

Alzheimer's Association. (2014). 2014 Alzheimer's disease facts and figures. *Alzheimers Dement.* 10, e47–e92. doi: 10.1016/j.jalz.2014.02.001

Baez, S., and Ibáñez, A. (2016). Dementia in Latin America: an emergent silent Tsunami. *Front. Aging Neurosci.* 8:253. doi: 10.3389/fnagi.2016.00253

Ballard, C., Gauthier, S., Corbett, A., Brayne, C., Aarsland, D., and Jones, E. (2011). Alzheimer's disease. *Lancet* 377, 1019–1031. doi: 10.1016/S0140-6736(10)61349-9

Baumgart, M., Snyder, H. M., Carrillo, M. C., Fazio, S., Kim, H., and Johns, H. (2015). Summary of the evidence on modifiable risk factors for cognitive decline and dementia: a population-based perspective. *Alzheimers Dement.* 11, 718–726. doi: 10.1016/j.jalz.2015.05.016

Bernardi, L., Frangipane, F., Smirne, N., Colao, R., Puccio, G., Curcio, S. A., et al. (2012). Epidemiology and genetics of frontotemporal dementia: a door-to-door survey in southern Italy. *Neurobiol. Aging* 33, 2948.e1–2948.e10. doi: 10.1016/j.neurobiolaging.2012.06.017

Bongaarts, J. (2009). Human population growth and the demographic transition. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 2985–2990. doi: 10.1098/rstb.2009.0137

Borroni, B., Alberici, A., Grassi, M., Turla, M., Zanetti, O., Bianchetti, A., et al. (2010). Is frontotemporal lobar degeneration a rare disorder? Evidence from a preliminary study in Brescia county, Italy. *J. Alzheimers Dis.* 19, 111–116. doi: 10.3233/JAD-2010-1208

Bossert, T. J., and Leisewitz, T. (2016). Innovation and change in the Chilean health system. *N. Engl. J. Med.* 374, 1–5. doi: 10.1056/NEJmp1514202

Boustani, M., Peterson, B., Hanson, L., Harris, R., Lohr, K. N., and U.S. Preventive services task force (2003). Screening for dementia in primary care: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann. Intern. Med.* 138, 927–937. doi: 10.7326/0003-4819-138-11-200306030-00015

Brodaty, H., Seeher, K., and Gibson, L. (2012). Dementia time to death: a systematic literature review on survival time and years of life lost in people with dementia. *Int. Psychogeriatr.* 24, 1034–1045. doi: 10.1017/s1041610211002924

Brooker, D., La Fontaine, J., Evans, S., Bray, J., and Saad, K. (2014). Public health guidance to facilitate timely diagnosis of dementia: Alzheimer's cooperative valuation in europe recommendations. *Int. J. Geriatr. Psychiatry* 29, 682–693. doi: 10.1002/gps.4066

Bupa, and Alzheimer's Disease International. (2013). *Dementia in the Americas: Current and Future Cost and Prevalence of Alzheimer's Disease and Other Dementias.* London: Bupa & Alzheimer's Disease International.

Caamaño-Isorna, F., Corral, M., Montes-Martínez, A., and Takkouche, B. (2006). Education and dementia: a meta-analytic study. *Neuroepidemiology* 26, 226–232. doi: 10.1159/000093378

CEPAL. (2014). *La Nueva era Demográfica en América Latina y el Caribe. Primera reunión de la mesa directiva de la conferencia regional sobre población y desarrollo de América Latina y el Caribe.* Santiago, Chile: Centro Latinoamericano y Caribeño de Población y Desarrollo (CELADE)-División de Población de la Comisión Económica para América Latina y el Caribe (CEPAL).

Cerquera Córdova, A. M., Pabón Poches, D. K., and Uribe Báez, D. M. (2012). Nivel de depresión experimentada por una muestra de cuidadores informales de pacientes con demencia de tipo Alzheimer. *Psicol. Caribe* 29, 360–384.

Conapam. (2014). *Plan Nacional para la Enfermedad de Alzheimer y Demencias Relacionadas 2014–2024 (National Plan for Alzheimer's Disease and Related Diseases 2014–2024).* Costa RIca: Consejo Nacional de la persona adulta mayor (National Council of Older Persons).

Cunha Folquitto, J., Gomes Marques, R. C., Franciosi Tatsch, M., and Campos Bottino, C. M. (2013). Correlation between neuropsychiatric symptoms and caregiver burden in population-based sample from São Paulo, Brazil: a preliminary report. *Dement. Neuropsychol.* 7, 258–262. doi: 10.1590/s1980-57642013dn70300005

Custodio, N., García, A., Montesinos, R., Escobar, J., and Bendezú, L. (2008). Prevalencia de demencia en una población urbana de Lima-Perú: un estudio puerta a puerta. *An. Fac. Med.* 69, 233–238. doi: 10.15381/anales.v69i4.1110

Custodio, N., Herrera-Perez, E., Lira, D., Montesinos, R., and Bendezú, L. (2013). Prevalence of frontotemporal dementia in community-based studies in Latin America: a systematic review. *Dement. Neuropsychol.* 7, 27–32. doi: 10.1590/s1980-57642013dn70100005

Custodio, N., Lira, D., Herrera-Perez, E., Nuñez Del Prado, L., Parodi, J., Guevara-Silva, E., et al. (2014). Informal caregiver burden in middle-income countries: results from Memory Centers in Lima—Peru. *Dement. Neuropsychol.* 8, 376–383. doi: 10.1590/s1980-57642014dn84000012

Custodio, N., Lira, D., Herrera-Perez, E., Nuñez Del Prado, L., Parodi, J., Guevara-Silva, E., et al. (2015). Cost-of-illness study in a retrospective cohort of patients with dementia in Lima, Peru. *Dement. Neuropsychol.* 9, 32–41. doi: 10.1590/s1980-57642015dn91000006

Dubois, B., Padovani, A., Scheltens, P., Rossi, A., and Dell'agnello, G. (2016). Timely diagnosis for Alzheimer's disease: a literature review on benefits and challenges. *J. Alzheimers Dis.* 49, 617–631. doi: 10.3233/JAD-150692

ENS. (2010). *Encuesta Nacional de Salud 2009—2010.* Santiago: Minsal.

Ferrario, S. R., Vitaliano, P., Zotti, A. M., Galante, E., and Fornara, R. (2003). Alzheimer's disease: usefulness of the family strain questionnaire and the screen for caregiver burden in the study of caregiving-related problems. *Int. J. Geriatr. Psychiatry* 18, 1110–1114. doi: 10.1002/gps.1021

Fitzpatrick, A. L., Kuller, L. H., Lopez, O. L., Kawas, C. H., and Jagust, W. (2005). Survival following dementia onset: Alzheimer's disease and vascular dementia. *J. Neurol. Sci.* 229–230, 43–49. doi: 10.1016/j.jns.2004.11.022

França-Resende, E., Costa Silva, L., Carmona, K., Machado, T., Machado, J., Cerqueira GuimarãEs, H., et al. (2016). Ischemic cerebrovascular burden evaluated by magnetic resonance imaging in an elderly Brazilian community: the Pietà study. *eNeurologicalSci* 5, 30–34. doi: 10.1016/j.ensci.2016.11.011

Fratiglioni, L., and Wang, H. X. (2007). Brain reserve hypothesis in dementia. *J. Alzheimers Dis.* 12, 11–22. doi: 10.3233/jad-2007-12103

Fuentes, P., and Albala, C. (2014). Aging and dementia in Chile. *Dement. Neuropsychol.* 8, 317–322. doi: 10.1590/s1980-57642014dn84000003

Gajardo, J., and Abuseleme, T. (2016). Plan nacional de demencias: antecedentes globales y síntesis de la estrategias chilena. *Rev. Med. Clin. Condes* 27, 286–296. doi: 10.1016/j.rmclc.2016.06.003

Garcia-Subirats, I., Vargas, I., Mogollón-Pérez, A. S., De Paepe, P., da Silva, M. R., Unger, J. P., et al. (2014). Barriers in access to healthcare in countries with different health systems. A cross-sectional study in municipalities of central Colombia and north-eastern Brazil. *Soc. Sci. Med.* 106, 204–213. doi: 10.1016/j.socscimed.2014.01.054

Georges, J., Jansen, S., Jackson, J., Meyrieux, A., Sadowska, A., and Selmes, M. (2008). Alzheimer's disease in real life—the dementia carer's survey. *Int. J. Geriatr. Psychiatry* 23, 546–551. doi: 10.1002/gps.1984

Gilberti, N., Turla, M., Alberici, A., Bertasi, V., Civelli, P., Archetti, S., et al. (2012). Prevalence of frontotemporal lobar degeneration in an isolated population: the Vallecamonica study. *Neurol. Sci.* 33, 899–904. doi: 10.1007/s10072-011-0865-0

Glassman, A., Gaziano, T. A., Bouillon Buendia, C. P., and Guanais de Aguiar, F. C. (2010). Confronting the chronic disease burden in Latin America and the Caribbean. *Health Aff.* 29, 2142–2148. doi: 10.1377/hlthaff.2010.1038

González, F., Massad, C., Lavanderos, F., Albala, C., Sanchez, H., Fuentes, A., et al. (2009). *Estudio Nacional de la Dependencia en las Personas Mayores.* Chile: SENAMA.

Grinberg, L. T., Ferretti, R. E., Farfel, J. M., Leite, R., Pasqualucci, C. A., Rosemberg, S., et al. (2007). Brain bank of the Brazilian aging brain study group—a milestone reached and more than 1,600 collected brains. *Cell Tissue Bank* 8, 151–162. doi: 10.1007/s10561-006-9022-z

Gutiérrez-Robledo, L., and Arrieta-Cruz, I. (2015). Demencias en México: la necesidad de un Plan de Acció. *Gac. Med. Mex.* 151, 667–673. Available online at: http://www.medigraphic.com/pdfs/gaceta/gm-2015/gm155p.pdf

Helzner, E. P., Scarmeas, N., Cosentino, S., Tang, M. X., Schupf, N., and Stern, Y. (2008). Survival in Alzheimer disease: a multiethnic, population-based study of incident cases. *Neurology* 71, 1489–1495. doi: 10.1212/01.wnl.0000334278.11022.42

Herrera, E. Jr., Caramelli, P., Silveira, A. S., and Nitrini, R. (2002). Epidemiologic survey of dementia in a community-dwelling Brazilian population. *Alzheimer Dis. Assoc. Disord.* 16, 103–108. doi: 10.1097/00002093-200204000-00007

Hojman, D., Duarte, F., Ruiz-Tagle, J., Troncoso, P., Budnich, M., and Slachevsky, A. (2017). The cost of dementia in an unequal country: the case of Chile. *PLoS One* 12:e0172204. doi: 10.1371/journal.pone.0172204

Ientile, L., De Pasquale, R., Monacelli, F., Odetti, P., Traverso, N., Cammarata, S., et al. (2013). Survival rate in patients affected by dementia followed by memory clinics (UVA) in Italy. *J. Alzheimers Dis.* 36, 303–309. doi: 10.3233/JAD-130002

Kua, E. H., Ho, E., Tan, H. H., Tsoi, C., Thng, C., and Mahendran, R. (2014). The natural history of dementia. *Psychogeriatrics* 14, 196–201. doi: 10.1111/psyg.12053

Lang, L., Clifford, A., Wei, L., Zhang, D., Leung, D., Augustine, G., et al. (2017). Prevalence and determinants of undetected dementia in the community: a systematic literature review and a meta-analysis. *BMJ Open* 7:e011146. doi: 10.1136/bmjopen-2016-011146

Le Couteur, D. G., Doust, J., Creasey, H., and Brayne, C. (2013). Political drive to screen for pre-dementia: not evidence based and ignores the harms of diagnosis. *BMJ* 347:f5125. doi: 10.1136/bmj.f5125

Liu, Z. (2013). *Economic Costs of Dementia in Low and Middle Income Countries.* PhD. Doctor of Philosophy, King's College, London.

Lobo, A., Launer, L. J., Fratiglioni, L., Andersen, K., Di Carlo, A., Breteler, M. M., et al. (2000). Prevalence of dementia and major subtypes in Europe: a collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. *Neurology* 54, S4–S9.

Lopes, M. (2006). *Estudo Epidemiológico de Prevalência de Demência em Ribeirão Preto; Epidemiological Survey of Prevalence of Dementia in Ribeirão Preto.* PhD thesis, Faculdade de Medicina, Universidade de Paulo, São Paulo. doi: 10.11606/T.5.2006.tde-18042007-110300

Lopes, M. A., Hototian, S. R., Reis, G. C., Elkis, H., and Bottino, M. C. (2007). Systematic review of dementia prevalence 1994 to 2000. *Dement. Neuropsychol.* 1, 230–240. doi: 10.1590/s1980-57642008dn10300003

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., et al. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380, 2095–2128. doi: 10.1016/S0140-6736(12)61728-0

Llibre, J. J., Guerra, M. A., Pérez-Cruz, H., Bayarre, H., Fernández-Ramírez, S., González-Rodríguez, M., et al. (1999). Dementia syndrome and risk factors in adults older than 60 years old residing in Habana. *Rev. Neurol.* 29, 908–911.

Maestre, G. E. (2012). Assessing dementia in resource-poor regions. *Curr. Neurol. Neurosci. Rep.* 12, 511–519. doi: 10.1007/s11910-012-0300-9

Maestre, G. E., Pino-Ramírez, G., Molero, A. E., Silva, E. R., Zambrano, R., Falque, L., et al. (2002). The maracaibo aging study: population and methodological issues. *Neuroepidemiology* 21, 194–201. doi: 10.1159/000059524

Manes, F. (2016). The huge burden of dementia in Latin America. *Lancet Neurol.* 15:29. doi: 10.1016/s1474-4422(15)00360-9

Manly, J., Schupf, N., Tang, M., Weiss, C., and Stern, Y. (2007). "Literacy and cognitive decline among ethnically diverse elder," in *Cognitive Reserve: Theory and Applications*, ed. Y. Stern (New York, NY: Taylor & Francis), 219–235.

Marmot, M. (2005). Social determinants of health inequalities. *Lancet* 365, 1099–1104. doi: 10.1016/S0140-6736(05)71146-6

Meng, X., and D'arcy, C. (2012). Education and dementia in the context of the cognitive reserve hypothesis: a systematic review with meta-analyses and qualitative analyses. *PLoS One* 7:e38268. doi: 10.1371/journal.pone.0038268

Ministerio de Salud, G. D. C. (2015). *Documento Preliminar Para la Elaboración del Plan Nacional Para las Demencias.* Argentina: Ministerio de Salud.

Mitchell, S. L., Teno, J. M., Kiely, D. K., Shaffer, M. L., Jones, R. N., Prigerson, H. G., et al. (2009). The clinical course of advanced dementia. *N. Engl. J. Med.* 361, 1529–1538. doi: 10.1056/NEJMoa0902234

Murray, C. J., Vos, T., Lozano, R., Naghavi, M., Flaxman, A. D., Michaud, C., et al. (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380, 2197–2223. doi: 10.1016/S0140-6736(12)61689-4

Nitrini, R., Bottino, C. M., Albala, C., Custodio Capuñay, N. S., Ketzoian, C., Llibre Rodriguez, J. J., et al. (2009). Prevalence of dementia in Latin America: a collaborative study of population-based cohorts. *Int. Psychogeriatr.* 21, 622–630. doi: 10.1017/S1041610209009430

Nitrini, R., Caramelli, P., Herrera, E. Jr., Bahia, V. S., Caixeta, L. F., Radanovic, M., et al. (2004). Incidence of dementia in a community-dwelling Brazilian population. *Alzheimer Dis. Assoc. Disord.* 18, 241–246.

Nitrini, R., Caramelli, P., Herrera, E. Jr., de Castro, I., Bahia, V. S., Anghinah, R., et al. (2005). Mortality from dementia in a community-dwelling Brazilian population. *Int. J. Geriatr. Psychiatry* 20, 247–253. doi: 10.1002/gps.1274

Olavarría, L., Mardones, C., Delgado, C., and Slachevsky Ch, A. (2016). Chilean health professionals perception of knowledge about dementia. *Rev. Med. Chil.* 144, 1365–1368. doi: 10.4067/S0034-98872016001000019

O'Neill, S., Richly, P., Prats, M., Mastadueno, R., Pontello, N., Christie, C., et al. (2013). Diagnosis and management of cognitive disorders in Latin America: new technologies or more knowledge needed? *Alzheimers Dement.* 9:P645. doi: 10.1016/j.jalz.2013.05.1315

PAMI. (2016). *Plan Estratégico Nacional Pami para un Cerebro Saludable, Enfermedad De Alzheimer Y Otras Demencias 2016—2019.* (Buenos Aires: PAMI.

Parra, M. A. (2014). Overcoming barriers in cognitive assessment of Alzheimer's disease. *Dement. Neuropsychol.* 8, 95–98. doi: 10.1590/s1980-57642014dn82000002

Prince, M., Ali, G. C., Guerchet, M., Prina, A. M., Albanese, E., and Wu, Y. T. (2016). Recent global trends in the prevalence and incidence of dementia, and survival with dementia. *Alzheimers Res. Ther.* 8:23. doi: 10.1186/s13195-016-0188-8

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., and Ferri, C. P. (2013). The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement.* 9, 63.e2–75.e2. doi: 10.1016/j.jalz.2012.11.007

Prince, M., Bryce, R., and Ferri, C. (2011). *Alzheimer's Disease International—World Alzheimer Report 2011: The Benefits of Early Diagnosis and Intervention.* London: Alzheimer's Disease International.

Prince, M., Wimo, A. M. G., Gemma-Claire, A., Yu-Tzu, W., and Prina, M. (2015). *World Alzheimer Report 2015 The Global Impact of Dementia An Analysis of Prevalence, Incidence, Cost and trends.* London: Alzheimer's Disease International (ADI).

Qiu, C., Backman, L., Winblad, B., Aguero-Torres, H., and Fratiglioni, L. (2001). The influence of education on clinically diagnosed dementia incidence and mortality data from the Kungsholmen Project. *Arch. Neurol.* 58, 2034–2039. doi: 10.1001/archneur.58.12.2034

Ramos-Cerqueira, A., Torres, A., Crepaldi, A., Oliveira, N., Scazufca, M., Menezes, P., et al. (2005). Identification of dementia cases in the community: a Brazilian experience. *J. Am. Geriatr. Soc.* 53, 1738–1742. doi: 10.1111/j.1532-5415.2005.53553.x

Romero, S., and Ge, M. (2007). Knowledge, belief and report of the practice of general practicioners in primary attention in Maracaibo, Venezuela about dementia. *Invest. Salud* 9, 10–15.

Rosso, S. M., Donker Kaat, L., Baks, T., Joosse, M., de Koning, I., Pijnenburg, Y., et al. (2003). Frontotemporal dementia in The Netherlands: patient

characteristics and prevalence estimates from a population-based study. *Brain* 126, 2016–2022. doi: 10.1093/brain/awg204

Russ, T. C., Murianni, L., Icaza, G., Slachevsky, A., and Starr, J. M. (2016). Geographical variation in dementia mortality in Italy, New Zealand, and Chile: evidence for an effect of lattitude and possible vitamin D. *Dement. Geriatr. Cogn. Disord.* 42, 31–41. doi: 10.1159/000447449

Satizabal, C. L., Beiser, A. S., Chouraki, V., Chêne, G., Dufouil, C., and Seshadri, S. (2016). Incidence of dementia over three decades in the framingham heart study. *N. Engl. J. Med.* 374, 523–532. doi: 10.1056/NEJMoa 1504327

Sharp, E. S., and Gatz, M. (2011). Relationship between education and dementia: an updated systematic review. *Alzheimer Dis. Assoc. Disord.* 25, 289–304. doi: 10.1097/WAD.0b013e318211c83c

Slachevsky, A., Abusleme Lama, M., and Arenas Massa, A. (2016). Cuidados paliativos en personas con demencia severa: reflexiones y desafios. Palliative care of patients with severe dementia. *Rev. Med. Chil.* 144, 94–101. doi: 10.4067/s0034-98872016000100012

Slachevsky, A., Budinich, M., Miranda-Castillo, C., Núñez-Huasaf, J., Silva, J. R., Muñoz-Neira, C., et al. (2013). The CUIDEME Study: determinants of burden in chilean primary caregivers of patients with dementia. *J. Alzheimers Dis.* 35, 297–306. doi: 10.3233/JAD-122086

Slachevsky, A., and Gajardo, J. (in press). "Dementia care in Chile: making dementia an urgent matter for public health," in *Dementia Care: International Perspectives*, eds A. Burns and P. Robertt (London: MHS IDEAL book).

Sorensen, S., Duberstein, P., Gill, D., and Pinquart, M. (2006). Dementia care: mental health effects, intervention strategies, and clinical implications. *Lancet Neurol.* 5, 961–973. doi: 10.1016/s1474-4422(06) 70599-3

Sousa, R. M., Ferri, C. P., Acosta, D., Guerra, M., Huang, Y., Jacob, K., et al. (2010). The contribution of chronic diseases to the prevalence of dependence among older people in Latin America, China and India: a 10/66 Dementia Research Group population-based survey. *BMC Geriatr.* 10:53. doi: 10.1186/1471-2318-10-53

Stern, Y. (2009). Cognitive reserve. *Neuropsychologia* 47, 2015–2028. doi: 10.1016/j.neuropsychologia.2009.03.004

Stern, Y. (2012). Cognitive reserve in ageing and Alzheimer's disease. *Lancet Neurol.* 11, 1006–1012. doi: 10.1016/s1474-4422(12)70191-6

Truzzi, A., Valente, L., Ulstein, I., Engelhardt, E., Laks, J., and Engedal, K. (2012). Burnout in familial caregivers of patients with dementia. *Rev. Bras. Psiquiatr.* 34, 405–412. doi: 10.1016/j.rbp.2012.02.006

Turtós Carbonell, L. B., Rodríguez Rosa, Y., Rodríguez Abreu, Y., and Martínez, E. O. (2016). Caracterización demográfica de cuidadores informales de ancianos con ictus y demencias en Santiago de Cuba. *Rev. Encuentros* 14, 61–72. doi: 10.15665/re.v14i2.795

United Nations. (2015). *World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241.World Population Prospects.* New York, NY: United Nations.

Veras, P., Caldas, C., Dantas, S., Sancho, L., Sicsú, B., and Motta, L. (2008). Demented elderly people living at home in Rio de Janeiro, Brazil: evaluation of expenditure care. *Psychogeriatrics* 8, 88–95. doi: 10.1111/j.1479-8301.2008. 00237.x

Wang, H. X., Gustafson, D. R., Kivipelto, M., Pedersen, N. L., Skoog, I., Windblad, B., et al. (2012). Education halves the risk of dementia due to apolipoprotein epsilon4 allele: a collaborative study from the Swedish brain power initiative. *Neurobiol. Aging* 33, 1007.e1–1007.e7. doi: 10.1016/j. neurobiolaging.2011.10.003

WHO. (2015). *World Report on Ageing And Health.* Washington, DC: World Health Organization's (WHO).

Wimo, A., Guerchet, M., Ali, G. C., Wu, Y. T., Prina, A. M., Winblad, B., et al. (2017). The worldwide costs of dementia 2015 and comparisons with 2010. *Alzheimers Dement.* 13, 1–7. doi: 10.1016/j.jalz. 2016.07.150

Wimo, A., Winblad, B., and Jönsson, L. (2010). The worldwide societal costs of dementia: estimates for 2009. *Alzheimers Dement.* 6, 98–103. doi: 10.1016/j.jalz. 2010.01.010

Winblad, B., Amouyel, P., Andrieu, S., Ballard, C., Brayne, C., Brodaty, H., et al. (2016). Defeating Alzheimer's disease and other dementias: a priority for European science and society. *Lancet Neurol.* 15, 455–532. doi: 10.1016/S1474-4422(16)00062-4

World Health Organization, and Alzheimer's Disease International. (2012). *Dementia: A Public Health Priority*. Geneva: World Health Organization (WHO).

Wu, Y. T., Beiser, A. S., Breteler, M. M. B., Fratiglioni, L., Helmer, C., Hendrie, H. C., et al. (2017). The changing prevalence and incidence of dementia over time—current evidence. *Nat. Rev. Neurol.* 13, 327–339. doi: 10.1038/nrneurol.2017.63

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: 20 December 2016
doi: 10.3389/fnagi.2016.00304

Dementia, Preclinical Studies in Neurodegeneration and its Potential for Translational Medicine in South America

Gloria Patricia Cardona-Gómez[1]* *and Francisco Lopera*[2]

[1]*Cellular and Molecular Neurobiology Area, Neuroscience Group of Antioquia, Faculty of Medicine, Sede de Investigación Universitaria (SIU), University of Antioquia, Medellin, Colombia,* [2]*Clinical Neuroscience Area, Neuroscience Group of Antioquia, Faculty of Medicine, Sede de Investigación Universitaria (SIU), University of Antioquia, Medellin, Colombia*

Latin-American people with dementia will increase to an astounding 368% in 2050, higher than USA and Europe. In addition, to sporadic dementia type like Alzheimer, and vascular dementia (VaD) progression after Cerebrovascular disease is also found. These incidences are increased in Colombia by specific populations affected with pure Neurodegenerative and VaDs like Autosomical Dominant familial Alzheimer's disease (AD) and Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL). In spite of the enormous human effort with and economical effort and investment costs, neither sporadic nor genetic kinds of dementia progression have been prevented or blocked yet. Currently, there exist several animal models that partially solve the understanding of the neurodegenerative etiopathogenesis and its treatment. However, when the potential therapies are translated to humans, those do not work or present a limited action. Main difficulties are the diverse comorbility associated to the cause and/or several affected brain regions, reducing the efficacy of some therapies which are limited to a tissue-specific action or modulating a kind of neurotransmission. Global investigation suggests that a general prevention could be achieved with the improvement in the quality of lifestyle, including healthy diet, physical and mental activity, and avoiding mechanical or chemical pro-inflammatory events in an early stage in the most of non-communicable diseases. In this review article, we present some molecular targets and preclinical studies in animal models to propose strategies that could be useful in a future translation to prevent or block neurodegeneration: one is gene therapy; silencing pathogenic genes in critical brain areas where excitotoxicity arise and spread. Another is to take advantage of the natural source and its wide biodiversity of natural products that are capable of identifying, by the blocking and prevention of neurodegeneration. On the other side, the casuistic of pure dementias in the Latin-American region gives an exceptional opportunity to understand the pathogenesis in these human populations. Further, this is in support of the basic and clinical researchers working on an interaction for a better understanding and medical care of mixed dementias, which have more complex factors than pure ones. However,

OPEN ACCESS

Edited by:
Agustin Ibanez, Institute of Cognitive and Translational Neuroscience, Argentina

Reviewed by:
Fiona Kumfor, Neuroscience Research Australia, Australia
Raluca Sandu Vintilescu, University of Medicine and Pharmacy of Craiova, Romania

***Correspondence:**
Gloria Patricia Cardona-Gómez
patricia.cardona@neurociencias.udea.edu.co

Received: *24 September 2016*
Accepted: *29 November 2016*
Published: *20 December 2016*

Citation:
Cardona-Gómez GP and Lopera F (2016) Dementia, Preclinical Studies in Neurodegeneration and its Potential for Translational Medicine in South America. Front. Aging Neurosci. 8:304. doi: 10.3389/fnagi.2016.00304

to promote the translation of any therapeutical alternative is necessary to clarify the normative and the protocols for developing clinical trials with original candidates or work upon strategies proposed from South-American countries.

Keywords: neurodegeneration, preclinical studies, pharmacological therapy, gene therapy, translational medicine

DEMENTIA EPIDEMIOLOGY: CHRONIC AND ACUTE BRAIN INJURY

Mental and neurological disorders are prevalent worldwide. Traditional epidemiological studies represent and indicate only mortality levels, and seriously underestimate the social impact of neurodegeneration; because it does not consider disability levels. The latest report on the global burden of disease shows that neurodegenerative disorders generate 1% of mortality; out of the 11.2% of disability reported worldwide. Life expectancy increase in industrialized and developing countries leads to neurological disorders and are becoming a major public health problem that could reach pandemic proportions in the coming years. It is anticipated that by 2020 the levels of disability due to neurological diseases will be above 14.7% (WHO, 2016).

Dementia (Alzheimer's disease (AD) type, vascular dementia (VaD), and other dementias) is a disease that affects more than 26 million people worldwide. Latin-American people with dementia will increase in a 368% in 2050, higher than USA and Europe (Informe ADI/Bupa, 2013; WHO, 2016). As a result of these statistical projections, clear and detailed programs are needed for capacity building in Latin-America to handle the volumes due to increased casuistic (Gonzalez et al., 2014). The prevalence of Dementia is South America exists in multiple variations due to Population, Age Structure, Genetics, life style of people who are older than 65 years. In South America there exists a big variation in the dementia prevalence, according to population indicators like population age structure, Genetics and Lifestyle of people older than 65 years, Whereas in Argentina (11.5%) and Venezuela (10.3%) present a higher prevalence of dementia, Cuba (8.2%), Brazil (5.3%), Perú (6.7%), Chile (4.3%) present an intermediate prevalence, although some lower prevalence exists in Colombia (1.8%), Uruguay (0.5%) and some cities in Brazil (1%–2%) and in rural Perú (<1%; Kalaria et al., 2008). In Colombia, people with dementia and other cognitive disorders are increasing due to the closed relationship with public health problems, such as metabolic disorders like diabetes, hypertension, cerebrovascular disease and accidents. In the Antioquia department in Colombia, the situation is more critical, due to the high incidence of familial AD. On an average more than 1000 individuals will develop the disease before reaching old age, apart from other dementias like Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) with more than 80 affected individuals per 1000. Also, an analog situation occurs in Venezuela, with an estimated Huntington's disease prevalence of 720 per 100,000 habitants in Maracaibo (Al-Jader et al., 2001).

AD is the most common cause of dementia in people over 65 years of age, including more than 20 million people are affected by sporadic AD worldwide. AD is characterized by aggregated protein that forms amyloid plaques and neurofibrillary tangles (NTFs), causing brain atrophy and cognitive impairment progression leading to dementia. Protein's aggregates are a common feature of several neurodegenerative diseases, however, some cellular accumulated proteins in the senescent brains, do not cause cognitive disorders, suggesting that, this might be the result of normal aging processes (Przedborski et al., 2003). AD has an important vascular component (Muresanu et al., 2014), and recently it has been considered that early detection of proto-fibrillar amyloid in young Familiar AD (FAD) patients by PET (Reiman et al., 2012), could be an important biomarker for the prevention of vascular deterioration, as the common cause of VaD. Nevertheless, aggregation of the tau protein is an exception. Studies that evaluate the brains of AD patients have found that the number of NTFs correlates with dementia more accurately than the amyloid plaques (Arriagada et al., 1992) in the later stage. This evidence generates great interest for therapeutic intervention in AD and other tauopathies (Ballatore et al., 2007), by chronic or acute injuries.

VaD is the second most common type of dementia, small-artery associated to hypertensive disease is a common cause around the world (73%), but presenting a low prevalence from 0.6% to 2.1% in people older to 65 years in developing countries, maybe due to poor early diagnosis and a subdiagnosis as mix dementia, which has a prevalence of 10% in Latin-American (Kalaria et al., 2008). Mix dementia combines more than one type of dementia presenting deposits associated to AD and vascular affection, which may coexist with Lewy bodies, typical of Parkinson's dementia (Alzheimer's association, 2016). Therefore, it is difficult to determine its prevalence, based upon post-morten determination. However it is closely correlated with the increased age and is also higher in the senior patients in age above >85 year old. In addition these symptoms depend upon the affected brain regions, but are also commonly diagnosed as Alzheimer's patients (94%), coexisting with vascular disease and Lewy bodies and other such nonspecific treatments have also been included (Alzheimer's association, 2016).

On the other hand, developing countries have a higher level of exposure to cerebrovascular risk factors, such as hypertension, smoking, obesity and diabetes, than in developed countries (Rizzi et al., 2014); which increase the probability for suffering an ischemic stroke, which is a major risk factor for developing VaD and AD (Vijayan and Reddy, 2016). Cerebral ischemia is a type of stroke characterized by transient or permanent decreas in blood flow due to thrombotic occlusion or embolic

in one or more cerebral arteries. This results in the deprivation of trophic factors, metabolic substrates and the activation of cell death pathways (Roberts et al., 2013). Depending on the impact of ischemia (duration of reduced blood flow and infarct location), this disease can cause various clinical manifestations. Common afflictions are Paralysis or Hemiplegia, Aphasia, problems associated with processes of memory and learning and similar other effects (Kemp and McKernan, 2002). Stroke is responsible for more than five million deaths each year worldwide, making it the second highest leading cause of death and a major cause of permanent mental and physical disability (Wen et al., 2004; Zheng et al., 2010). Accordingly to the World Health Organization (WHO) Argentina, Chile, Brazil and Uruguay present 6–11 per 1000 affected persons in comparison with 5–6 per 1000 habitants in South-America; being higher in Brazil (11 per 1000) and lower in rural Bolivia (1 per 1000; Lavados et al., 2007). Cerebrovascular accident is the second cause of death in people over 45 years of age in Colombia (Ministry of health and social protection, Colombia 2015). It is estimated that one in six people may suffer at least one episode of cerebral ischemia in life and only one in three survive as patients that too in a state of physical dependence, such cases could also develop dementia (Durukan and Tatlisumak, 2007). Stroke remains one of the cause of high social and individual cost of employee disability, leading to high spending on health and generating a negative impact on the economy of the region itself, therefore cerebral ischemia is a priority within the current scientific research.

Several studies have demonstrated presence of senile plaques (βA) and amyloid precursor protein (APP) in close proximity to the ischemic focus (Ikeda et al., 2000; Shi et al., 2006), suggesting a degree of convergence in the neuropathogenesis of cerebral ischemia and AD (Zekry et al., 2003; Snyder et al., 2015; Kalaria et al., 2016; Nelson et al., 2016). In stroke pathophysiology, changes in the phosphorylation pattern of the Tau protein during and after the ischemic event are observed (Wen et al., 2004; Gutiérrez-Vargas et al., 2016). After infarction, the rapid dephosphorylation of Tau occurs, and after blood reperfusion, there is evidence of slow but steady hyperphosphorylation, which causes an accumulation of the tau protein, resulting in long-term brain damage (Wen et al., 2004). Additionally, cerebral ischemia contributes to the development of cognitive decline and dementia, which is induced by a sedentary lifestyle, unhealthy eating habits, diabetes and other metabolic diseases (Vijayan and Reddy, 2016). Epidemiological studies have also shown that the prevalence of cognitive impairment in ischemic stroke patients is nine-fold higher and controls at 3 months and 4–12 times higher and controls 4 years after stroke, as a result three out of four patients are probable to develop dementia (Wen et al., 2004), predominantly AD-type (Pluta, 2004).

However, with respect to the cognitive impairment definition, it is necessary to clarify that it is a clinical sign related to cerebrovascular disease, but it has not been precisely quantified as a vascular cognitive impairment (VCI) incidence (Chui, 2005). Hence a vascular cognitive disorder spectrum exists between VCI to VaD (Román et al., 2004). In general, VaD is considered a deficit of new learning and memory issues more than the pattern of motor slowing and executive deficit. Some researchers suggest separated definitions, but epidemiological studies suggest a continuum of AD's disease and vascular brain disease. Also, microvascular disease is correlated with the advanced age, but not completely correlated with cognitive impairment (Selnes and Vinters, 2006). Therefore, a better understanding between vascular disease to cognitive decline and dementia risk is necessary. Maybe an early treatment of vascular affection may reduce the probability to develop dementia (Stephan et al., 2009). Also, it is necessary to unify some criteria for defining differential treatments, depending upon the ethiopathogenesis of VCI, because most of the risk factors are common to AD (Venkat et al., 2015).

Recent scientific advances support that vascular risk factors linked to cerebrovascular disease and stroke in the elderly, significantly increase the risk of developing AD. These include atherosclerosis, atrial fibrillation, coronary artery disease, hypertension and diabetes mellitus. Moreover, review of various autopsy series shows that 60%–90% of AD cases exhibit variable cerebrovascular pathology. Although some vascular lesions such as cerebral amyloid angiopathy, endothelial degeneration, and periventricular white matter lesions are evident in most cases of AD and a third would exhibit cerebral infarction (Kalaria, 2000; Snyder et al., 2015; Kalaria et al., 2016). The development and progression of both AD neuropathology and cerebrovascular lesions in the central nervous system are characterized by excitotoxicity. Activation of glial cells and upregulation of inflammatory mediators (Sandu et al., 2015), in a particular temporal, causes the disease progression converging in neurovascular affection, loss of connectivity and dementia. Indeed, maybe an anti-spreading of excitotoxicity and anti-inflammatory approaches have proven to be beneficial in the prevention and treatment of a wider spectrum of vascular cognitive impairment and dementia (VCID).

CURRENT THERAPEUTICAL APPROACH: AN INVITATION TO CHANGE THE LIFESTYLE

Currently, exist several animal models that partially solve the understanding of the neurodegenerative etiopathogenesis and its treatment. However, when the potential therapies are translated to humans it did not work or presented only a limited action. Main difficulties are the diverse comorbility associated to the cause and/or multiple affected brain regions. This reduces the efficacy of some therapies that are limited to a tissue-specific action or neurotransmission modulating types. Global investigation suggests that a general prevention could be achieved with the improvement in the quality of lifestyle, including healthy diet, physical and mental activity, and avoiding mechanical or chemical pro-inflammatory events in an early stage of the most of non-communicable diseases (NIH, 2016; WHO, 2016). Sedentary lifestyle, high calories ingest, little or nil physical and mental activity, lead to metabolic disorders

such as, hyperglycemia, dyslipidemia, hyperthension, diabetes, tissue pro-immflamatory environment. These unhealthy circumstances are a common cause of neurodegeneration due to loss of kinases/phosphatases balance and reduces brain plasticity, independently of the etiopathogenesis, which leads to Sickness, Aging and high probability to develop VCID (**Figure 1A**). Therefore, the best prevention of brain diseases is to promote a healthy lifestyle, including balanced nutrition, exercise, mental activity and social relationships apart from providing motivation and integrated health to the people (**Figure 1B**).

However, once it has started the disease must be defined how and when to treat, which continues to being a big challenge. Important progress has been made in understanding the neurodegeneration progress in AD and its treatment, for example; some medications have been approved by the FDA. But therapeutical approaches provide only a modest relief of cognitive symptoms, including disturbances in memory and perception (Bassil and Grossberg, 2009; Neugroschl and Sano, 2009); ameliorating symptoms but do not alter the course of disease progression and have even shown some undesired side effects (Soreq and Seidman, 2001; Bassil and Grossberg, 2009; Mimica and Presecki, 2009). Currently, available drugs for AD's treatment are glutamate modulators and acetyl-cholinesterase (AChE) inhibitors with just palliative outreach. Glutamate modulators continue being studied for improving its action (Danysz and Parsons, 2012), because they have poor effect in severe AD patients and carry several side effects. AChE has been detected in senile plaques (Gomez-Ramos et al., 1992), and its inhibition reduces the metabolic degradation of ACh slowing the progression of cognitive dysfunction. In consequence, AChE inhibitors are indicated in the early stage of the disease, delaying the memory and attention deterioration. This treatment is combined with other drugs for associated AD symptons, such as depression, agitation, sleep disturbances, or later complications like sphincter incontinence, urinary infections, ulcers and thrombophlebitis caused by immobility. However, many of these drugs have several side effects, such as diarrhea, dizziness, loss of appetite, muscle cramps, nausea, fatigue, insomnia, vomit, weight loss and hepatotoxicity, reducing the use of those medicaments.

Other research have applied immunotherapy for AD treatment, for example it has been developed that the AN-1792 vaccine (Tabira et al., 2011), which is a synthetic form of βA protein, that could stimulate the immune system by eliminating βA plaques and preventing emergence of new ones. But adverse effects like development of meningoencephalitis have been identified, resulting in non-approval of the treatment (Delrieu et al., 2012). Currently, there are several immunotherapy clinical trials in phase II and III, which evaluate new strategies for blocking βA, or at least for slowing the disease progression (Mangialasche et al., 2010). For example, the placebo-controlled first prevention trial of crenezumab immunotherapy developed by Genentech, Banner and our research group "GNle of A", is being evaluated with high risk members from families carrying the E280A-PS1 AD mutation, before onset of mild cognitive impairment (MCI) stage (Neurology, 2012). However, new therapeutical agents capable of blocking cognitive impairment progress in AD must be identified. At the same time it is necessary to determine the critical event that triggers the disease's development, and for trying to offer not only a slower or a palliative approach, but ideally to offer a preventive or curative treatment without side effects on the patient's health. Currently these are not available on the market.

On the other hand, today, according to the National Institute of Infarction in USA, the only therapy approved by the FDA for using in the first 4.5 h post-ischemic stroke is the thrombolytic therapy with recombinant tissue plasmilógeno (tPA; Hanger et al., 2007). This fibrinolytic process is a cost-effective treatment: which does not increase process costs and is efficient because it reduces post-infarction failure, resulting in a better quality of life for patients and reduced health costs on the long term (Rajan et al., 2015). However, the main limitation of this treatment is the availability of short therapeutic window between 3 h and 4.5 h post-stroke, also due to the stringent inclusion and exclusion criteria very few patients have benefited. Only between 20% and 25% of patients arrive on time to a hospital, and between 3% and 5% of them are candidates for thrombolysis, out of which approximately 50% are reperfused (Auriel and Bornstein, 2010). Due to the complexity of cerebrovascular disease, no effective therapies for neuroprotection after acute window are available, that could prevent or block or to reverse the progressive degeneration on long term, as cognitive impairment and dementia. Therefore, new therapeutical approaches are necessaries to find effective and safety neuroprotective agents. In this review article, we highlight gene therapy for a specific gene intervention, and natural products and their derivatives as promising candidates.

PRECLINICAL STUDIES AND MOLECULAR TARGETS

For the last two decades, neuroprotective agents designed to block death cascade have been investigated in animal models. Numerous agents have been proposed to neurodegeneration in rodent, rabbit and primate models (Yuede et al., 2007; Van Dam and De Deyn, 2011). However, trying to solve difficulties from different kind of risk factors in VCID, as age, diabetes, hypertension, metabolic syndrome and some specific models for VCID has been proposed (Helman and Murphy, 2016; Madigan et al., 2016). Although there exist a big variability between them (Venkat et al., 2015), and the most common affection is the cognitive impairment. Therefore, an "ideal VCID animal model" is not possible, because of the existence of a wide spectrum in the definition and variation in animal models of VCID. But each preclinical animal model under controlled variable based in specific human VCI pathogenesis could give light for intervention (Jiwa et al., 2010; Helman and Murphy, 2016). Also, considering that there exist a primary or secondary convergence in the hippocampus alteration, the question could

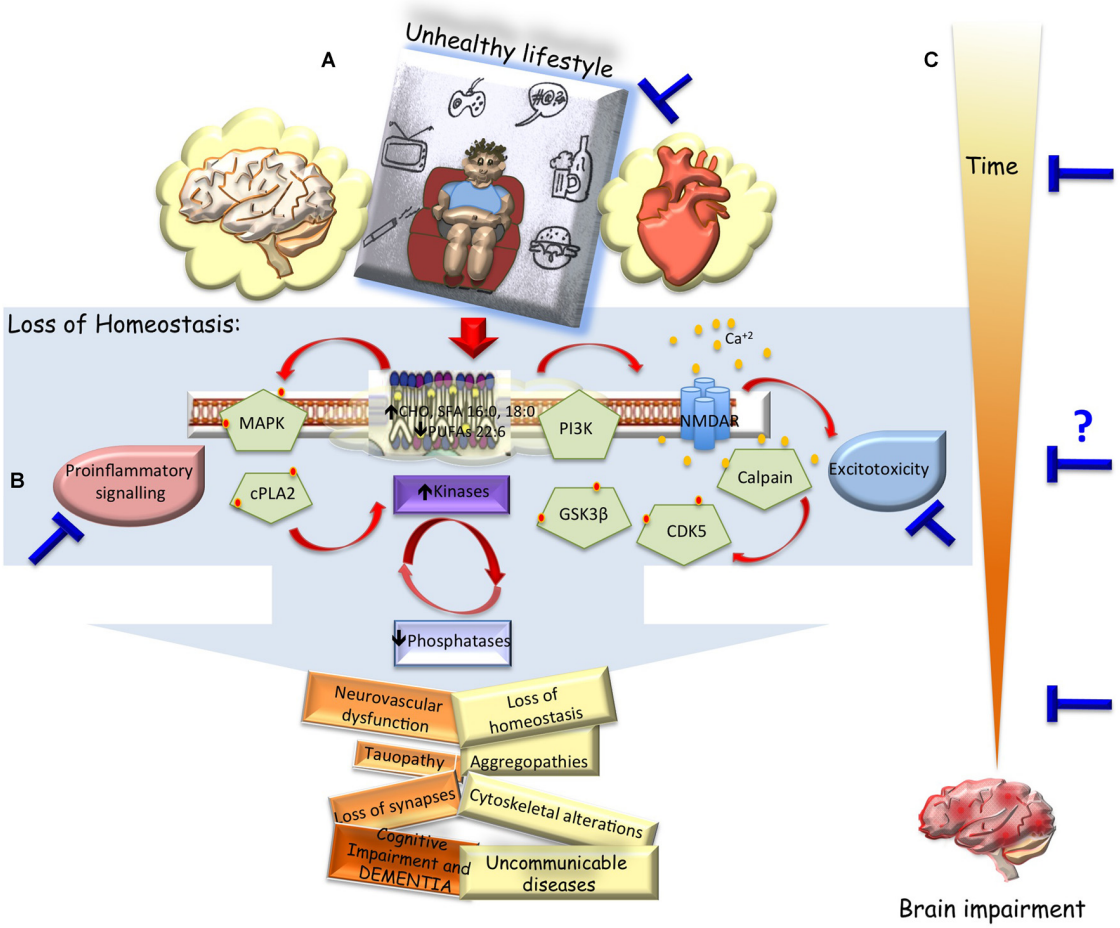


FIGURE 1 | Unhealthy lifestyle vs. dementia and therapeutical approach. (A) Hypothetical scheme representing how an unhealthy lifestyle could change the cell environment, triggering imbalanced cell signaling and lossing intrinsic regulation (altered saturated/unsaturated phospholipid composition, spreading of excitotoxicity, kinases/phosphates disbalance, pro-inflammatory response). Which could generate progressive affection of systemic functions (v.gr. metabolic disorders, diabetes, hypertension, atherosclerosis), in general; and cerebral dysfunction in particular, inducing loss of connectivity, vascular cogntivie impairment and dementia. **(B)** Convergent disbalance events in the brain after acute or chronic injury, which could be blocked by change of habits, or pharmacological and gene therapies in pivotal events (blue bars). **(C)** Being the main difficulty to define the inclusion criteria and the intervention time for translational approach, but highlighting the diverse therapeutical windows opportunities and phases of prevention of brain impairment.

be focused in to analyze the *in vitro* and *in vivo* vascular implications.

On the other side, diverse kind of targets could be good options for blocking the progression of brain impairment and dementia (Anand et al., 2012), but this raises difficult questions like; How to define the stem event, Temporal sequence of the neurodegenerative processes, Sex and age dependent effects, when and how to carry out the intervention as well as the best analog situation in humans for a better safety and efficacy properties?

Several research groups in SouthAmerica are working in order to answer some of these questions, for example in Brazil and Argentina. Further Chile is working on methods to better understanding of neurodegeneration and looking for new treatment strategies and biomarkers for early detection of AD (Rojo et al., 2010; Beauquis et al., 2014; Vargas et al., 2014; Martino Adami et al., 2015; Nunes et al., 2015) and VaD (Valério Romanini et al., 2013; Schiavon et al., 2014; Silva et al., 2015; Primo et al., 2016). In Colombia, some experimental designs have been developed on ischemic stroke and Alzheimer's animal models trying to propose therapeutical approach that would be versatile in the time (prevention, blocking progress and reversion) as mentioned later.

For AD studies, triple-transgenic AD mice model (3 × Tg-AD) is being used. The 3 × Tg-AD model harbors the PS1 (M146V), APP (Swe), and tau (P301L) transgenes (Oddo et al., 2003). The 3 × Tg-AD mice progressively develops Aβ plaques (3–6 months) and NFTs (12 months). In addition, 3 × Tg-AD exhibits synaptic dysfunction, including LTP deficits, like those present in an age-related manner. These deficits in long-term synaptic plasticity correlate with the accumulation of intraneuronal βA and NTFs formation. Interestingly, silenced BACE1 (main enzyme involved in the production of βA), in the hippocampus using adenoassociated viral vectors reduces

the hyperphosphorilation of tau (Piedrahita et al., 2016) and improve cognitive function at 6 months and 12 months post-treatment, presenting a balanced phospholipid composition, by the reduction of saturated fatty acid (stearic acid (18:0), palmitic acid (16:0)) and increase of poli-unsaturated fatty acid (docosahexaenoic acid, DHA (22:6)), which prevented the activation of pro-inflammatory signaling cPLA2/AA/COX2 in old AD mice to long-term post-therapy (Villamil-Ortiz et al., 2016). Also, the silencing of CDK5 in the CA1 hippocampal area prevents the spreading of excitoxicity to other areas of the neuronal circuit (Piedrahita et al., 2010; Castro-Alvarez et al., 2014, 2015; Posada-Duque et al., 2015a) also reversing (Piedrahita et al., 2010) or preventing neurodegeneration by reduction of paired helicoidal formations (Castro-Alvarez et al., 2014) and further, decreases the β amyloidosis production (Castro-Alvarez et al., 2015). The result could be assumed as it involves the prevention of Calpain activation by reduction of the active p25/CDK5 complex formation (Posada-Duque et al., 2015a) with a clear impact on the down-regulation of phosphorilation rate of tau (Castro-Alvarez et al., 2015), as well as regulation of phosphatases and GSK3β activity (Castro-Alvarez et al., 2015). All of this, would lead to the control of both histopathological hallmarks, improving the neurotransmission and synaptic remodeling (Posada-Duque et al., 2016); at the same time recovering the complex synaptic molecular adhesion (p120 ctn/PSD95), which has a consequential long-term effect (12 months) in protection and improvement of cognitive function (Castro-Alvarez et al., 2015; Uribe-Arias et al., 2016). Therefore, safe and effective studies suggest that the BACE1 and CDK5 RNAi-based therapy would be ready for a potential translational trial in a patient with advanced or mild stage of Alzheimer disease, without additional option for a curative treatment.

On the other side, we take global and focal models of cerebral ischemia. In the global model occlusion of two vessels analog to a cardiac arrest is used, which develops hyperphosphorilated tau and cognitive impairment (Gutiérrez-Vargas et al., 2010; Castro-Alvarez et al., 2011). In the case of focal ischemia, the occlusion of the middle cerebral artery (t-MCAO), is useful for determining the infarct volume and the ischemic/reperfusion stroke analog to human ischemic event. There is prevelance of neurodegenerative hallmarks such as hyperphosphorilated tau, microgliosis and cognitive impairment (Gutiérrez-Vargas et al., 2015b, 2016).

Other previous research has been focused on the proposal of therapeutical alternatives with atorvastatin after the first 6 h (Céspedes-Rubio et al., 2010) and to long term post-cerebral infarction (1 month; Gutiérrez-Vargas et al., 2014), this remains valid and is potentially transferable to humans without hemorrhagic risk. As an alternative to reduceing neurological sequelae to a short and long term post-ischemia (Gutiérrez-Vargas et al., 2015a). However, CDK5 silencing in the hippocampus of ischemic rats has very impacting benefits, because it reverses learning and relearning impairment caused by cerebral ischemia, correlated with the prevention of neuronal loss, decreased hyperphosphorylated tau, reactive astroglia and microglia, within a month of post-ischemia/reperfusion (Gutiérrez-Vargas et al., 2015b). CDK5 silencing effects remain at 4 months post-ischemic, avoiding cognitive disturbance and reversing neuropathological markers, showing a potentially versatile use out of the current approved therapeutical window or in any advance stage of the VCI post-ischemic stroke. CDK5 RNAi-induced neurotransmission was a BDNF (brain derivate neutrophic factor) dependent (Gutiérrez-Vargas et al., 2016). Additional advantage shows that the targeting of CDK5 in astrocytes has protective benefits, because CDK5 RNAi induced a morphological-functional that changes in the branching and BDNF release from astrocytes protecting co-cultured neurons (Posada-Duque et al., 2015b) and increases the endotelial adhesion in ischemic brain of rats (Becerra-Calixto and Cardona-Gómez, 2016). In general, these findings suggesting that CDK5 based therapy recover the neurovascular unit integrity post-stroke (Posada-Duque et al., 2014) and also, those findings support the potential in translational studies in demented post-ischemic patients as well.

Nevertheless, looking for other translational alternatives, pharmacological therapy using natural products has a big potential. Natural products have been widely used as antioxidants or antiradicals agents, so that there are reports of inverse relationship between a diet with foods rich in antioxidants and the incidence of diseases (Sies, 1993). However, synthetic antioxidants in the food industry have proven to be responsible for liver damage and carcinogenesis and are less effective. For this reason, the use of natural antioxidants investigation has increased recently (Krishnaiah et al., 2010; Mecocci and Polidori, 2012). Natural products have a wide variety of biological effects: anti-inflammatory, anticancer, antiviral, anti-thrombotic and among others (Lee et al., 2011; Yang et al., 2014). In CNS diseases natural products have anti-convulsant antioxidant, analgesic, anxiolytic, anti-depressant, being effective in various pathologies. In addition, natural products are considered as a source of potential molecules in the field of neuroprotection (Lara-Guzman et al., 2012; Ho et al., 2013; Sumi et al., 2013; Dong et al., 2014). Therefore, in order to find pharmacological alternatives for a more agile translation to humans, a systemic administration during 3 months in a late stage of the AD mice model has been evaluated, using various natural products as flavonoids by intraperitoneal administration and monoterpenes by oral administration, which surpresively reduced the β-amyloidosis, tauopathy, cognitive disorder and anxiety present in old triple transgenic mice model of AD (Sabogal-Guáqueta et al., 2015, 2016), which could be useful in different kind of neurodegenerative environment and different advanced stages with proinflammatory markers and synaptic deterioration as VCID. Bioavailability analysis and scaling the production will increase the possibility to develop a clinical trial from South America. In summary, those experimental evidences using gene and pharmacological therapies have suggested that the neuroprotective capacity of different therapeutical strategies, convergent in blocking the change of the membrane phospholipid composition avoids the spreading of excitatory

wave phenomenon and/or stopps the pro-inflammatory response in the time (**Figure 1B**).

SENSOR OF NEURODEGENERATION: A CONVERGENT CELL PHENOMENA IN COGNITIVE IMPAIRMENT AND DEMENTIA

Many targets are viable as strategy for preventing neurodegeneration, however based upon several evidences; the hyperphosphorilation of tau is a key phenomena that reflect cell imbalance associated to progressive brain impairment. Especially in cognitive disorder and dementia by diferent ethiologies. Tauopathy is a sensor of excitotoxicity, loss of homoeostasis, metabolic disbalance, loss of kinases/phosphateses regulation, consequences of pro-inflammatory environment, inductor of plasticity and synaptic remodeling failure are the main hallmarks of cognitive impairment and dementia (**Figure 1**).

AD and other tauopathies are progressive supranuclear palsy (PSP), linked to chromosome 17 Parkinson, frontotemporal dementia and cerebral ischemia, are characterized by a phenomenon of hyperphosphorylation of tau protein, causing changes in the microtubule (MT) assemblying-disassemblying dynamics (Avila et al., 2004). Tau is dissociated from MTs under the abnormal action of kinases and cytosolic accumulated as paired helical filaments (PHFs) can be bound forming NTFs (Grundke-Iqbal et al., 1986); However the condition that facilitate aggregation and formation of those structures are still unknown, stressing the importance for deeper understanding of the cellular contexts involved in such phenomenon.

Currently, 45 phosphorylation sites of tau in the brain of AD have been identified along with its 441 amino acids—that are involved in the formation of PHFs (Augustinack et al., 2002; Hanger et al., 2009). *In vitro* experiments have shown that tau is a substrate for multiple kinases including PKA, CaMKII, PKC, MAPK, MARK, CDK5 and GSK3. Among these kinases, CDK5 and GSK3 (along with their activators p35, p39, p25 and p29), has been shown to phosphorylate tau in AD related epitopes (Kobayashi et al., 1993; Paudel et al., 1993; Patrick et al., 1999). Also, the hyperphosphorilation of tau is increased by transient focal cerebral ischemia (Céspedes et al., 2013) and overall, causes impaired spatial memory (Castro-Alvarez et al., 2011), triggering in this process alteration of proteins that regulate MT assemblying. Such as CDK5, GSK3 and actin cytoskeleton, as RhoA (Céspedes-Rubio et al., 2010) and Rac (Gutiérrez-Vargas et al., 2010), small GTPases proteins and phosphatases as well. Damage of the neuronal cytoskeleton can be considered as the main cause of the loss of protein transport and neuronal instability in cerebral ischemia. MT disassemblying occurs after ischemia and plays an important role in the pathophysiology of this neurovascular disease (Pettigrew et al., 1996). Alterations in the cytoskeleton partly reflect degradation and protein aggregation after ischemia (Kühn et al., 2005). Degradation and aggregation of MAP2 and Tau hyperphosphorylation are biomarkers for the progression of ischemic damage (Pettigrew et al., 1996), and are supported by some studies showing immunoreactivity of tau in neurons and glia from thalamus, hippocampus and cerebral cortex in brain slices from people who suffered an ischemic event (Uchihara et al., 1995, 2000; Irving et al., 1996) and also in experimental cerebral ischemia models (Geddes et al., 1994; Dewar and Dawson, 1995; Irving et al., 1996).

In general, recent studies suggest that the brain pathophysiology of different kinds of VCID has a common commitment of the kinases/phosphatases balance and neurovascular integrity (Posada-Duque et al., 2014). And as a potential marker of the tissue disequilibrium progression and as a consequence of the chronic membrane composition and cell signaling alteration, inducing cognitive impairment (Llorens et al., 2016; Viswanathan et al., 2016), could be the taupathy (**Figure 1**).

HUMAN PRECLINICAL STUDIES

Other strategic approach could be based in the casuistic of pure dementias in the Latin-American region, which give an exceptional opportunity to understand the pathogenesis in these human populations with genetic risk, and go in favor of the basic and clinical researchers interaction for a better understanding and medical care of mix dementias, which have more complex factors than pure ones.

The pure dementias are very rare. Most patients with dementia have mixed factors leading to dementia. Therefore, It is very common to notice AD patients with brain atrophy having vascular changes characterized by cortical infarcts, subcortical or cortico-suborticales and lecucoencefalopatía or signs of vascular suffering of the white substance which correlates with mixed dementias. Similarly, patients with VaD, also present atrophy and neurodegenerative changes that are also difficult to identify as pure case of dementia. Alzheimer's disease and a pure case of vascular dementia.

Therefore, it is important to note that autosomal dominant AD is a pure Mendelian, neurodegenerative dementia and the autosomal dominant VaD by CADASIL is a pure VaD, the rest are vascular changes. The Mendelian autosomal dominant forms of AD and CADASIL model are two ends of a single chain. One is a model of neurodegeneration, while the other is a model of vascular disease. The comparison between these two types of dementias in clinical, neuro and pathophysiological levels will allow us to differentiate a vascular vs. a neurodegenerative dementia. But most importantly, these two models of dementia allows genetic preclinical studies providing the possibility to identify the healthy relatives carrying out the mutation in presenilin or NOTCH3 respectively and is also ideal for evaluating preventive therapies. But the more exceptional point is that these affected populations with genetic AD and CADASIL in Latin America come from large families with high average number of children per family, allowing prevention studies in population with those genetic risks.

On the other side, the search of AD treatments have evolved in the recent years. Therapeutical strategies have changed from palliative and symptomatic approach toward preventive therapies, which are considered of three types: (a) tertiary prevention therapies, consisting of treating affected population

with MCI for preventing its conversion to dementia. Therefore, tertiary prevention is not properly a preclinical therapy, but it is an early intervention of the clinical phase of the disease. (b) Secondary prevention studies are aimed to prevent the onset of symptoms of AD in people who already have the disease's neuropathological changes. Currently, few studies of secondary prevention therapies are being conducted, such as: Dominantly Inherited Alzheimer's Network Trials Unit (DIAN-TU), testing Gantenerumab, an aggregated anti-βA antibody, and A4 (Anti-Amyloid treatment in Asymptomatic Alzheimer's) trial, using Solanezumab (a soluble anti-βA antibody); which invite to clinically normal individuals of 65–85 years old with elevated fibrillar amyloid in brain detected by PET Amyloid to be treated (Doody et al., 2014), each drug compared to the pooled placebo. API COLOMBIA, Alzheimer's Prevention Initiative (API-Colombia) in Latin America, which is evaluating the passive β-amyloid (βA) immunization therapy Crenezumab in cognitively unaffected persons 30–60 years old from the world's largest known ADAD kindred in Antioquia, Colombia (Reiman et al., 2011); and API APOE4 Trial in USA will evaluate an immunization therapy in 60–75 year-old cognitively unimpaired APOE4 homozygotes. And finally, in studies of primary prevention (c), the aim is to prevent the onset of neuropathological changes of the people with high risk. However, nobody is conducting studies of primary prevention for AD or other kind of dementia. Therefore, the challenge for future is to design and to develop primary prevention studies in people with high risk for developing the disease, without symptoms or neuropathological changes. Here exist a big opportunity for developing this kind of studies, because it has been identified that there are more than 400 young carriers in the E280A FAD population.

TRANSLATIONAL PERSPECTIVE IN SOUTH AMERICA: CHALLENGES AND PITFALLS

Despite having identified potential molecular targets, there are considerable limitations inherent to the nervous system, such as crossing the blood-brain barrier and the difficulty for targeting specific neuronal populations. The presence of these obstacles has prompted the search for new strategies for the treatment of neurodegenerative diseases, using viral vectors, design of nanoparticle for improving delivery, and future approaches by systemic administration vectors or functionalized nutrition, but including safety and efficacy of the treatment remains a major challenge.

In addition to those inherent difficulties of the central nervous system, general translation of drug discovery to clinical usefulness needs to overcome several limitations, such as sensibility, specificity, well stratified patients, correct analytical approaches and differentiation from others (Drucker and Krapfenbauer, 2013). However, to promove the translation of therapeutical alternatives impacting morbility and mortality is an urgent necessity around the world and mainly in South American countries. More specifically in Colombia, there is a need to formulate policies and procedures on the subject in addition to developing a quality emergency for medical care for acute injuries (Razzak and Kellermann, 2002). Also, we need to catch up in normatives and protocols for developing clinical trials with original candidates or strategies proposed from our South American countries; and a concerted effort from scientists, medical specialists, pharmaceutical industry and government, accompanied by economical and social support. We should be able to offer experimental treatments, without false expectations to the patient/family, based in the rigorousness of the scientific preclinical evidences, a close and direct dialog with the medical team, peer-review vigilance of the I, II and III phases, and strict long-term monitoring of the potential unwanted side-effects (Main et al., 2014), are also essential. This would progressively strengthen the contribution for solving the increased mental health problems.

Now, focused in the pathogenesis, one of the most difficult aspect for developing a new strategy to block or to prevent neurodegeneration is to define a therapeutical window and its versatility to similar disorders such as VCID. For example, AD is a progressive disorder apparently preceded by a dormant period of several years, sometimes even decades before cognitive decline is evident. This normally makes it difficult to establish a suitable time window for initiating treatment. But after ischemic stroke there exist a high probability to develop cognitive impairment within weeks or months (Snyder et al., 2015; Kalaria et al., 2016), but there are no available preventive therapy. Interestingly, both neurological disorders present common phenomena like cognitive impairment and dementia that could be blocked using similar strategies. The challenge is when to intervent in each type of neurodegenerative process? (**Figure 1C**). Some experimental studies comparing tauopathy in Alzheimer's animal model and ischemic stroke in rats, show that in both models exist an intrinsic homeostasis in an early period itself. But, due to loss of time this intrinsic balance is lost (Villamil-Ortiz and Cardona-Gómez, 2015; Madigan et al., 2016), generating intermediate and late period of degenerative progress that reflect in hyperphosphorylation of tau leading to loss of connectivity, therefore, this could be the common phenomenon to attack in any potential moment of the triggered cascade (**Figure 1C**).

In Colombia, the development of clinical trials with our own discovered therapeutical candidates have not been developed yet. A primary prevention therapy would impact the affected population and would be a boost for solving related diseases, for example like in young carriers of AD or CADASIL mutation. However, trying to put together the different and existing barriers, these are necessary: (a) discuss the inclusion and exclusion criteria of the translational medicine by interdisciplinary team of a project or institution; (b) it is necessary to prepare and to present a clinical trial protocol to a national regulatory entity, for its approval, seeking particular adjustment in each Latin American country; (c) to convince an investment agency of the need to support a clinical trial with support from original proposal from developing countries; (d) proceed step by step in the advancement of the clinical phase

to the benefit as many people as possible. Finally, if this could be achieved, then it would result in providing a boost to the scientific and biotechnology development of the region.

CONCLUSION

Society is being more exposed to dangerous environment and habits that induces chronic diseases, physical and mental disabilities. A Stronger Public Health normatives and social programs for prevention of unhealthy lifestyle must be developed. Non transmisible disorder triggering cognitive impairment and dementia and existing rigorous preclinical evidences that block or reverse the brain pathology disorders as presented above either by using silencing gene therapy or by systemic treatments with natural products should be further studied and researched. Colombia and South American countries should invest and support clinical trials with the understanding of the casuistic of pure dementias in patients for developing primary prevention methods. But at the same time should moderate it by normative and adequate vigilance from peer-reviewers for long-term monitoring of the potential unwanted side-effects. This action would be a great strategy for the scientific and biotechnology development of the region, and a worthy contribution to solve a major health problem.

AUTHOR CONTRIBUTIONS

GPC-G and FL discussed, wrote and accepted the final version of the manuscript.

ACKNOWLEDGMENTS

We would like to thank Cellular and Molecular Neurobiology team from group of Neuroscience of Antioquia (GNA) and to the Bioactive Substances group (GISB), from University of Antioquia, for their scientific advice and constructive academic discussion. This research was supported by Colciencias projects (Departamento Administrativo de Ciencia, Tecnología e Innovación) (code #111554531478, #111551928905 and #111554531400) (GPC-G).

REFERENCES

Al-Jader, L. N., Harper, P. S., Krawczak, M., and Palmer, S. R. (2001). The frequency of inherited disorders database: Prevalence of Huntington disease. *Community Genet.* 4, 148–157. doi: 10.1159/000051175

Alzheimer's association. (2016). Available online at: http://www.alz.org/dementia/mixed-dementia-symptoms.asp

Anand, A., Banik, A., Thakur, K., and Masters, C. L. (2012). The animal models of dementia and Alzheimer's disease for pre-clinical testing and clinical translation. *Curr. Alzheimer Res.* 9, 1010–1029. doi: 10.2174/156720512803569055

Arriagada, P. V., Growdon, J. H., Hedley-Whyte, E. T., and Hyman, B. T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* 42, 631–639. doi: 10.1212/wnl.42.3.631

Augustinack, J. C., Schneider, A., Mandelkow, E. M., and Hyman, B. T. (2002). Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol.* 103, 26–35. doi: 10.1007/s004010100423

Auriel, E., and Bornstein, N. M. (2010). Neuroprotection in acute ischemic stroke—current status. *J. Cell. Mol. Med.* 14, 2200–2202. doi: 10.1111/j.1582-4934.2010.01135.x

Avila, J., Lucas, J. J., Perez, M., and Hernandez, F. (2004). Role of tau protein in both physiological and pathological conditions. *Physiol Rev.* 84, 361–384. doi: 10.1152/physrev.00024.2003

Ballatore, C., Lee, V. M.-Y., and Trojanowski, J. Q. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat. Rev. Neurosci.* 8, 663–672. doi: 10.1038/nrn2194

Bassil, N., and Grossberg, G. T. (2009). Novel regimens and delivery systems in the pharmacological treatment of Alzheimer's disease. *CNS Drugs* 23, 293–307. doi: 10.2165/00023210-200923040-00003

Beauquis, J., Vinuesa, A., Pomilio, C., Pavía, P., Galván, V., and Saravia, F. (2014). Neuronal and glial alterations, increased anxiety and cognitive impairment before hippocampal amyloid deposition in PDAPP mice, model of Alzheimer's disease. *Hippocampus* 24, 257–269. doi: 10.1002/hipo.22219

Becerra-Calixto, A., and Cardona-Gómez, G. P. (2016). Neuroprotection induced by transplanted CDK5 knockdown astrocytes in global cerebral ischemic rats. *Mol. Neurobiol.* doi: 10.1007/s12035-016-0162-2 [Epub ahead of print].

Castro-Alvarez, J. F., Gutiérrez-Vargas, J., Darnaudéry, M., and Cardona-Gómez, G. P. (2011). Rock inhibition prevent hyperphosphorylation of Tau and p25/CDK5 increase after global cerebral ischemia. *Behav. Neurosci.* 125, 465–472. doi: 10.1037/a0023167

Castro-Alvarez, J. F., Uribe-Arias, A., and Cardona-Gómez, G. P. (2015). Cyclin-Dependent kinase 5 targeting prevents β-Amyloid aggregation involving glycogen synthase kinase 3β and phosphatases. *J. Neurosci. Res.* 93, 1258–1266. doi: 10.1002/jnr.23576

Castro-Alvarez, J. F., Uribe-Arias, S. A., Kosik, K. S., and Cardona-Gómez, G. P. (2014). Long- and short-term CDK5 knockdown prevents spatial memory dysfunction and tau pathology of triple transgenic Alzheimer's mice. *Front. Aging Neurosci.* 6:243. doi: 10.3389/fnagi.2014.00243

Céspedes, A. E., Arango, C. A., and Cardona-Gómez, G. P. (2013). Injury markers in two models of cerebral ischemia. *Biomedica* 33, 292–305. doi: 10.7705/biomedica.v33i2.830

Céspedes-Rubio, Á., Wandosell Jurado, F., and Cardona-Gómez, G. P. (2010). p120 catenin/α N-catenin are molecular targets in the neuroprotection and neuronal plasticity mediated by atorvastatin after focal cerebral ischemia. *J. Neurosci. Res.* 88, 3621–3634. doi: 10.1002/jnr.22511

Chui, H. (2005). Neuropathology lessons in vascular dementia. *Alzheimer Dis. Assoc. Disord.* 19, 45–52. doi: 10.1097/01.wad.0000155065.31561.0a

Danysz, W., and Parsons, C. G. (2012). Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine—searching for the connections. *Br. J. Pharmacol.* 167, 324–352. doi: 10.1111/j.1476-5381.2012.02057.x

Delrieu, J., Ousset, P. J., Caillaud, C., and Vellas, B. (2012). "Clinical trials in Alzheimer's disease": immunotherapy approaches. *J. Neurochem.* 120, 186–193. doi: 10.1111/j.1471-4159.2011. 07458.x

Dewar, D., and Dawson, D. (1995). Tau protein is altered by focal cerebral ischaemia in the rat: an immunohistochemical and immunoblotting study. *Brain Res.* 684, 70–78. doi: 10.1016/0006-8993(95)00417-o

Dong, Y. S., Wang, J. L., Feng, D. Y., Qin, H. Z., Wen, H., Yin, Z. M., et al. (2014). Protective effect of quercetin against oxidative stress and brain edema in an experimental rat model of subarachnoid hemorrhage. *Int. J. Med. Sci.* 11, 282–290. doi: 10.7150/ijms.7634

Doody, R. S., Thomas, R. G., Farlow, M., Iwatsubo, T., Vellas, B., Joffe, S., et al. (2014). Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N. Engl. J. Med.* 370, 311–321. doi: 10.1056/NEJMoa1312889

Drucker, E., and Krapfenbauer, K. (2013). Pitfalls and limitations in translation from biomarker discovery to clinical utility in predictive and personalised medicine. *EPMA J.* 4:7. doi: 10.1186/1878-5085-4-7

Durukan, A., and Tatlisumak, T. (2007). Acute ischemic stroke: overview of major experimental rodent models, pathophysiology and therapy of focal cerebral ischemia. *Pharmacol. Biochem. Behav.* 87, 179–197. doi: 10.1016/j.pbb.2007.04.015

Geddes, J. W., Schwab, C., Craddock, S., Wilson, J. L., and Pettigrew, L. C. (1994). Alterations in τ immunostaining in the rat hippocampus following transient cerebral ischemia. *J. Cereb. Blood Flow Metab.* 14, 554–564. doi: 10.1038/jcbfm.1994.69

Gomez-Ramos, P., Mufson, E. J., and Moran, M. A. (1992). Ultrastructural localization of acetylcholinesterase in neurofibrillary tangles, neuropil threads and senile plaques in aged and Alzheimer's brain. *Brain Res.* 569, 229–237. doi: 10.1016/0006-8993(92)90634-l

Gonzalez, F. J., Gaona, C., Quintero, M., Chavez, C. A., Selga, J., and Maestre, G. E. (2014). Building capacity for dementia care in latin america and the caribbean. *Dement. Neuropsychol.* 8, 310–316. doi: 10.1590/s1980-57642014dn84000002

Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M., and Binder, L. I. (1986). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U S A* 83, 4913–4917. doi: 10.1073/pnas.83.13.4913

Gutiérrez-Vargas, J., Castro-Álvarez, J. F., Velásquez-Carvajal, D., Montañez-Velásquez, M. N., Céspedes-Rubio, Á., and Cardona-Gómez, G. P. (2010). Rac1 activity changes are associated with neuronal pathology and spatial memory long-term recovery after global cerebral ischemia. *Neurochem Int.* 57, 762–773. doi: 10.1016/j.neuint.2010.08.014

Gutiérrez-Vargas, J. A., Cespedes-Rubio, Á., and Cardona-Gómez, G. P. (2015a). Perspective of synaptic protection after post-infarction treatment with statins. *J. Transl. Med.* 13:118. doi: 10.1186/s12967-015-0472-6

Gutiérrez-Vargas, J. A., Múnera, A., and Cardona-Gómez, G. P. (2015b). CDK5 knockdown prevents hippocampal degeneration and cognitive dysfunction produced by cerebral ischemia. *J. Cereb. Blood Flow Metab.* 35, 1937–1949. doi: 10.1038/jcbfm.2015.150

Gutiérrez-Vargas, J. A., Moreno, H., and Cardona-Gómez, G. P. (2016). Targeting CDK5 post-stroke provides long-term neuroprotection and rescues synaptic plasticity. *J. Cereb. Blood Flow Metab.* doi: 10.1177/0271678x16662476 [Epub ahead of print].

Gutiérrez-Vargas, J. A., Muñoz-Manco, J. I., Garcia-Segura, L. M., and Cardona-Gómez, G. P. (2014). GluN2B N-methyl-D-aspartic acid receptor subunit mediates atorvastatin-Induced neuroprotection after focal cerebral ischemia. *J. Neurosci. Res.* 92, 1529–1548. doi: 10.1002/jnr.23426

Hanger, D. P., Anderton, B. H., and Noble, W. (2009). Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol. Med.* 15, 112–119. doi: 10.1016/j.molmed.2009.01.003

Hanger, C., Fletcher, V., Fink, J., Sidwell, A., and Roche, A. (2007). Improving care to stroke patients: adding an acute stroke unit helps. *N Z Med. J.* 120:U2450.

Helman, A. M., and Murphy, M. P. (2016). Vascular cognitive impairment: modeling a critical neurologic disease *in vitro* and *in vivo*. *Biochim. Biophys. Acta* 1862, 975–982. doi: 10.1016/j.bbadis.2015.12.009

Ho, L., Ferruzzi, M. G., Janle, E. M., Wang, J., Gong, B., and Chen, T. Y. (2013). Identification of brain-targeted bioactive dietary quercetin-3-O-glucuronide as a novel intervention for Alzheimer's disease. *FASEB J.* 27, 769–781. doi: 10.1096/fj.12-212118

Ikeda, K., Akiyama, H., Arai, T., Kondo, H., Haga, C., Tsuchiya, K., et al. (2000). Neurons containing Alz-50-immunoreactive granules around the cerebral infarction: evidence for the lysosomal degradation of altered tau in human brain? *Neurosci. Lett.* 284, 187–189. doi: 10.1016/s0304-3940(00)01009-0

Informe ADI/Bupa. (2013). La demencia en América: El coste y la prevalencia del Alzheimer y otros tipos de demencia.

Irving, E. A., Nicoll, J., Graham, D. I., and Dewar, D. (1996). Increased tau immunoreactivity in oligodendrocytes following human stroke and head injury. *Neurosci. Lett.* 213, 189–192. doi: 10.1016/s0304-3940(96)12856-1

Jiwa, N. S., Garrard, P., and Hainsworth, A. H. (2010). Experimental models of vascular dementia and vascular cognitive impairment: a systematic review. *J. Neurochem.* 115, 814–828. doi: 10.1111/j.1471-4159.2010.06958.x

Kalaria, R. N. (2000). The role of cerebral ischemia in Alzheimer's disease. *Neurobiol. Aging* 21, 321–330. doi: 10.1016/s0197-4580(00)00125-1

Kalaria, R. N., Akinyemi, R., and Ihara, M. (2016). Stroke injury, cognitive impairment and vascular dementia. *Biochim. Biophys. Acta* 1862, 915–925. doi: 10.1016/j.bbadis.2016.01.015

Kalaria, R. N., Maestre, G. E., Arizaga, R., Friedland, R. P., Galasko, D., Hall, K., et al. (2008). Alzheimer's disease and vascular dementia in developing countries: prevalence, management and risk factors. *Lancet Neurol.* 7, 812–826. doi: 10.1016/S1474-4422(08)70169-8

Kemp, J. A., and McKernan, R. M. (2002). NMDA receptor pathways as drug targets. *Nat. Neurosci.* 5, 1039–1042. doi: 10.1038/nn936

Kobayashi, S., Ishiguro, K., Omori, A., Takamatsu, M., Arioka, M., Imahori, K., et al. (1993). A cdc2-related kinase PSSALRE/cdk5 is homologous with the 30 kDa subunit of tau protein kinase II, a proline-directed protein kinase associated with microtubule. *FEBS Lett.* 335, 171–175. doi: 10.1016/0014-5793(93)80723-8

Krishnaiah, D., Sarbatly, R., and Nithyanandam, R. (2010). A review of the antioxidant potential of medicinal plant species. *Food Bioproducts Process.* 89, 217–233. doi: 10.1016/j.fbp.2010.04.008

Kühn, J., Meissner, C., and Oehmichen, M. (2005). Microtubule-associated protein 2 (MAP2)—a promising approach to diagnosis of forensic types of hypoxia-ischemia. *Acta Neuropathol.* 110, 579–586. doi: 10.1007/s00401-005-1090-9

Lara-Guzman, O. J., Tabares-Guevara, J. H., Leon-Varela, Y. M., Álvarez, R. M., Roldan, M., Sierra, J. A., et al. (2012). Proatherogenic macrophage activities are targeted by the flavonoid quercetin. *J. Pharmacol. Exp. Ther.* 343, 296–306. doi: 10.1124/jpet.112.196147

Lavados, P. M., Hennis, A. J., Fernandes, J. G., Medina, M. T., Legetic, B., Hoppe, A., et al. (2007). Stroke epidemiology, prevention and management strategies at a regional level: latin america and the caribbean. *Lancet Neurol.* 6, 362–372. doi: 10.1016/s1474-4422(07)70003-0

Lee, J. K., Kwak, H. J., Piao, M. S., Jang, J. W., Kim, S. H., and Kim, H. S. (2011). Quercetin reduces the elevated matrix metalloproteinases-9 level and improves functional outcome after cerebral focal ischemia in rats. *Acta Neurochir. (Wien)* 153, 1321–1329; discussion 1329. doi: 10.1007/s00701-010-0889-x

Llorens, F., Schmitz, M., Ferrer, I., and Zerr, I. (2016). CSF biomarkers in neurodegenerative and vascular dementias. *Prog. Neurobiol.* 138–140, 36–53. doi: 10.1016/j.pneurobio.2016.03.003

Madigan, J. B., Wilcock, D. M., and Hainsworth, A. H. (2016). Vascular contributions to cognitive impairment and dementia: topical review of animal models. *Stroke* 47, 1953–1959. doi: 10.1161/STROKEAHA.116.012066

Main, H., Munsie, M., and O'Connor, M. D. (2014). Managing the potential and pitfalls during clinical translation of emerging stem cell therapies. *Clin. Transl. Med.* 3:10. doi: 10.1186/2001-1326-3-10

Mangialasche, F., Solomon, A., Winblad, B., Mecocci, P., and Kivipelto, M. (2010). Alzheimer's disease: clinical trials and drug development. *Lancet Neurol.* 9, 702–716. doi: 10.1016/S1474-4422(10)70119-8

Martino Adami, P. V., Quijano, C., Magnani, N., Galeano, P., Evelson, P., Cassina, A., et al. (2015). Synaptosomal bioenergetic defects are associated with cognitive impairment in a transgenic rat model of early Alzheimer's disease. *J. Cereb. Blood Flow Metab.* 9:0271678X15615132. doi: 10.1177/0271678x15615132

Mecocci, P., and Polidori, M. C. (2012). Antioxidant clinical trials in mild cognitive impairment and Alzheimer's disease. *Biochim. Biophys. Acta* 1822, 631–638. doi: 10.1016/j.bbadis.2011.10.006

Mimica, N., and Presecki, P. (2009). Side effects of approved antidementives. *Psychiatr. Danub.* 21, 108–113. doi: 10.4172/2329-6488.1000213

Muresanu, D. F., Popa-Wagner, A., Stan, A., Buga, A. M., and Popescu, B. O. (2014). The vascular component of Alzheimer's disease. *Curr. Neurovasc. Res.* 11, 168–176. doi: 10.2174/1567202611666140408105333

Nelson, A. R., Sweeney, M. D., Sagare, A. P., and Zlokovic, B. V. (2016). Neurovascular dysfunction and neurodegeneration in dementia and Alzheimer's disease. *Biochim. Biophys. Acta* 1862, 887–900. doi: 10.1016/j.bbadis.2015.12.016

Neugroschl, J., and Sano, M. (2009). An update on treatment and prevention strategies for Alzheimer's disease. *Curr. Neurol. Neurosci. Rep.* 9, 368–376. doi: 10.1385/1-59259-661-4:253

Neurology, T. L. (2012). Colombia at the centre of preclinical AD research. *Lancet Neurol.* 11:567. doi: 10.1016/s1474-4422(12)70144-8

NIH. (2016). The dementias: hope through research: risk factors for dementia. Available online at: https://www.nia.nih.gov/alzheimers/publication/dementias/risk-factors-dementia

Nunes, M. A., Schöwe, N. M., Monteiro-Silva, K. C., Baraldi-Tornisielo, T., Souza, S. I., Balthazar, J., et al. (2015). Chronic microdose lithium treatment prevented memory loss and neurohistopathological changes in a transgenic

mouse model of Alzheimer's disease. *PLoS One* 10:e0142267. doi: 10.1371/journal.pone.0142267

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 Abeta and synaptic dysfunction. *Neuron* 39, 409–421. doi: 10.1016/S0896-6273(03)00434-3

Patrick, G. N., Zukerberg, L., Nikolic, M., de la Monte, S., Dikkes, P., and Tsai, L. H. (1999). Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature* 402, 615–622. doi: 10.1038/45159

Paudel, H. K., Lew, J., Ali, Z., and Wang, J. H. (1993). Brain proline-directed protein kinase phosphorylates tau on sites that are abnormally phosphorylated in tau associated with Alzheimer's paired helical filaments. *J. Biol. Chem.* 268, 23512–23518.

Pettigrew, L. C., Holtz, M. L., Craddock, S. D., Minger, S. L., Hall, N., and Geddes, J. W. (1996). Microtubular proteolysis in focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* 16, 1189–1202. doi: 10.1097/00004647-199611000-00013

Piedrahita, D., Castro-Alvarez, J. F., Boudreau, R., Villegas-Lanau, C. A., Kosik, K. S., Gallego-Gomez, J. C., et al. (2016). β-secretase 1's targeting reduces hyperphosphorilated tau, implying autophagy actors in 3xTg-AD mice. *Front. Cell. Neurosci.* 9:498. doi: 10.3389/fncel.2015.00498

Piedrahita, D., Hernandez, I., López-Tobón, A., Fedorov, D., Obara, B., Manjunath, B. S., et al. (2010). Silencing of CDK5 reduces neurofibrillary tangles in transgenic alzheimer's mice. *J. Neurosci.* 30, 13966–13976. doi: 10.1523/JNEUROSCI.3637-10.2010

Pluta, R. (2004). From brain ischemia-reperfusion injury to possible sporadic Alzheimer's disease. *Curr. Neurovasc. Res.* 1, 441–453. doi: 10.2174/1567202043361839

Posada-Duque, R. A., Barreto, G. E., and Cardona-Gómez, G. P. (2014). Protection after stroke: celular effectors of neurovascular unit integrity. *Front. Cell. Neurosci.* 8:231. doi: 10.3389/fncel.2014.00231

Posada-Duque, R. A., López-Tobón, A., Piedrahita, D., González-Billault, C., and Cardona-Gómez, G. P. (2015a). p35 and Rac1 underlie the neuroprotection and cognitive improvement induced by CDK5 silencing. *J. Neurochem.* 134, 354–370. doi: 10.1111/jnc.13127

Posada-Duque, R. A., Palacio-Castañeda, V., and Cardona-Gómez, G. P. (2015b). CDK5 knockdown in astrocytes provide neuroprotection as a trophic source via Rac1. *Mol. Cell. Neurosci.* 68, 151–166. doi: 10.1016/j.mcn.2015.07.001

Posada-Duque, R. A., Ramirez, O., Härtel, S., Inestrosa, N. C., Bodaleo, F., González-Billault, C., et al. (2016). CDK5 downregulation enhances synaptic plasticity. *Cell. Mol. Life Sci.* doi: 10.14711/thesis-b1029261 [Epub ahead of print].

Primo, V., Graham, M., Bigger-Allen, A. A., Chick, J. M., Ospina, C., Quiroz, Y. T., et al. (2016). Blood biomarkers in a mouse model of CADASIL. *Brain Res.* 1644, 118–126. doi: 10.14800/ttnd.434

Przedborski, S., Vila, M., and Jackson-Lewis, V. (2003). Neurodegeneration: what is it and where are we? *J. Clin. Invest.* 111, 3–10. doi: 10.1172/JCI200317522

Rajan, S. S., Baraniuk, S., Parker, S., Wu, T. C., Bowry, R., and Grotta, J. C. (2015). Implementing a mobile stroke unit program in the United States: why, how and how much? *JAMA Neurol.* 72, 229–234. doi: 10.1001/jamaneurol.2014.3618

Razzak, J., and Kellermann, A. (2002). Emergency medical care in developing countries is it worthwhile? *Bull. World Health Organ.* 80, 900–905.

Reiman, E. M., Langbaum, J. B., Fleisher, A. S., Caselli, R. J., Chen, K., Ayutyanont, N., et al. (2011). Alzheimer's prevention initiative: a plan to accelerate the evaluation of presymptomatic treatments. *J. Alzheimers Dis.* 26, 321–329. doi: 10.3233/JAD-2011-0059

Reiman, E. M., Quiroz, Y. T., Fleisher, A. S., Chen, K., Velez-Pardo, C., Jimenez-Del-Rio, M., et al. (2012). Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study. *Lancet Neurol.* 11, 1048–1056. doi: 10.1016/S1474-4422(12)70228-4

Rizzi, L., Rosset, I., and Roriz-Cruz, M. (2014). Global epidemiology of dementia: Alzheimer's and vascular types. *Biomed Res. Int.* 2014:908915. doi: 10.1155/2014/908915

Roberts, B. W., Mitchell, J., Kilgannon, J. H., Chansky, M. E., and Trzeciak, S. (2013). Nitric oxide donor agents for the treatment of ischemia/reperfusion injury in human subjects: a systematic review. *Shock* 39, 229–239. doi: 10.1097/SHK.0b013e31827f565b

Rojo, L. E., Alzate-Morales, J., Saavedra, I. N., Davies, P., and Maccioni, R. B. (2010). Selective interaction of lansoprazole and astemizole with tau polymers: potential new clinical use in diagnosis of Alzheimer's disease. *J. Alzheimers Dis.* 19, 573–589. doi: 10.3233/JAD-2010-1262

Román, G. C., Sachdev, P., Royall, D. R., Bullock, R. A., Orgogozo, J. M., and López-Pousa, S. (2004). Vascular cognitive disorder: a new diagnostic category updating vascular cognitive impairment and vascular dementia. *J. Neurol. Sci.* 226, 81–87. doi: 10.1016/j.jns.2004.09.016

Sabogal-Guáqueta, A. M., Muñoz-Manco, J. I., Ramírez-Pineda, J. R., Lamprea-Rodriguez, M., Osorio, E., and Cardona-Gómez, G. P. (2015). The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer's disease model mice. *Neuropharmacology* 93, 134–145. doi: 10.1016/j.neuropharm.2015.01.027

Sabogal-Guáqueta, A. M., Osorio, E., and Cardona-Gómez, G. P. (2016). Linalool reverses neuropathological and behavioral impairments in old triple transgenic Alzheimer's mice. *Neuropharmacology* 102, 111–120. doi: 10.1016/j.neuropharm.2015.11.002

Sandu, R. E., Buga, A. M., Uzoni, A., Petcu, E. B., and Popa-Wagner, A. (2015). Neuroinflammation and comorbidities are frequently ignored factors in CNS pathology. *Neural Regen. Res.* 10, 1349–1355. doi: 10.4103/1673-5374.165208

Schiavon, A. P., Soares, L. M., Bonato, J. M., Milani, H., Guimarães, F. S., and Weffort de Oliveira, R. M. (2014). Protective effects of cannabidiol against hippocampal cell death and cognitive impairment induced by bilateral common carotid artery occlusion in mice. *Neurotox. Res.* 26, 307–316. doi: 10.1007/s12640-014-9457-0

Selnes, O. A., and Vinters, H. V. (2006). Vascular cognitive impairment. *Nat. Clin. Pract. Neurol.* 2, 538–547. doi: 10.1038/ncpneuro0294

Shi, D. H., Bae, Y. C., Kim-Han, J. S., Lee, J. H., Choi, I. Y., Son, K. H., et al. (2006). Polyphenol amentoflavone affords neuroprotection against neonatal hypoxic-ischemic brain damage via multiple mechanisms. *J. Neurochem.* 96, 561–572. doi: 10.1111/j.1471-4159.2005.03582.x

Sies, H. (1993). Strategies of antioxidant defense. *Eur. J. Biochem.* 215, 213–219. doi: 10.1111/j.1432-1033.1993.tb18025.x

Silva, B., Sousa, L., Miranda, A., Vasconcelos, A., Reis, H., Barcelos, L., et al. (2015). Memory deficit associated with increased brain proinflammatory cytokine levels and neurodegeneration in acute ischemic stroke. *Arq. Neuropsiquiatr.* 73, 655–659. doi: 10.1590/0004-282X20150083

Snyder, H. M., Corriveau, R. A., Craft, S., Faber, J. E., Greenberg, S. M., Knopman, D., et al. (2015). Vascular contributions to cognitive impairment and dementia including Alzheimer's disease. *Alzheimers Dement.* 11, 710–717. doi: 10.1016/j.jalz.2014.10.008

Soreq, H., and Seidman, S. (2001). Acetylcholinesterase–new roles for an old actor. *Nat. Rev. Neurosci.* 2, 294–302. doi: 10.1138/2001032

Stephan, B. C., Matthews, F. E., Khaw, K. T., Dufouil, C., and Brayne, C. (2009). Beyond mild cognitive impairment: vascular cognitive impairment, no dementia (VCIND). *Alzheimers Res. Ther.* 1:4. doi: 10.1186/alzrt4

Sumi, M., Tateishi, N., Shibata, H., Ohki, T., and Sata, M. (2013). Quercetin glucosides promote ischemia-induced angiogenesis, but do not promote tumor growth. *Life Sci.* 93, 814–819. doi: 10.1016/j.lfs.2013.09.005

Tabira, T., Matsumoto, S. E., and Jin, H. (2011). Antibody therapy for Alzheimer's disease. *Rinsho Shinkeigaku* 51, 1160–1161. doi: 10.5692/clinicalneurol.51.1160

Uchihara, T., Tsuchiya, K., Kondo, H., Hayama, T., and Ikeda, K. (1995). Widespread appearance of Alz-50 immunoreactive neurons in the human brain with cerebral infarction. *Stroke* 26, 2145–2148. doi: 10.1161/01.str.26.11.2145

Uchihara, T., Tsuchiya, K., Nakamura, A., and Ikeda, K. (2000). Appearance of tau-2 immunoreactivity in glial cells in human brain with cerebral infarction. *Neurosci. Lett.* 286, 99–102. doi: 10.1016/s0304-3940(00)01103-4

Uribe-Arias, A., Posada-Duque, R. A., González-Billault, C., Villegas, A., Lopera, F., and Cardona-Gómez, G. P. (2016). p120-catenin is necessary for neuroprotection induced by CDK5 silencing in models of Alzheimer's disease. *J. Neurochem.* 138, 624–639. doi: 10.1111/jnc.13697

Valério Romanini, C., Dias Fiuza Ferreira, E., Correia Bacarin, C., Verussa, M. H., Weffort de Oliveira, R. M., and Milani, H. (2013). Neurohistological and behavioral changes following the four-vessel occlusion/internal carotid artery model of chronic cerebral hypoperfusion comparison between normotensive and spontaneously hypertensive rats. *Behav. Brain Res.* 252, 214–221. doi: 10.1016/j.bbr.2013.05.043

Van Dam, D., and De Deyn, P. P. (2011). Animal models in the drug discovery pipeline for Alzheimer's disease. *Br. J. Pharmacol.* 164, 1285–1300. doi: 10.1111/j.1476-5381.2011.01299.x

Vargas, J. Y., Fuenzalida, M., and Inestrosa, N. C. (2014). *in vivo* activation of Wnt signaling pathway enhances cognitive function of adult mice and reverses cognitive déficits in an Alzheimer's disease model. *J. Neurosci.* 34, 2191–2202. doi: 10.1523/JNEUROSCI.0862-13.2014

Venkat, P. B. S., Chopp, M., and Chen, J. (2015). Models and mechanisms of vascular dementia. *Exp. Neurol.* 272, 97–108. doi: 10.1016/j.expneurol.2015.05.006

Vijayan, M., and Reddy, P. H. (2016). Stroke, vascular dementia and Alzheimer's disease: molecular links. *J. Alzheiemrs Dis.* 54, 427–443. doi: 10.3233/JAD-160527

Villamil-Ortiz, J. G., Barrera-Ocampo, A., Piedrahita, D., Velásquez-Rodriguez, C. M., Arias-Londoño, J. D., and Cardona-Gómez, G. P. (2016). BACE1 RNAi restores the composition of phosphatidylethanolamine-derivates related to memory improvement in aged 3xTg-AD mice. *Front. Cell. Neurosci.* 10:260. doi: 10.3389/fncel.2016.00260

Villamil-Ortiz, J. G., and Cardona-Gómez, G. P. (2015). Comparative analysis of autophagy and tauopathy related markers in cerebral ischemia and Alzheimer's disease animal models. *Front. Aging Neurosci.* 7:84. doi: 10.3389/fnagi.2015.00084

Viswanathan, A., Greenberg, S. M., and Scheltens, P. (2016). Role of vascular disease in alzheimer-like progressive cognitive impairment. *Stroke* 47, 577–580. doi: 10.1161/STROKEAHA.115.009010

Wen, H. M., Lam, W. W., Rainer, T., Fan, Y. H., Leung, T. W., Chan, Y. L., et al. (2004). Multiple acute cerebral infarcts on diffusion-weighted imaging and risk of recurrent stroke. *Neurology* 63, 1317–1319. doi: 10.1212/01.wnl.0000140490.22251.b6

WHO. (2016). Dementia. Available online at: http://www.who.int/mediacentre/factsheets/fs362/en/

Yang, M. H., Kim, J., Khan, I. A., Walker, L. A., and Khan, S. I. (2014). Nonsteroidal anti-inflammatory drug activated gene-1 (NAG-1) modulators from natural products as anti-cancer agents. *Life Sci.* 100, 75–84. doi: 10.1016/j.lfs.2014.01.075

Yuede, C. M., Dong, H., and Csernansky, J. G. (2007). Anti-dementia drugs and hippocampal-dependent memory in rodents. *Behav. Pharmacol.* 18, 347–363. doi: 10.1097/fbp.0b013e3282da278d

Zekry, D., Duyckaerts, C., Belmin, J., Geoffre, C., Herrmann, F., Moulias, R., et al. (2003). The vascular lesions in vascular and mixed dementia: the weight of functional neuroanatomy. *Neurobiol. Aging* 24, 213–219. doi: 10.1016/s0197-4580(02)00066-0

Zheng, G. Q., Wang, X. M., Wang, Y., and Wang, X. T. (2010). Tau as a potential novel therapeutic target in ischemic stroke. *J. Cell. Biochem.* 109, 26–29. doi: 10.1002/jcb.22408

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: 22 August 2017
doi: 10.3389/fnagi.2017.00278

Memory Alteration Test to Detect Amnestic Mild Cognitive Impairment and Early Alzheimer's Dementia in Population with Low Educational Level

Nilton Custodio[1,2,3], David Lira[1,2,3], Eder Herrera-Perez[3,4,5]*, Rosa Montesinos[2,3,6], Sheila Castro-Suarez[1,3,7], José Cuenca-Alfaro[3,8] and Lucía Valeriano-Lorenzo[3,8]*

[1]*Servicio de Neurología, Instituto Peruano de Neurociencias, Lima, Peru,* [2]*Unidad de Diagnóstico de Deterioro Cognitivo y Prevención de Demencia, Clínica Internacional, Lima, Peru,* [3]*Unidad de Investigación, Instituto Peruano de Neurociencias, Lima, Peru,* [4]*GESID, Lima, Peru,* [5]*Instituto Nacional de Salud del Niño, Lima, Peru,* [6]*Servicio de Medicina de Rehabilitación, Instituto Peruano de Neurociencias, Lima, Peru,* [7]*Servicio de Neurología de la Conducta, Instituto Nacional de Ciencias Neurológicas, Lima, Peru,* [8]*Unidad de Neuropsicología, Instituto Peruano de Neurociencias, Lima, Peru*

Background/Aims: Short tests to early detection of the cognitive impairment are necessary in primary care setting, particularly in populations with low educational level. The aim of this study was to assess the performance of Memory Alteration Test (M@T) to discriminate controls, patients with amnestic Mild Cognitive Impairment (aMCI) and patients with early Alzheimer's Dementia (AD) in a sample of individuals with low level of education.

Methods: Cross-sectional study to assess the performance of the M@T (study test), compared to the neuropsychological evaluation (gold standard test) scores in 247 elderly subjects with low education level from Lima-Peru. The cognitive evaluation included three sequential stages: (1) screening (to detect cases with cognitive impairment); (2) nosological diagnosis (to determinate specific disease); and (3) classification (to differentiate disease subtypes). The subjects with negative results for all stages were considered as cognitively normal (controls). The test performance was assessed by means of area under the receiver operating characteristic (ROC) curve. We calculated validity measures (sensitivity, specificity and correctly classified percentage), the internal consistency (Cronbach's alpha coefficient), and concurrent validity (Pearson's ratio coefficient between the M@T and Clinical Dementia Rating (CDR) scores).

Results: The Cronbach's alpha coefficient was 0.79 and Pearson's ratio coefficient was 0.79 ($p < 0.01$). The AUC of M@T to discriminate between early AD and aMCI was 99.60% (sensitivity = 100.00%, specificity = 97.53% and correctly classified = 98.41%) and to discriminate between aMCI and controls was 99.56% (sensitivity = 99.17%, specificity = 91.11%, and correctly classified = 96.99%).

Conclusions: The M@T is a short test with a good performance to discriminate controls, aMCI and early AD in individuals with low level of education from urban settings.

Keywords: memory alteration test, mild cognitive impairment, dementia, Alzheimer's disease, neuropsychological assessment, validity and reliability, diagnostic test accuracy

OPEN ACCESS

Edited by:
Agustin Ibanez, Institute of Cognitive and Translational Neuroscience (INCYT), Argentina

Reviewed by:
Alfredo Ardila, Florida International University, United States
Arun Bokde, Trinity College, Dublin, Ireland
Thais Helena Machado, Universidade Federal de Minas Gerais, Brazil

****Correspondence:***
Nilton Custodio
ncustodio@ipn.pe
Eder Herrera-Perez
eder.herrera.p@outlook.com

Received: *06 December 2016*
Accepted: *07 August 2017*
Published: *22 August 2017*

Citation:
Custodio N, Lira D, Herrera-Perez E, Montesinos R, Castro-Suarez S, Cuenca-Alfaro J and Valeriano-Lorenzo L (2017) Memory Alteration Test to Detect Amnestic Mild Cognitive Impairment and Early Alzheimer's Dementia in Population with Low Educational Level. Front. Aging Neurosci. 9:278. doi: 10.3389/fnagi.2017.00278

INTRODUCTION

Mild cognitive impairment (MCI) is a well recognized risk factor for Alzheimer's disease (AD), and for the predemential phase of this and other dementias (Albert et al., 2011; Li et al., 2011; Cooper et al., 2015). The need for research aimed to AD early diagnosis have been highlighted in several studies directed towards the prevention and control of the worldwide progression of the disease (Richard et al., 2012; Barnett et al., 2013). Thus, it is necessary to have brief and reliable instruments to early diagnosis in primary care settings (Custodio et al., 2017).

Globally, there is a generalized low detection of dementia in the community. This is a real challenge in Latin America (LA; Lang et al., 2017), where previous studies showed that the majority of medical doctors perceive that their practices for diagnosis and treatment of dementia are inadequate, underscoring that this deficiency is higher in general practitioners than in specialists (Olavarria et al., 2015). In addition, other challenge in LA countries is the lack of validated and standardized instruments to assess cognition and functionality in indigenous populations, in rural areas, with a language other than Spanish, or with low levels of education (Maestre, 2012; Parra, 2014).

Various instruments have been developed to detect dementia (Folstein et al., 1975; Mattis, 1976; Roth et al., 1986), but there is not still gold standard short test. The Mini-Mental State Examination (MMSE), the most widely used short test, is especially inadequate in less-educated populations (Rosselli et al., 2000; Scazufca et al., 2009) because its low validity and diagnostic accuracy in this populations (Lonie et al., 2009; Mitchell, 2009; Carnero-Pardo et al., 2011b). Other short tests include task that require reading and writing abilities or involve the use of pencil and paper, which affects its use in populations with a low educational level (Carnero-Pardo et al., 2011a).

In Peru, several short tests have been validated in urban samples from Lima, including the clock drawing test (CDT)—Mano's version (Custodio et al., 2011), the Addenbrooke's cognitive examination (ACE; Custodio et al., 2011), the memory alteration test (M@T; Custodio et al., 2014), the INECO frontal screening (IFS; Custodio et al., 2016b) and the Peruvian version of the Eurotest (Oscanoa et al., 2016). However, neither of these tests were validated in LA low-educated populations (Paddick et al., 2017).

The M@T is a short cognitive test to detect dementia, able to discriminate between controls, patients with amnestic MCI (aMCI), and patients with early AD (Rami et al., 2007, 2010; Custodio et al., 2014; Ozer et al., 2016). It has been reported the utility of M@T in patients with low level of education (Sousa et al., 2015), however, validation studies of short cognitive tests for detecting aMCI and AD in population with low-level education are scarce (Paddick et al., 2017). Thus, the aim of the present study is to assess the validity of M@T to discriminate between controls, patients with aMCI and patients with early AD in a sample of individuals with low level of education.

MATERIALS AND METHODS

Design of the Study

Diagnostic test cross-sectional study to evaluate the performance of the M@T (study test), compared to the neuropsychological evaluation (gold standard test).

The Study Test

The M@T is a valid screening test that assess the temporal orientation and different types of memory (episodic, textual and semantic) and discriminates between healthy elderly subjects, patients with aMCI and patients with early AD. This is a cognitive test with high internal consistency and validity, short application (5–10 min), easy to perform and to interpret, developed in Spain (Rami et al., 2007) and validated in Peru (Custodio et al., 2014). Its results are mildly influenced by educational level, thereby the cutoff points are 36/37 and 37/38 for subjects with <8 years and ≥ 8 years of education, respectively (Carnero-Pardo et al., 2011a).

This test is totally oral and do not require reading or writing skills or the use of pencil and paper, allowing the evaluation of very low educated subjects. All the questions of M@T have a single correct answers, and covering five domains: temporal orientation (5), short term memory (10), semantic memory (15), free recall (10) and facilitated recall (10). Thus, the maximum score of this test is 50 points.

The Gold Standard Test

The neuropsychological assessment is the detailed evaluation of the cognitive functions, by means of a neuropsychological battery adapted to Peruvian population. The battery included the following tests: Rey Auditory Verbal Learning Test (RAVLT; Rey, 1941), Logical Memory—Subtest of Wechsler Memory Scale Revised (Wechsler, 1997), Trail Making Test A and B (Partington and Leiter, 1949), Rey-Osterrieth Complex Figure Test (ROCF; Rey, 1941), Boston Naming Test (Kaplan et al., 1983), Wisconsin Card Sorting Test (WCST; Nelson, 1976), Letter-Number and Digit Span, subtests of Wechsler Adult Intelligent Scale III (Wechsler, 1997).

Following the order of the tests mentioned above, the neuropsychological battery has the main purpose to explore cognitive skills such as verbal memory and verbal learning through retention and evocation of verbal stimuli, immediate recall and delayed recall of stories, scanning and visuomotor tracking, divided attention, cognitive flexibility, visual memory and visuospatial construction skill. Also it appraises language skills like naming ability and word retrieval, executive functioning like forming concepts, conceptual flexibility as well attentional control, working memory and span of immediate verbal recall.

The decision criterion is two standard deviations below the mean in order to establish deficit in the cognitive domain assessed. These values were collected from the original articles for each selected test. Throughout the study, the neuropsychologists were blinded to results of M@T.

TABLE 1 | Demographic characteristics and cognitive test scores in 247 low-level education individuals from Lima-Peru, according to definitive diagnosis.

	Study group				
	Early Alzheimer's dementia (*n* = 81)	**amnestic mild cognitive impairment (*n* = 45)**	**Control (*n* = 121)**	***p*-value 1[†] (early AD vs. aMCI)**	***p*-value 2[‡] (aMCI vs. control)**
Sex: female	52 (64.20%)	30 (66.67%)	68 (56.20%)	0.781	0.223
Age, years[§]	74.18 (3.81)	71.09 (4.20)	69.53 (4.11)	0.000**	0.032*
Education, years[§]	2.65 (1.28)	2.53 (1.46)	2.57 (1.45)	0.629	0.885
MMSE, score[§]	18.32 (2.78)	21.36 (0.98)	22.02 (1.26)	0.000**	0.056
CDT, score[§]	2.42 (1.69)	8.02 (1.06)	8.75 (0.91)	0.000**	0.000**
M@T, score[§]	17.54 (4.67)	30.53 (2.54)	41.97 (2.68)	0.000**	0.000**

*AD, Alzheimer's dementia; aMCI, amnestic mild cognitive impairment; MMSE, Mini Mental State of Examination; CDT, Clock Drawing Test—Mano's version; M@T, Memory Alteration Test; [§]Data showed as mean (standard deviation); [†]p-value for comparation between early AD and aMCI; [‡]p-value for comparation between aMCI and control; *p-value < 0.05; **p-value < 0.001.*

Population and Sample

The study was carried out in elderly care home centers of two districts of Lima (four from "Carabayllo" and two from "Cercado de Lima") between March and September of 2015. We included subjects older than 60 years, Spanish speakers with low educational level (<4 years of completed formal education), excluding those with any condition that might cause cognitive impairment non-related to neurodegenerative etiology (history of substances addiction or abuse, depression, hypothyroidism, vitamin B12 deficiency, chronic hepatopathy or nephropathy, neuroinfections by HIV or syphilis, severe brain injury, sub-dural hematoma, cerebrovascular illness, vascular dementia suggestion (Hachinski Ischemic Score >4), etc.) or that could affect their performance to realize the cognitive tests (auditory, visual or other physical deficits).

Additionally, we excluded to patients that consumed any of following drugs: opioid analgesics, decongestants, anti-spasmodics, anti-cholinergics, anti-depressants, antiarrhythmics, antipsychotics, anti-emetics, anxiolytics and valproate.

Procedures

We requested the list of regular users (i.e., assistance frequency >3 times/week) of the elderly care home centers. By means of simple random sampling (table of random numbers), the potential participants were selected until completing a quota of half of available population (sample size = 0.5 N), consented to participate, and provided information necessary to assess compliance with eligibility criteria. The evaluation of cognitive impairment was performed in three successive stages: (1) screening (to detect cases with cognitive impairment); (2) nosological diagnosis (to determinate specific disease that is the cause of cognitive impairment); and (3) final classification (to differentiate disease subtypes).

In the screening phase, an integral clinical evaluation was performed, including measurement of anthropometry and blood pressure, application of Pfeffer Functional Activities Questionnaire (PFAQ) and cognitive screening tests (MMSE and CDT). If any cognitive test was positive for impairment, it was repeated by a different evaluator. The confirmed cases were considered as patients with cognitive impairment (PCI). According to educational level, the cutoff score used was 23 for subjects with 4 years of education, 21 for subjects with 1–3 years of education, and 18 for subjects with less than 1 year of education (Custodio and Lira, 2014). The MMSE and CDT was applied to study subjects, and PFAQ was applied to their caregivers/accompanist.

In the second stage, the PCIs were assessed using blood tests (hemogram, glucose, electrolytes, transaminases, rapid plasma

TABLE 2 | Results of the neuropsychological assessment in 247 low-level education individuals from Lima-Peru, according to definitive diagnosis.

		Study group		
Test	**Sub-test**	**Early Alzheimer's dementia (*n* = 81)**	**amnestic mild cognitive impairment (*n* = 45)**	**Control (*n* = 121)**
RAVLT	Free-recall	3.22 (0.72)	5.33 (0.67)	6.17 (0.90)
	Recognition	5.84 (1.01)	9.93 (0.98)	13.02 (1.05)
Logical memory	Immediate recall	1.89 (1.07)	6.51 (0.94)	12.00 (1.38)
	Delayed recall	1.42 (0.91)	6.18 (0.74)	11.83 (1.24)
Trail making test	Test A (s)	80.93 (8.30)	67.96 (7.80)	54.00 (8.74)
	Test B (s)	188.98 (21.37)	115.09 (12.39)	99.31 (14.92)
ROCF	Copy	16.62 (2.29)	24.93 (2.23)	28.35 (1.93)
	Recall	6.30 (1.65)	9.67 (1.72)	13.97 (2.81)
Test of denomination of Boston		13.85 (3.70)	28.67 (5.44)	51.59 (3.37)
WCST	Categories	2.73 (0.63)	4.16 (0.64)	4.97 (0.53)
	Perseverations	13.07 (2.76)	6.13 (1.79)	1.86 (0.73)
Letter—Number		4.83 (0.75)	7.13 (0.94)	10.10 (1.66)
Digit span		2.40 (0.72)	4.24 (0.43)	4.90 (0.55)

RAVLT, Rey Auditory Verbal Learning Test; ROCF, Rey-Osterrieth Complex Figure; WCST, Wisconsin Card Sorting Test.

reagin (RPR), urea, creatinine, vitamin B12, folic acid, free T3 and T4, and ultra-sensitive TSH), images studies (brain tomography and/or magnetic resonance imaging), and Beck Depression Inventory II (BDI-II) for discarding non-neurodegenerative causes of cognitive impairment. We applied the DSM-IV (American Psychiatric Association, 2000) criteria to diagnosis dementia, and the Clinical Dementia Rating (CDR; Hughes et al., 1982) for staging dementia. The CDR was applied to both study subjects and caregivers/accompanist.

Finally, in the third stage, we performed the neuropsychological evaluation of patients with MCI or dementia to typify its subtype. We applied the criteria of Petersen (Petersen et al., 1999) and NINCDS-ADRDA (McKhann et al., 1984) to classify as aMCI or AD, respectively. The doubtful cases (regarding typification) were resolved by researchers consensus.

The subjects with negative results in all tests for cognitive assessment were considered as cognitively normal (controls). The M@T was applied to study subjects in first stage and the evaluators were blinded to the results of this psychometric. The results of M@T were not used as part of the neuropsychological battery for diagnosis. The team of evaluators of the second and third phases (expert neurologists and neuropsichologists) was different from the team of the first phase (students of medicine and psychology supervised by expert neurologists).

Statistical Methods

The corresponding descriptive statistics were performed. The analysis was performed comparing the cognitive groups (controls, aMCI and AD) by pairs. For this purpose we applied *T* tests (for quantitative variables) and Chi Square (for categorical variables). We assessed the internal consistency (Cronbach's alpha coefficient) and the concurrent validity (Pearson's ratio coefficient between the M@T and CDR scores).

We performed a logistic regression (logit) for each pair of study groups (early AD/aMCI, aMCI/control, and early AD/control), using a model of two variables: final diagnosis as dependent variable and test as independent variable. We applied postestimation analysis to compute area under receiver operating characteristics (ROC) curve and graph ROC curve, and calculate validity measures (sensitivity, specificity and positive and negative predictive values).

Additionally, we calculated the diagnostic accuracy (percentage of correctly classified individuals) for M@T, MMSE and CDT. The maximum values of this measure were the standard for the cut-off scores selection of sensitivity, specificity and predictive values. Finally, we compared the AUC of this tests using the method of Hanley and McNeil. The tests were performed at 95% confidence using the STATA software (version 12.0).

Ethical Aspects

This study was carried out in accordance with the recommendations of the Council for International Organizations and Medical Sciences (CIOMS). A written informed consent was obtained from all participants or their carers in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the *Universidad de San Martin de Porres*.

RESULTS

Flow of Participants

The first stage started with 346 participants, but 41 were missed (14 due to withdrawal of informed consent, 21 due to difficulty in attending scheduled appointments and six due to caregiver or evaluator illness). In the second stage, 22 of 305 participants were missed (seven due to difficulty in attending scheduled appointments, four for lack of blood tests results and 11 for lack of brain tomography).

Finally, 283 participants completed the third stage. However, 36 participants were not included in the present analysis because they were classified as non-amnesic MCI (16), vascular dementia (6), Frontotemporal dementia (4), dementia associated with Parkinson's disease (2) and other unspecified dementias (8).

Data of Participants

Statistical analysis of the sociodemographic data, MMSE scores and M@T scores were performed according to the comparison groups. In patients with AD, compared to those with aMCI, age was significantly higher and test scores (MMSE, CDT and M@T) were significantly lower. On the other hand, in the patients with aMCI the age was significantly higher and the M@T and CDT scores were significantly lower, compared to control subjects (**Table 1**). The M@T and CDT scores showed a differential distribution according to the comparison group, behaving as a trend (**Figure 1**). The results of the neuropsychological assessment are detailed in **Table 2**.

Psychometric Properties of M@T

Internal consistency (Cronbach's alpha coefficient: 0.79) and concurrent validity ($r = 0.79$; $p < 0.01$) were good. In relation to the M@T cutoff, a score of 26 allows to discriminate between early AD and aMCI (sensitivity = 100.00% and specificity = 97.53%), with an accuracy of 98.41%. Similarly, a score of 35 allows discriminating between aMCI and controls

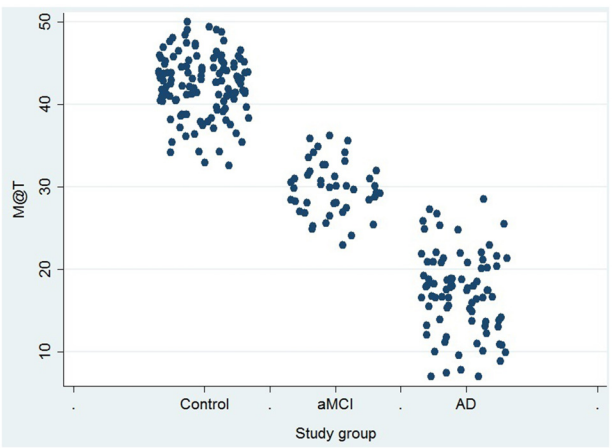


FIGURE 1 | Score in Memory Alteration Test (M@T) in 247 low-level education individuals from Lima-Peru, according to definitive diagnosis. AD, Alzheimer's dementia; aMCI, amnestic mild cognitive impairment.

(sensitivity = 99.17% and specificity = 91.11%), with an accuracy of 96.99% (**Table 3**).

The performance of the M@T to discriminate between early AD and aMCI was 0.9960 (**Figure 2**) and to discriminate between aMCI and controls was 0.9956 (**Figure 3**). The discriminatory performance of M@T was significantly higher than the MMSE (p = 0.000) for all combinations of analyzed group pairs. Furthermore, the performance of M@T was significantly higher than CDT to discriminate between patients with aMCI from controls (**Table 3**). Additionally, we performed an analysis for assessing if the score M@T is statistically associated with clinical diagnosis (early AD or aMCI; Supplementary Table S1).

DISCUSSION

Implications

This study shows a good performance of M@T to discriminate between early AD and aMCI in subjects with less than 4 years of education. These results are similar to those previously obtained with a sample of 6.5 years of average education (AUC: 0.9986; Custodio et al., 2014) and slightly higher than those obtained in a Spanish sample with 8 years of average education (AUC: 0.9300; Rami et al., 2007).

Similarly, we found a good performance to discriminate between patients with aMCI and controls (AUC: 0.9956), which was slightly lower than that reported previously (AUC: 0.9986; Custodio et al., 2014), but also higher than that obtained in a Spanish sample (AUC: 0.932; Rami et al., 2007). Our research has also shown a good correlation coefficient between M@T and MMSE, which suggests convergent validity. This is a finding similar to that previously obtained with the Portuguese version of the M@T (Sousa et al., 2015).

Additionally, we found that the performance of M@T is higher than MMSE and CDT for discriminating both AD vs. aMCI and aMCI vs. controls. This findings can be explained because M@T evaluates episodic and semantic memory, which have their biological substrate in the hippocampus, the medial temporal lobe and temporal neocortex, areas that are early affected in AD (Rami et al., 2007). In contrast, the MMSE evaluates orientation, language, praxia and general aspects of memory and the CDT evaluates planning, visuospatial and constructive functions. Thus, MMSE is not able to discriminate between AD and aMCI (Tombaugh and McIntyre, 1992; Wind et al., 1997; Rami et al., 2009), and CDT is more appropriate to detect advanced stages of AD (Custodio et al., 2016a).

According to recent UNESCO data, 16% of adults have emerged from education systems without basic literacy skills, which is a major problem in the regions of Sub-Saharan Africa and South Asia, where more than 1/3 of adults are illiterate. Around the world, at least 20 countries have adult literacy rates less than 60% and 43 countries have adult literacy rates less than 75% (UNESCO Institute for Statistics, 2016). Thus, this population constitutes an important group and their needs emerge as public health focus. In this context, valid diagnostic tests for its use in people with low educational level are required.

There are evidence about the demographic influences (e.g., age, gender, education, and residence rural/urban) on the

TABLE 3 | Cut-off points and diagnostic performance of M@T and MMSE to discriminate between AD, aMCI.

	Discrimination between early AD and aMCI			Discrimination between aMCI and controls			Discrimination between early AD and controls		
	M@T	MMSE	CDT	M@T	MMSE	CDT	M@T	MMSE	CDT
Optimal cut-off [§]	26	21	5	35	21	8	29	21	5
Sensitivity	100.00	86.67	100.00	99.17	90.91	95.04	100.00	90.91	100.00
Specificity	97.53	75.31	87.65	91.11	13.33	31.11	98.77	75.31	87.65
Correctly classified (%)	98.41	79.37	92.06	96.99	69.88	77.71	99.50	84.65	95.05
Likelihood ratio +	40.500	3.51	8.10	11.16	1.05	1.38	81.00	3.68	8.10
Likelihood ratio −	0.000	2.07	0.00	0.009	0.68	0.16	0.000	0.12	0.00
Area under curve [95% CI]	0.9960[†] [0.99–1.00]	0.8278 [0.76–0.90]	1.0000 [1.00–1.00]	0.9956[†‡] [0.99–1.00]	0.6536 [0.57–0.74]	0.6869 [0.60–0.78]	1.0000[†] [1.00–1.00]	0.8820 [0.83–0.93]	1.0000 [1.00–1.00]

AD, Alzheimer's dementia; aMCI, amnestic mild cognitive impairment; MMSE, Mini Mental State of Examination; M@T, Memory Alteration Test; CI, Confidence interval; [§]Cut-off base on maximum value of correctly classified. [†]Significant difference regarding the MMSE (p-value < 0.05); [‡]Significant difference regarding the CDT (p-value < 0.05).

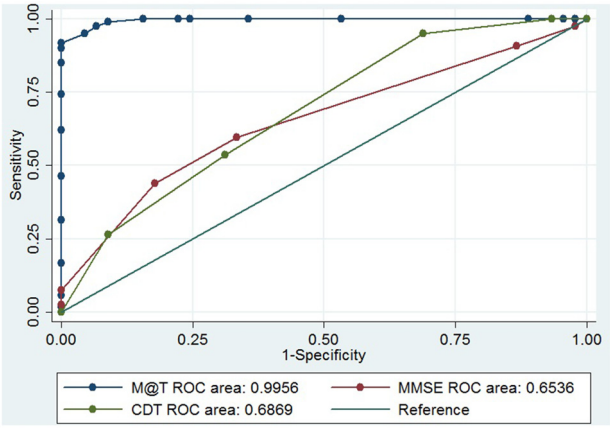


FIGURE 2 | Receiver operating characteristics (ROC) curve of M@T, MMSE and CDT to discriminate between patients with aMCI and controls in 166 low-level education individuals from Lima-Peru. MMSE, Mini Mental State of Examination; CDT, Clock Drawing Test—Mano's version; M@T, Memory Alteration Test.

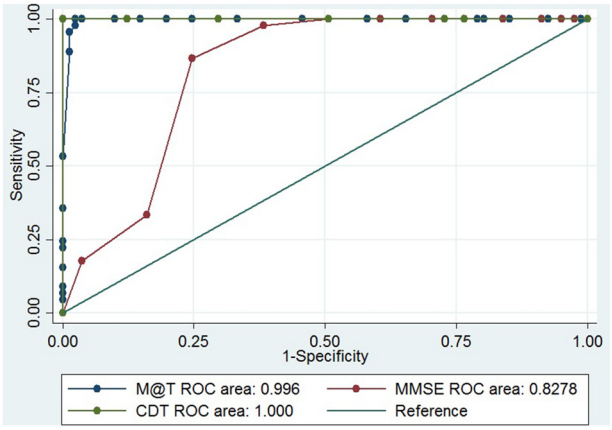


FIGURE 3 | ROC curve of M@T, MMSE and CDT to discriminate between patients with aMCI and early AD in 126 low-level education individuals from Lima-Peru. MMSE, Mini Mental State of Examination; CDT, Clock Drawing Test—Mano's version; M@T, Memory Alteration Test.

performance of several cognitive tests (Freitas et al., 2015; Li et al., 2016; Xie et al., 2016). Particularly, the education is a key factor since dementia is under-recognized among people with low education levels (Xie et al., 2016). Thereby the international norms of MMSE, the most broadly used cognitive screening instrument, consider different optimal cut-off points depending of educational level to improve screening precision for cognitive impairment (Moraes et al., 2010; Kim et al., 2012; Freitas et al., 2015; Li et al., 2016; Xie et al., 2016). Regarding previous results in Peruvian subjects with at least 6 years of education (Custodio et al., 2014), our data showed that performance with M@T is affected by education and cut-off points should be adjusted.

Additionally, previous studies have shown that non-specialist physicians have difficulties in effectively identifying aMCI and early AD. Thus, it is necessary to develop clinically useful, non-invasive and/or cost-effective, screening tools (Connolly et al., 2011), which must be applicable in primary care centers (Laske et al., 2015). In Peru, M@T has been shown to be a reliable test with high precision to discriminate between early AD, aMCI and normal cognition in samples of low educational level (Custodio et al., 2014) and, according to the results of this study, in samples with very low educational level. There are evidence suggesting a progression between various clinical states, beginning with MCI and, after a period of up to 5 years, evolving to dementia in its various sequential stages of severity (De Meyer et al., 2010; Derby et al., 2013). Our results show that, in fact, the average age is higher among patients with AD compared to patients with aMCI and, in turn, they are older than the control subjects.

In addition to age, another important sociodemographic variable is the sex. Several population-based studies have shown nearly two-thirds of individuals diagnosed with AD are females (Dal Forno et al., 2005). In this sense, the sociodemographic profile of the patients included in this study is consistent with that previously reported in the world literature.

In our sample, MMSE and CDT showed a suboptimal performance for discriminating between aMCI and healthy controls. This findings contrasts with previous studies, which found an AUC values higher than 0.80 and 0.70 with the use of MMSE and CDT, respectively (Cacho et al., 2010; Kato et al., 2013). However, a brazilian study showed a low performance of these tests (0.63 and 0.59, respectively; Ladeira et al., 2009). Similarly, other study in high educated sample showed same results (0.70 and 0.61, respectively; Rubínová et al., 2014). Thus, the discrepancy in these topic could be explained for the differences in educational level of participants and, potentially, other regional features.

Limitations

We have not included rural populations or with native language other than Spanish. Consequently, the results of this study may not be applicable to these population subgroups. The comparison groups were statistically different for the age, a potential confounding variable. However, we performed a secondary sub-analysis for checking that the performance of the logistic regression model is not affected by the age.

Conclusion

The psychometric properties of M@T allow its application in subjects with less than 4 years of primary education in urban settings. Cut-off points should be corrected for educational level and, according our data, values of 35 and 26 are useful for distinguishing patients with aMCI and early AD, respectively, in patients with low level of education. However, M@T should not be used in isolation to define dementia, since it measures memory impairment (episodic and semantic) and orientation well, but no other types of cognitive impairment nor functionality. Therefore, the simultaneous use of brief functional tests to compensate for this deficiency is required.

Recommendations

Recent studies in European populations have evaluated the ability of M@T to discriminate between aMCI and subjective memory complaints (SMC), showing an optimal performance in subjects with medium (Rami et al., 2010) and low educational level (Sousa et al., 2015). Our study did not incorporate this study group. However, we consider that future research should do so because SMC has been reported as a predictor of cognitive decline and AD (Mendonça et al., 2016).

Additionally, the future studies should include population with a broad variability of educational level and higher sample size. Thus, multivariate models could be applied to assess the factors that is statistically associated with clinical diagnosis, which includes the years of education.

The M@T constitutes a brief, non-invasive and reliable cognitive test, which could be applicable for non-specialist physicians to support the discrimination between aMCI and early AD in primary care centers.

AUTHOR CONTRIBUTIONS

NC performed the conception of the study. NC, DL, RM and EH-P designed the study. NC, DL, RM, SC-S, JC-A and LV-L collected the data. NC and EH-P analyzed and interpreted the data of the work. EH-P and NC drafted the first draft of the article. All authors critically revised the manuscript and approved the version to be published.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2017.00278/full#supplementary-material

REFERENCES

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., et al. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the national institute on aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 270–279. doi: 10.1016/j.jalz.2011.03.008

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition: DSM-IV-TR®*. Washington, DC: American Psychiatric Association.

Barnett, J. H., Hachinski, V., and Blackwell, A. D. (2013). Cognitive health begins at conception: addressing dementia as a lifelong and preventable condition. *BMC Med.* 11:246. doi: 10.1186/1741-7015-11-246

Cacho, J., Benito-León, J., García-García, R., Fernández-Calvo, B., Vicente-Villardón, J. L., and Mitchell, A. J. (2010). Does the combination of the MMSE and clock drawing test (mini-clock) improve the detection of mild Alzheimer's disease and mild cognitive impairment? *J. Alzheimers Dis.* 22, 889–896. doi: 10.3233/JAD-2010-101182

Carnero-Pardo, C., Espejo-Martínez, B., López-Alcalde, S., Espinosa-García, M., Sáez-Zea, C., Hernández-Torres, E., et al. (2011a). Diagnostic accuracy, effectiveness and cost for cognitive impairment and dementia screening of three short cognitive tests applicable to illiterates. *PLoS One* 6:e27069. doi: 10.1371/journal.pone.0027069

Carnero-Pardo, C., Espejo-Martinez, B., Lopez-Alcalde, S., Espinosa-Garcia, M., Saez-Zea, C., Vilchez-Carrillo, R., et al. (2011b). Effectiveness and costs of phototest in dementia and cognitive impairment screening. *BMC Neurol.* 11:92. doi: 10.1186/1471-2377-11-92

Connolly, A., Gaehl, E., Martin, H., Morris, J., and Purandare, N. (2011). Underdiagnosis of dementia in primary care: variations in the observed prevalence and comparisons to the expected prevalence. *Aging Ment. Health* 15, 978–984. doi: 10.1080/13607863.2011.596805

Cooper, C., Sommerlad, A., Lyketsos, C. G., and Livingston, G. (2015). Modifiable predictors of dementia in mild cognitive impairment: a systematic review and meta-analysis. *Am. J. Psychiatry* 172, 323–334. doi: 10.1176/appi.ajp.2014.14070878

Custodio, N., Alva-Diaz, C., Becerra-Becerra, Y., Montesinos, R., Lira, D., Herrera-Pérez, E., et al. (2016a). Rendimiento en pruebas cognitivas breves, de adultos mayores con demencia en estadios avanzados, residentes de una comunidad urbana de Lima, Perú. *Rev. Peru. Med. Exp. Salud Pública* 33, 662–669. doi: 10.17843/rpmesp.2016.334.2549

Custodio, N., Herrera-Perez, E., Lira, D., Roca, M., Manes, F., Báez, S., et al. (2016b). Evaluation of the INECO frontal screening and the frontal assessment battery in peruvian patients with Alzheimer's disease and behavioral variant Frontotemporal dementia. *eNeurologicalSci* 5, 25–29. doi: 10.1016/j.ensci.2016.11.001

Custodio, N., García, A., Montesinos, R., Lira, D., and Bendezú, L. (2011). Validation of the clock drawing test—Manos' version—as a screening test for detection of dementia in older persons of Lima, Peru. *Rev. Peru. Med. Exp. Salud Publica* 28, 29–34. doi: 10.1590/S1726-46342011000100005

Custodio, N., and Lira, D. (2014). Adaptación peruana del minimental state examination (MMSE). *An. Fac. Med.* 75:69. doi: 10.15381/anales.v75i1.6951

Custodio, N., Lira, D., Herrera-Perez, E., Nuñez Del Prado, L., Parodi, J., Guevara-Silva, E., et al. (2014). The memory alteration test discriminates between cognitively healthy status, mild cognitive impairment and Alzheimer's disease. *Dement. Geriatr. Cogn. Dis. Extra* 4, 314–321. doi: 10.1159/000365280

Custodio, N., Wheelock, A., Thumala, D., and Slachevsky, A. (2017). Dementia in latin america: epidemiological evidence and implications for public policy. *Front. Aging Neurosci.* 9:221. doi: 10.3389/fnagi.2017.00221

Dal Forno, G., Palermo, M. T., Donohue, J. E., Karagiozis, H., Zonderman, A. B., and Kawas, C. H. (2005). Depressive symptoms, sex, and risk for Alzheimer's disease. *Ann. Neurol.* 57, 381–387. doi: 10.1002/ana.20405

De Meyer, G., Shapiro, F., Vanderstichele, H., Vanmechelen, E., Engelborghs, S., De Deyn, P. P., et al. (2010). Diagnosis-independent Alzheimer disease biomarker signature in cognitively normal elderly people. *Arch. Neurol.* 67, 949–956. doi: 10.1001/archneurol.2010.179

Derby, C. A., Burns, L. C., Wang, C., Katz, M. J., Zimmerman, M. E., L'italien, G., et al. (2013). Screening for predementia AD: time-dependent operating characteristics of episodic memory tests. *Neurology* 80, 1307–1314. doi: 10.1212/WNL.0b013e31828ab2c9

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

Freitas, S., Simões, M. R., Alves, L., and Santana, I. (2015). The relevance of sociodemographic and health variables on MMSE normative data. *Appl. Neuropsychol. Adult* 22, 311–319. doi: 10.1080/23279095.2014.926455

Hughes, C. P., Berg, L., Danziger, W. L., Coben, L. A., and Martin, R. (1982). A new clinical scale for the staging of dementia. *Br. J. Psychiatry* 140, 566–572. doi: 10.1192/bjp.140.6.566

Kaplan, E., Goodglass, H., and Weintraub, S. (1983). *The Boston Naming Test*. 2nd Edn. Philadelphia, PA: Lea & Febiger.

Kato, Y., Narumoto, J., Matsuoka, T., Okamura, A., Koumi, H., Kishikawa, Y., et al. (2013). Diagnostic performance of a combination of Mini-mental state examination and clock drawing test in detecting Alzheimer's disease. *Neuropsychiatr. Dis. Treat.* 9, 581–586. doi: 10.2147/NDT.S42209

Kim, J. L., Park, J. H., Kim, B. J., Kim, M. D., Kim, S.-K., Chi, Y. K., et al. (2012). Interactive influences of demographics on the mini-mental state examination (MMSE) and the demographics-adjusted norms for MMSE in elderly Koreans. *Int. Psychogeriatr.* 24, 642–650. doi: 10.1017/s1041610211002456

Ladeira, R. B., Diniz, B. S., Nunes, P. V., and Forlenza, O. V. (2009). Combining cognitive screening tests for the evaluation of mild cognitive impairment in the elderly. *Clinics* 64, 967–973. doi: 10.1590/s1807-59322009001000006

Lang, L., Clifford, A., Wei, L., Zhang, D., Leung, D., Augustine, G., et al. (2017). Prevalence and determinants of undetected dementia in the community: a systematic literature review and a meta-analysis. *BMJ Open* 7:e011146. doi: 10.1136/bmjopen-2016-011146

Laske, C., Sohrabi, H. R., Frost, S. M., López-de-Ipiña, K., Garrard, P., Buscema, M., et al. (2015). Innovative diagnostic tools for early detection of Alzheimer's disease. *Alzheimers Dement.* 11, 561–578. doi: 10.1016/j.jalz.2014.06.004

Li, H., Jia, J., and Yang, Z. (2016). Mini-mental state examination in elderly chinese: a population-based normative study. *J. Alzheimers Dis.* 53, 487–496. doi: 10.3233/jad-160119

Li, J., Wang, Y. J., Zhang, M., Xu, Z. Q., Gao, C. Y., Fang, C. Q., et al. (2011). Vascular risk factors promote conversion from mild cognitive impairment to Alzheimer disease. *Neurology* 76, 1485–1491. doi: 10.1212/WNL.0b013e318217e7a4

Lonie, J. A., Tierney, K. M., and Ebmeier, K. P. (2009). Screening for mild cognitive impairment: a systematic review. *Int. J. Geriatr. Psychiatry* 24, 902–915. doi: 10.1002/gps.2208

Maestre, G. E. (2012). Assessing dementia in resource-poor regions. *Curr. Neurol. Neurosci. Rep.* 12, 511–519. doi: 10.1007/s11910-012-0300-9

Mattis, S. (1976). Mental status examination for organic mental syndrome in the elderly patient. *Geriatr. Psychiatry* 11:e121.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. *Neurology* 34, 939–944. doi: 10.1212/wnl.34.7.939

Mendonça, M. D., Alves, L., and Bugalho, P. (2016). From subjective cognitive complaints to dementia: who is at risk? A systematic review. *Am. J. Alzheimers Dis. Other Demen.* 31, 105–114. doi: 10.1177/1533317515592331

Mitchell, A. J. (2009). A meta-analysis of the accuracy of the mini-mental state examination in the detection of dementia and mild cognitive impairment. *J. Psychiatr. Res.* 43, 411–431. doi: 10.1016/j.jpsychires.2008.04.014

Moraes, C., Pinto, J. A., Lopes, M. A., Litvoc, J., and Bottino, C. M. C. (2010). Impact of sociodemographic and health variables on mini-mental state examination in a community-based sample of older people. *Eur. Arch. Psychiatry Clin. Neurosci.* 260, 535–542. doi: 10.1007/s00406-010-0104-3

Nelson, H. E. (1976). A modified card sorting test sensitive to frontal lobe defects. *Cortex* 12, 313–324. doi: 10.1016/s0010-9452(76)80035-4

Olavarria, L., Mardones, C., Delgado, C., and Slachevsky, A. (2015). Chilean health professionals perception of knowledge about dementia. *J. Neurol. Sci.* 357:e134. doi: 10.1016/j.jns.2015.08.429

Oscanoa, T. J., Cieza, E., Parodi, J. F., and Paredes, N. (2016). Evaluación de la prueba de la moneda peruana en el tamizaje de trastorno cognitivo en adultos mayores. *Rev. Peru. Med. Exp. Salud Publica* 33, 67–73. doi: 10.17843/rpmesp.2016.331.2009

Ozer, S., Noonan, K., Burke, M., Young, J., Barber, S., Forster, A., et al. (2016). The validity of the memory alteration test and the test your memory test for community-based identification of amnestic mild cognitive impairment. *Alzheimers Dement.* 12, 987–995. doi: 10.1016/j.jalz.2016.03.014

Paddick, S.-M., Gray, W. K., McGuire, J., Richardson, J., Dotchin, C., and Walker, R. W. (2017). Cognitive screening tools for identification of dementia in illiterate and low-educated older adults, a systematic review and meta-analysis. *Int. Psychogeriatr.* 29, 897–929. doi: 10.1017/s1041610216001976

Parra, M. A. (2014). Overcoming barriers in cognitive assessment of Alzheimer's disease. *Dement. Neuropsychol.* 8, 95–98. doi: 10.1590/s1980-57642014dn82000002

Partington, J. E., and Leiter, R. G. (1949). Partington's pathways test. *Psychol. Serv. Cent. J.* 1, 9–20.

Petersen, R. C., Smith, G. E., Waring, S. C., Ivnik, R. J., Tangalos, E. G., and Kokmen, E. (1999). Mild cognitive impairment: clinical characterization and outcome. *Arch. Neurol.* 56, 303–308. doi: 10.1001/archneur.56.3.303

Rami, L., Bosch, B., Sanchez-Valle, R., and Molinuevo, J. L. (2010). The memory alteration test (M@T) discriminates between subjective memory complaints, mild cognitive impairment and Alzheimer's disease. *Arch. Gerontol. Geriatr.* 50, 171–174. doi: 10.1016/j.archger.2009.03.005

Rami, L., Bosch, B., Valls-Pedret, C., Caprile, C., Sánchez-Valle Díaz, R., and Molinuevo, J. L. (2009). Discriminatory validity and association of the mini-mental test (MMSE) and the memory alteration test (M@T) with a neuropsychological battery in patients with amnestic mild cognitive impairment and Alzheimer's disease. *Rev. Neurol.* 49, 169–174.

Rami, L., Molinuevo, J. L., Sanchez-Valle, R., Bosch, B., and Villar, A. (2007). Screening for amnestic mild cognitive impairment and early Alzheimer's disease with M@T (Memory Alteration Test) in the primary care population. *Int. J. Geriatr. Psychiatry* 22, 294–304. doi: 10.1002/gps.1672

Rey, A. (1941). L'examen psychologique dans les cas d'encéphalopathie traumatique (Les problems). *Arch. Psychol.* 28, 286–340.

Richard, E., Andrieu, S., Solomon, A., Mangialasche, F., Ahtiluoto, S., Moll van Charante, E. P., et al. (2012). Methodological challenges in designing dementia prevention trials—The European Dementia Prevention Initiative (EDPI). *J. Neurol. Sci.* 322, 64–70. doi: 10.1016/j.jns.2012.06.012

Rosselli, D., Ardila, A., Pradilla, G., Morillo, L., Bautista, L., Rey, O., et al. (2000). The mini-mental state examination as a selected diagnostic test for dementia: a Colombian population study. GENECO. *Rev. Neurol.* 30, 428–432.

Roth, M., Tym, E., Mountjoy, C. Q., Huppert, F. A., Hendrie, H., Verma, S., et al. (1986). CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. *Br. J. Psychiatry* 149, 698–709. doi: 10.1192/bjp.149.6.698

Rubínová, E., Nikolai, T., Marková, H., Siffelová, K., Laczó, J., Hort, J., et al. (2014). Clock drawing test and the diagnosis of amnestic mild cognitive impairment: can more detailed scoring systems do the work? *J. Clin. Exp. Neuropsychol.* 36, 1076–1083. doi: 10.1080/13803395.2014.977233

Scazufca, M., Almeida, O. P., Vallada, H. P., Tasse, W. A., and Menezes, P. R. (2009). Limitations of the mini-mental state examination for screening dementia in a community with low socioeconomic status: results from the Sao Paulo Ageing & Health Study. *Eur. Arch. Psychiatry Clin. Neurosci.* 259, 8–15. doi: 10.1007/s00406-008-0827-6

Sousa, M., Pereira, A., Costa, R., and Rami, L. (2015). Initial phase of adaptation of memory alteration test (M@T) in a portuguese sample. *Arch. Gerontol. Geriatr.* 61, 103–108. doi: 10.1016/j.archger.2015.03.008

Tombaugh, T. N., and McIntyre, N. J. (1992). The mini-mental state examination: a comprehensive review. *J. Am. Geriatr. Soc.* 40, 922–935. doi: 10.1111/j.1532-5415.1992.tb01992.x

UNESCO Institute for Statistics. (2016). 50th anniversary of international literacy day: literacy rates are on the rise but millions remain illiterate. Available online at: http://uis.unesco.org/sites/default/files/documents/fs38-50th-anniversary-of-international-literacy-day-literacy-rates-are-on-the-rise-but-millions-remain-illiterate-2016-en.pdf [Accessed February 26, 2017].

Wechsler, D. (1997). *WAIS-III: Wechsler Adult Intelligence Scale*. 3rd Edn. San Antonio, TX: Psychological Corporation.

Wind, A. W., Schellevis, F. G., Van Staveren, G., Scholten, R. P., Jonker, C., and Van Eijk, J. T. (1997). Limitations of the mini-mental state examination in diagnosing dementia in general practice. *Int. J. Geriatr. Psychiatry* 12, 101–108. doi: 10.1002/(SICI)1099-1166(199701)12:1<101::AID-GPS469>3.0.CO;2-R

Xie, H., Zhang, C., Wang, Y., Huang, S., Cui, W., Yang, W., et al. (2016). Distinct patterns of cognitive aging modified by education level and gender among adults with limited or no formal education: a normative study of the mini-mental state examination. *J. Alzheimers Dis.* 49, 961–969. doi: 10.3233/jad-143066

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: 10 May 2017
doi: 10.3389/fnagi.2017.00122

Feature Binding of Common Everyday Items Is Not Affected by Age

Serge Hoefeijzers[1†], Alfredis González Hernández[2†], Angela Magnolia Rios[2] and Mario A. Parra[1, 2, 3, 4, 5, 6]*

[1] Department of Psychology, School of Social Sciences, Heriot-Watt University, Edinburgh, UK, [2] DENEUROPSY, Surcolombiana University, Huila, Colombia, [3] Department of Psychology, Human Cognitive Neuroscience and Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, UK, [4] Alzheimer Scotland Dementia Research Centre, University of Edinburgh, Edinburgh, UK, [5] Scottish Dementia Clinical Research Network, University of Edinburgh, Edinburgh, UK, [6] Facultad de Psicologia, Universidad Autónoma del Caribe, Barranquilla, Colombia

There is a surge of studies confirming that old age spares the ability to bind in visual working memory (VWM) multiple features within singular object representations. Furthermore, it has been suggested that such ability may also be independent of the cultural background of the assessed individual. However, this evidence has been gathered with tasks that use arbitrary bindings of unfamiliar features. Whether age spares memory binding functions when the memoranda are features of everyday life objects remains less well explored. The present study investigated the influence of age, memory delay, and education, on conjunctive binding functions responsible for representing everyday items in VWM. We asked 32 healthy young and 41 healthy older adults to perform a memory binding task. During the task, participants saw visual arrays of objects, colours, or coloured objects presented for 6 s. Immediately after they were asked either to select the objects or the colours that were presented during the study display from larger sets of objects or colours, or to recombine them by selecting from such sets the objects and their corresponding colours. This procedure was repeated immediately after but this time providing a 30 s unfiled delay. We manipulated familiarity by presenting congruent and incongruent object-colour pairings. The results showed that the ability to bind intrinsic features in VWM does not decline with age even when these features belong to everyday items and form novel or well-known associations. Such preserved memory binding abilities held across memory delays. The impact of feature congruency on item-recognition appears to be greater in older than in younger adults. This suggests that long-term memory (LTM) supports binding functions carried out in VWM for familiar everyday items and older adults still benefit from this LTM support. We have expanded the evidence supporting the lack of age effects on VWM binding functions to new feature and object domains (i.e., everyday items). We have confirmed that education does not negatively impact on such ability at old age. Such results have important implications for the selection of culturally unbiased tests to screen for abnormal ageing trajectories.

Keywords: visual working memory binding, ageing, cross-cultural validity, cognition, neuropsychological assessment, Feature binding, Common everyday items

OPEN ACCESS

Edited by:
Lucas Sedeño, Instute of Cognitive Neurology, Argentina

Reviewed by:
Louise A. Brown, University of Strathclyde, UK
Zaifeng Gao, Zhejiang University, China

***Correspondence:**
Mario A. Parra
m.parra_rodriguez@hw.ac.uk

[†] These authors have contributed equally to this work.

Received: *04 December 2016*
Accepted: *12 April 2017*
Published: *10 May 2017*

Citation:
Hoefeijzers S, González Hernández A, Magnolia Rios A and Parra MA (2017) Feature Binding of Common Everyday Items Is Not Affected by Age. Front. Aging Neurosci. 9:122. doi: 10.3389/fnagi.2017.00122

INTRODUCTION

Associative memory functions, such as those needed to remember the location of objects, decline with age. This effect is thought to underlie age-related impairments in retaining associations in long-term memory (LTM) (Chalfonte and Johnson, 1996), which in turn affects older people's episodic memory (Naveh-Benjamin et al., 2004, 2007; Old and Naveh-Benjamin, 2008; Shing et al., 2010; Peterson and Naveh-Benjamin, 2016). However, age-related associative memory deficits have also been observed over intervals of seconds, suggesting that the ability to form and maintain associations in short-term memory (STM) also declines with age (Cowan et al., 2006; Mitchell et al., 2006; Chen and Naveh-Benjamin, 2012; Cowan, 2016; Peterson and Naveh-Benjamin, 2016).

Whereas age affects the retention of associations both in LTM and in STM (but see Bopp and Verhaeghen, 2009; Pertzov et al., 2015), it seems to spare the ability to hold surface features, such as shapes and colours, bound within integrated objects in STM (Brockmole et al., 2008; Parra et al., 2009b; Brown and Brockmole, 2010; Brockmole and Logie, 2013; Isella et al., 2015; Read et al., 2016; Brown et al., 2017). Rhodes and colleagues argued for a distinction between binding of extrinsic features (i.e., linking of distinct items or contextual features accompanying an item) and binding of features that define the intrinsic characteristics of an object (i.e., within-item binding) (see also Allen et al., 2013; Rhodes et al., 2015). Binding objects' intrinsic features appears to be an automatic process (Allen et al., 2006, 2009; Karlsen et al., 2010; but see Shen et al., 2015; Gao et al., 2017) that is largely spared by age (Parra et al., 2009b; Brown and Brockmole, 2010; Isella et al., 2015; Rhodes et al., 2015; Read et al., 2016; Brown et al., 2017). In contrast, binding extrinsic features requires more cognitive resources (e.g., associative functions of the medial temporal lobe), which appear to be more susceptible to the effects of age (Mitchell et al., 2006).

Although general visual STM (VSTM) abilities do decline with older age (Reuter-Lorenz and Sylvester, 2005), the ability to integrate multiple surface features into singular object representations remains preserved across the lifespan (Brockmole et al., 2008; Parra et al., 2009b,c; Brown and Brockmole, 2010; Brockmole and Logie, 2013; Isella et al., 2015; Rhodes et al., 2015; Read et al., 2016; Brown et al., 2017). To ascertain that binding is selectively compromised, one needs to demonstrate that memory for the constituent parts is preserved. A psychometrically valid memory binding paradigm should assess the cost of binding against the cost of processing single features (shape and colour). Such a cost has proved to remain stable across the lifespan.

However, the same VSTM binding functions that have proved insensitive to normal ageing have been found to be dramatically affected in patients with Alzheimer's disease (AD) both when they recall (Parra et al., 2009a) or recognise (Parra et al., 2010a) feature conjunctions. Patients with AD show a disproportional cost of binding relative to healthy controls. This impairment becomes apparent in otherwise asymptomatic carriers of the E280A presenilin-1 gene mutation who will inevitably develop familial AD more than 10 years prior to the onset of dementia (Parra et al., 2010b, 2011). This indicates that VSTM binding holds marker properties to identify the pre-clinical stages of AD. It is worth noting that binding intrinsic features in VSTM is not affected by depression (Parra et al., 2010a) or other non-AD dementias (i.e., FTD, PD, VasD, DLB) (Della Sala et al., 2012), making it both sensitive and specific for AD. For a cognitive test to be considered a reliable marker for AD, it should also be insensitive to the cultural background of the assessed individual (Logie et al., 2015). Parra et al. (2011) suggested that feature binding in VSTM also appears to meet this criterion. The authors compared data from a change detection task of patients with sporadic and familial AD in samples recruited in Scotland and in Colombia, as well as data from healthy controls also recruited in both countries. Mean performance of patients and controls across countries did not differ significantly despite significant differences of age and education between samples across countries. In that earlier study, as well as in most of the above-mentioned studies investigating VSTM binding, meaningless combinations of random shapes and colours were the memoranda. It remains unknown whether age and education impair feature binding of common everyday items.

The present study investigated whether the ability to process in memory congruent and incongruent combinations of common surface features over short and long retention intervals is differentially affected in older adults with a low educational background. The results from studies by Parra and colleagues in healthy ageing (Brockmole et al., 2008; Parra et al., 2009b) and those in AD (Parra et al., 2010a,b) involved tasks assessing memory for coloured-shapes which are unfamiliar making them difficult to name or rehearse. Whether binding functions carried out in visual working memory (VWM) which support the integration of common features into familiar everyday items are also insensitive to age and education is yet unknown. Moreover, whether such factors (i.e., age and education) spare the representations of complex items in memory over longer memory delays is another question that needs investigation. Recent evidence from Chen and Naveh-Benjamin (2012) shows that age-related associative memory deficits for extrinsic features (i.e., face-scene association) are evident over both short (seconds) and longer delay intervals (minutes). As we used familiar everyday items, the support that VWM binding functions would receive from LTM would be greater in the context of the present study than in that of earlier studies (Brockmole et al., 2008; Parra et al., 2009b). Such a support is expected to render the influence of factors, such as age and education even more informative. We further manipulated the availability of such a support by presenting congruent (e.g., typical red apple) and incongruent object-colour pairings. We used a paradigm which assesses reconstruction rather than simple recognition (Parra et al., 2009c). Previous studies using similar versions of this paradigm have reported a lack of age effects on VSTM binding (Brockmole and Logie, 2013; van Geldorp et al., 2015). Reconstruction is a more challenging task than recognition as it involves aspects of both recall and recognition of previous experiences (van Geldorp et al., 2015). Such a paradigm would increase the likelihood of identifying effects of age on VWM binding functions under the different experimental conditions investigated here.

Based on core features of the memory binding paradigm used in this study we made the following predictions. Reconstruction accuracy would be significantly better for congruent object-colour bindings than for incongruent bindings regardless of age, as VWM binding functions would receive more support from LTM. In line with previous studies, no age-related binding deficits would be observed for familiar everyday items. Longer delay periods would have lesser impact on younger than on older adults. This is because the use of familiar items in our task may facilitate access to LTM and rehearsal over longer delays, functions known to be sensitive to normal ageing (Poon and Fozard, 1980; Nielsen-Bohlman and Knight, 1995; Brown et al., 2012).

MATERIALS AND METHODS

We investigated the ability of healthy young adults and healthy older adults to hold congruent (i.e., white hen) and incongruent (i.e., purple bread) combinations of common surface features and to recognise them immediately after encoding (i.e., 0 ms delay) and after a long retention interval (i.e., 30 s delay). To this aim, we relied on a mixed design (i.e., ANOVA) with age as the between-subjects factor and congruency and delay as the repeated measures, each with two levels. Moreover, due to the heterogeneity of this population with regard to their educational background, we capitalised on this opportunity to explore whether and to what extent such a demographic factor affected performance across conditions and age groups. This was achieved via ANCOVA.

Participants

A group of 32 healthy young adults (25 female) and a group of 41 healthy older adults (27 female) were recruited for the study. The study took place at the Psychology Department of the Surcolombiana University, Colombia. Younger and older participants were recruited from the university setting and from the community. They were invited via local media advertisements or were approached directly by members of the research group. All the participants were fully informed about the study. Informed consent was obtained from each participant according to the Declaration of Helsinki (World Medical Association, 2001). The study was reviewed and approved by the University's Ethics Committee.

Younger and older adults' groups significantly differed in age and education. Healthy older adults had significantly fewer years of education than younger adults. The healthy condition of our sample was ascertained via a brief neuropsychological assessment (see **Table 1**). In fact, the sample of healthy older adults collected for this study outperformed the local norms (Aguirre-Acevedo et al., 2007).

The VSTM Task

The paradigm applied in this study was based on paradigms used by (Parra et al., 2009c). Two sets of 10 nameable colours (Red, Blue, Green, Brown, Orange, Yellow, Purple, Silver, Turquoise, and Pink) and 20 nameable objects were used. The set of objects consisted of 10 man-made objects (saw, bread, cross, shoe, glove, hammer, hat, lamp, light bulb, frying pan) and 10 living objects (duck, crocodile, arm, baby, bear, bee, cat, cow, dog, hen). Objects were taken from the International Picture Naming Project (http://crl.ucsd.edu/∼aszekely/ipnp/). The stimuli were presented on a 15″ computer screen. As shown in **Figure 1**, the task consisted of three test conditions: the Object Only condition, the Colour Only condition and the Object-Colour Binding condition. For each test condition, participants were presented with a first study array which was followed immediately after by the first test array (immediate recognition). After participants' response, the same study array was presented again this time followed by a 30 s unfilled retention interval before the second test array was presented.

First Study Array

In the study array, participants were presented with objects, colours or coloured objects. To control for memory load, as determined by the number of to-be-remembered features, we kept the number of features constant for each test condition and varied the number of objects. Participants were presented with study arrays consisting of 4 objects (Object Only condition), 4 colours (Colour Only condition), or 2 combinations of objects and colours (Object-Colour Binding condition). The study array was presented for 1.5 s per feature (6 s in total). Participants were instructed to pay close attention to the presented items and to try to memorise them.

Test Array (Immediate)

Immediately after the study array a test array was presented. In the condition assessing memory for single features (i.e., the Object Only condition and the Colour Only condition) the test array presented twice as many items as the study array. Half of these items corresponded to those previously seen while the other half were items not presented in the study array. Participants were requested to select, using the mouse, the objects or the colours they had seen in the study array. In the condition assessing memory for Object-Colour Binding, the test display presented two separate arrays of items. One array consisted of the same objects previously seen plus the same number of new distracter objects, and the second array consisted of the same colours previously seen plus the same number of new distracter colours. Participants were requested to select, using the mouse, the objects they had seen in the study array with their corresponding colours (i.e., choose each object and its colour). For the object-colour binding condition we designed two separate blocks of trials. One block presented congruent combinations of objects and colours (e.g., yellow hen, brown bear, black shoes, silver frying pan) and the other presented incongruent combinations (e.g., pink bee, red crocodile, green hammer, purple saw). Stimuli congruency ratings was high for both groups (see post VSTM Task Congruency Test below).

Second Study Array

After immediate recognition, for which feedback was not provided, the same study display was presented again as described above. This was followed by a 30 s unfilled retention interval. Presenting the same study array a second time assured

TABLE 1 | Demographic and neuropsychological data and results from the statistical comparisons.

Variable	Healthy young adults (n = 32)/Or Norms (*) Mean (SD)	Healthy elderly (n = 41) Mean (SD)	t (p); Effect size (r); Power (β)
Age (years)	33.44 (8.36)	70.22 (7.34)	−19.99 (<0.001); 0.92; 1.00
Education (years)	12.03 (5.26)	4.85 (3.21)	6.80 (<0.001); 0.70; 1.00
MMSE*	27.76 (1.77)	27.70 (2.10)	0.16 (0.877); 0.01
World List Learning (Total Recall)*	3.55 (1.99)	6.03 (2.20)	6.21 (<0.001); 0.43
World List Learning Recognition*	7.70 (2.31)	9.8 (0.48)	23.73 (<0.001); 0.52
ROF-Copy*	18.08 (6.16)	27.12 (8.56)	5.78 (<0.001); 0.43
ROF-Recall*	6.51 (3.88)	9.77 (7.42)	2.40 (<0.023); 0.20

** Values taken from the norms (Aguirre-Acevedo et al., 2007), these were compared with the sample's values using one-sample t-tests.*

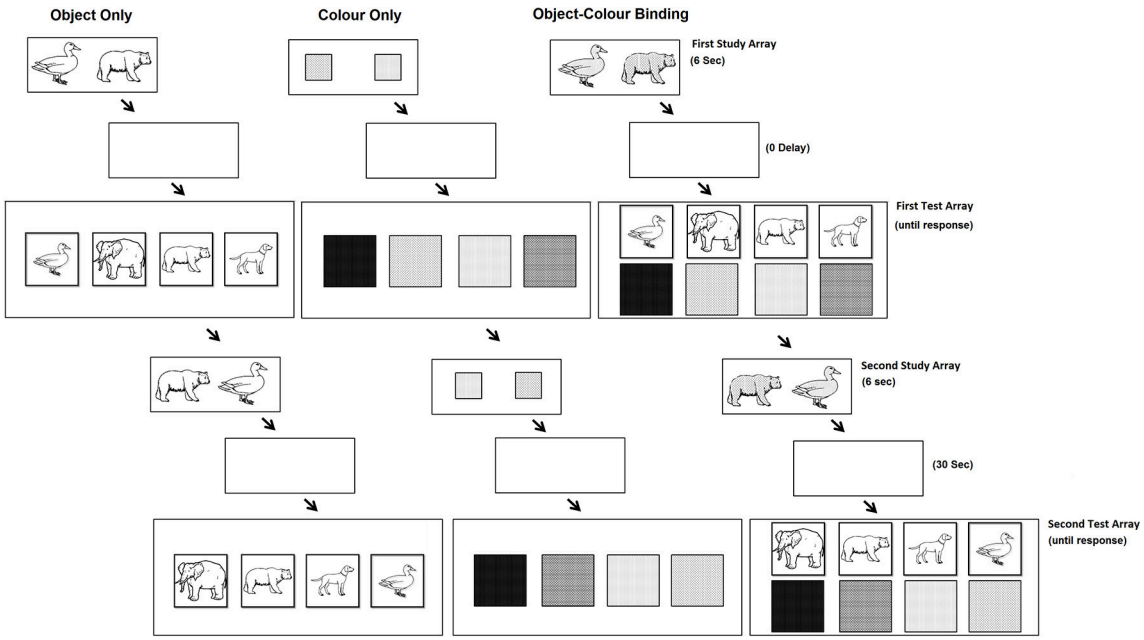


FIGURE 1 | The experimental conditions and trial sequences used in our experiment (see text for description).

that the to-be-remembered items were well presented in VWM prior to the 30 s delay interval as they could have been overwritten by items shown in the First Test array. As bindings held in VWM are fragile (quickly overwritten), we can assume that the information held during this longer retention interval was likely sourced from the Second Study array rather than from cumulative knowledge originating through the First and Second Study arrays (see Colzato et al., 2006; Logie et al., 2009).

Second Test Array (30 s Memory Delay)

After this longer delay, participants were presented with the test display again. In the Second Study array and delayed Test array, items were presented in new random locations. This manipulation was introduced to prevent the use of location as a memory cue.

Each test condition consisted of 8 trials (i.e., 4 blocks of 8 trials each). For the Objects only condition and the Object-colour binding condition, 4 of the 8 trials consisted of man-made objects. The remaining 4 trials consisted of living objects. Trials were randomised and experimental conditions were delivered in a counterbalanced order across participants. It is worth noting that the presentation time of the encoding display and delay periods (0 s and 30 s) used in our paradigm are not features of a typical working memory task. Long encoding times (1.5 s per feature) were used to enable an accurate representation of the to-be-remembered information. Such a procedure has been extensively used in previous studies (Parra et al., 2009c, 2015). Active recognition is not susceptible to the influence of iconic memory as change detection tasks may be. Moreover, retrieval functions during active recognition take longer to operate than during "same/different" change detection tasks, making immediate retention scores (0 ms delay) accurate measures of VWM (see Brockmole and Logie, 2013). Lastly, we included a 30 s delay interval which we left unfilled to create opportunity for rehearsal. As this function declines with age (Brown et al., 2012), we were interested in investigating whether such a reliance would

reveal dissociations of VWM for features and bindings of familiar everyday objects.

Post VSTM Task Congruency Test

After the VWM task, participants were presented with a screening test which assessed their perceived congruency of the coloured objects. To this aim each participant was simultaneously presented with two coloured objects. The objects and the colours had all been previously used in the VWM Task. The object presented in each screen was the same but it was shown with two different colours. For all the trials the object was paired with a congruent and an incongruent colour (side to side counterbalancing their position). In half of the trials one of the two combinations was that defined as congruent in the VWM task. In the other half one of the two combinations was that defined as incongruent in the VWM task. The participants were asked to select, using the mouse, the object of the pair that they thought was presented in its typical colour. We calculated the percentage of congruency. This task enabled us to ascertain that the intended congruency of the to-be-remembered bindings was indeed the perceived congruency. Independent-sample *t*-tests confirmed that this was the case. Both young and older adults obtained high congruency scores for the congruent and incongruent object-colour bindings that were used in the VWM task (90.63%, SD = 8.40%; 86.72%, SD = 12.91%, respectively) which did not differ statistically [$t_{(70)} = 1.479$, $p = 0.144$, $r = 0.17$, $\beta = 0.34$].

Scoring Procedures

Percentage Correct responses. The participant's percentage of correct responses was calculated for each test condition for both the immediate and 30 s delay test. Percentage Correct responses was calculated via the following equation: (# of objects, colour or object-colour combination – correctly recognised/total studied items) × 100. For the Object-Colour Binding condition, items were scored (i.e., a correct response) if the object and its corresponding colour were correctly selected. For this condition we generated two scores, one for congruent bindings and one for incongruent bindings.

While participants' judgment of congruent items was higher for living items than for man-made items, there was neither an effect of Group nor a Group × Category interaction (see Supplementary Table 1). Moreover, further exploratory analysis revealed that response accuracy (i.e., the Percentage correct responses) for congruent and incongruent items did not differ significantly across living or man-made items (see Supplementary Figure 1). We therefore collapsed the data across living and man-made categories and focused on the congruency effect only.

Statistical Analyses

Independent-sample or one-sample *t*-tests were conducted to compare demographic and neuropsychological data across groups (see **Table 1**). We applied a mixed ANOVA to examine the Percentage Correct responses across the within-subjects factors Test Condition (Objects Only vs. Colour Only vs. Congruent Object-Colour Binding vs. Incongruent Object-Colour Binding) and Delay (Immediate vs. 30 s Delay), and the between-subjects factor Group (Younger vs. Older). When the interactions were found to be significant, *post-hoc* contrasts were carried out across groups for each test condition separately (i.e., 4 comparisons) and across conditions for each group separately (6 comparisons per group). This was done for both the immediate and 30 s delay test (see **Table 2**). To avoid type-I error we used Bonferroni correction (alpha level: 0.05/32 = 0.001). We also implemented a mixed ANOVA collapsing performance across Delay (within-subjects factor: Test Condition; between-subjects factor: Group). Again, when the interactions were found to be significant, *post-hoc* contrasts were carried out across groups for each test condition separately (i.e., 4 comparisons) and across conditions for each group separately (6 comparisons per group). Bonferroni correction (alpha level: 0.05/16 = 0.003) were used to avoid type-I error. To further investigate the effect of congruency on memory binding, additional mixed ANOVAs (within-subjects factor: Test Condition; between-subjects factor: Group) were conducted with one of the 2 binding conditions (Incongruent Object-Colour Binding or Congruent Object-Colour binding) excluded from the analysis (see Supplementary Table 2). Moreover, an additional ANCOVA was carried out by adding "Education" as a covariate to the above described models. For the four *post-hoc* comparisons investigating the Percentage Correct responses between groups for each test condition, we adjusted the alpha level to 0.0125. The effect of Education on binding congruency was further examined by carrying out both a mixed ANOVA and an ANCOVA controlling for Education using Group (Younger vs. Older) as between-subjects factor and Test Condition (Congruent Object-Colour Binding vs. Incongruent Object-Colour Binding) as within-subjects factor (see Supplementary Table 3). Effect sizes for the ANOVAs and ANCOVAs were determined using partial eta-squared η_p^2, where 0.14 is a large effect (Stevens, 2002). For *t*-tests we used *r*, where 0.37 reflects a large effect size (Cohen, 1988). The alpha level was set to 0.05 for all analyses (except for the *post-hoc* comparisons), which were conducted in IBM SPSS Statistics 22.

RESULTS

Table 1 shows the age and years of education of the two groups. Healthy older adults had significantly fewer years of education than the younger adults [$t_{(48.52)} = 6.80$, $p < 0.001$, $r = 0.70$, $\beta = 1.00$].

Short-Term Memory Tasks

The ANOVA model (Group × Test Condition × Delay) revealed a significant effect of Group [$F_{(1, 70)} = 46.559$, $p < 0.001$, $\eta_p^2 = 0.399$, $\beta = 1.00$] whereby healthy older adults performed significantly poorer than young adults (see **Figure 2**). Test Condition also resulted in a significant main effect [$F_{(3, 210)} = 15.368$, $p < 0.001$, $\eta_p^2 = 0.180$, $\beta = 1.00$] whereby the percentage of correct responses was the highest for congruent Object-Colour Binding (91.88%, SE = 1.11%) followed by incongruent Object-Colour Binding (88.25%, SE = 1.46%), and Object Only (86.36%, SE = 1.05%). The percentage of correct responses for the Colour Only condition was the lowest (83.96%, SE = 1.22%). There was a

TABLE 2 | Results from paired-sample *t*-tests contrasting performance between test conditions across healthy young and older adults for the immediate and 30 s test delay.

PERFORMANCE ACROSS GROUPS FOR EACH TEST CONDITION AT THE IMMEDIATE AND 30 S TEST DELAY		
Test condition	**Immediate delay**	**30 s delay**
	t (p); Effect size (*r*); Power (β)	*t* (p); Effect size (*r*); Power (β)
Colours	**6.74 (<0.001); 0.66; 1.00**	**6.05 (<0.001); 0.61; 1.00**
Objects	**6.61 (<0.001); 0.62; 1.00**	**5.65 (<0.001); 0.57; 1.00**
Binding Congruent Objects	2.21 (0.030); 0.26; 0.64	**3.59 (0.001); 0.41; 0.95**
Binding Incongruent Objects	**4.11 (<0.001); 0.47; 0.98**	**6.30 (<0.001); 0.65; 1.00**

PERFORMANCE ACROSS TEST CONDITION FOR EACH GROUP AT THE IMMEDIATE AND 30 S TEST DELAY				
	Immediate delay		**30 s delay**	
Test condition	**Healthy young adults**	**Healthy older adults**	**Healthy young adults**	**Healthy elderly**
	t (p); Effect size (*r*); Power (β)	*t* (p); Effect size (*r*); Power (β)	*t* (p); Effect size (*r*); Power (β)	*t* (p); Effect size (*r*); Power (β)
Colours vs. Objects	−0.78 (0.443); 0.14; 0.11	−2.27 (0.029); 0.34; 0.61	−1.07 (0.295); 0.19; 0.17	−1.29 (0.205); 0.20; 0.25
Colours vs. Binding Congruent objects	−1.06 (0.299); 0.19; 0.17	**−5.28 (<0.001); 0.65; 1.00**	−3.04 (0.005); 0.48; 0.82	**−5.59 (<0.001); 0.67; 1.00**
Colours vs. Binding Incongruent objects	−0.77 (0.447); 0.14; 0.11	−1.32 (0.195); 0.21; 0.25	**−4.80 (<0.001); 0.65; 1.00**	−2.86 (0.007); 0.42; 0.81
Objects vs. Binding Congruent objects	−0.27 (0.793); 0.05; 0.04	**−3.98 (<0.001); 0.54; 0.98**	−2.15 (0.040); 0.36; 0.53	**−3.88 (<0.001); 0.53; 0.97**
Objects vs. Binding Incongruent objects	0.05 (0.958); 0.01; 0.03	−0.03 (0.980); 0.00; 0.03	**−4.02 (<0.001); 0.59; 0.97**	−1.39 (0.174); 0.22; 0.28
Binding Congruent objects vs. Binding Incongruent objects	0.34 (0.738); 0.06; 0.05	**3.67 (0.001); 0.51; 0.95**	−0.97 (0.340); 0.17; 0.15	**3.54 (0.001); 0.49; 0.94**

The alpha level was set to 0.05 for all mixed-factor ANOVAs. In order to avoid type-I error, we adjusted the alpha level to 0.001, using Bonferroni correction, i.e., 0.05/32 comparisons (For each delay interval: 6 within-group comparisons per test group, i.e., each comparison is between 2 of the 4 test conditions; 4 between group comparisons for each test condition–see statistical analysis). The values in bold represent statistical significant findings.

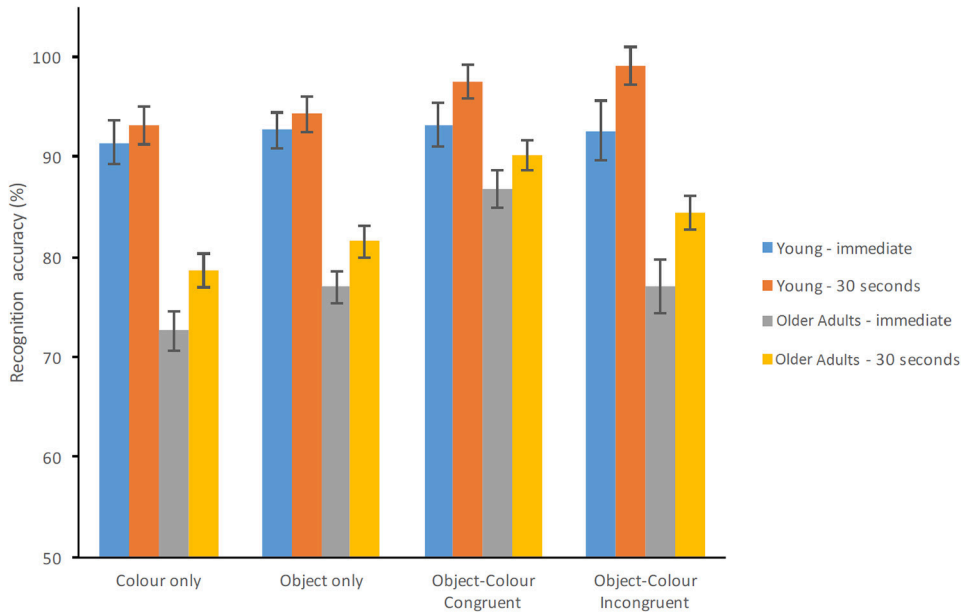


FIGURE 2 | Percentage of correct recognition for healthy young and older adults for each test condition and test delay (Error bars represent the standard error of the mean).

main effect of Delay [$F_{(1, 70)} = 29.042$, $p < 0.001$, $\eta_p^2 = 0.293$, $\beta = 1.00$] whereby response accuracy improved significantly after the 30 s delay compared to immediate recognition. The interaction between Group and Test condition was significant [$F_{(3, 210)} = 6.483$, $p < 0.001$, $\eta_p^2 = 0.085$, $\beta = 0.969$]. Neither the Group × Delay [$F_{(1, 70)} = 1.224$, $p = 0.272$, $\eta_p^2 = 0.017$, $\beta = 0.194$] nor the Group × Test Condition × Delay interaction [$F_{(3, 210)} = 0.766$, $p = 0.514$, $\eta_p^2 = 0.011$, $\beta = 0.213$] were found to be significant.

Post-hoc comparisons carried out across Group for each Condition and Delay are shown in **Table 2**. Young adults

outperformed the older adults on all test conditions, except for congruent Colour-Object Binding with 0 s delay. **Table 2** also shows performance across Condition for each Group at each Delay. No significant differences between test conditions were observed for young adults at 0 s delay. However, young adults performed significantly better on the Incongruent Colour-Object Binding condition compared to the Colour only or Object Only condition with 30 s delay. Healthy older adults performed significantly better on the Congruent Colour-Object binding condition compared to the other 3 test conditions. This was the case regardless of delay. As Delay did not interact with Group nor did it modify the key Group × Test Condition interaction, we collapsed the data across this factor and ran a two way ANOVA with Group and Test Condition.

Figure 3 shows the average Percentage Correct responses of healthy young and older adults across the four conditions of the STM Task. There was a main effect of Group [$F_{(1, 70)} = 46.559$, $p < 0.001$, $\eta_p^2 = 0.399$, $\beta = 1.00$] whereby healthy older adults performed significantly poorer than younger adults. The main effect of Condition was also significant [$F_{(3, 210)} = 15.368$, $p < 0.001$, $\eta_p^2 = 0.18$, $\beta = 1.00$] whereby congruent Object-Colour Binding > incongruent Object-Colour Binding > Objects Only > Colour only (see previous analysis for recognition scores). Crucially, the Group × Test Condition interaction was significant [$F_{(3, 210)} = 6.483$, $p < 0.001$, $\eta_p^2 = 0.085$, $\beta = 0.969$].

Post-hoc comparisons carried out across Group for each Test Condition separately showed that young adults outperformed the older adults on all tests (see **Table 3**). Performance across Test Condition for each Group (6 comparisons per group) showed that for healthy young adults performance did only differ significantly between the Colour Only condition and the Object–Colour Binding condition for incongruent bindings, with the percentage of correct responses for incongruent bindings being better than for colours only (see **Figure 3**). In contrast, for healthy older adults, performance on the Object-Colour binding condition for congruent object-colour bindings was significantly better than on all the other test conditions. No other contrasts revealed significant differences (see **Table 3**). In sum, these results suggest that the interaction was driven by a significantly better performance of older adults on the Object-Colour binding condition congruent trials relative to the other conditions, an effect that was not apparent in younger adults. This assumption is further supported by the findings of an additional 2 (age group) × 3 (test condition) ANOVAs shown in Supplementary Table 2. Here we included either the Congruent Object-Colour binding condition or the Incongruent Object-Colour binding condition as "binding" variable in the ANOVA (i.e., the included test conditions were: Objects only, Colour-only and either Incongruent Object-Colour binding or Congruent Object-Colour binding). The Group × Condition interaction was observed if the Congruent Object-Colour binding condition [$F_{(2, 140)} = 9.082$, $p < 0.001$, $\eta_p^2 = 0.115$, $\beta = 0.973$] was included in the analysis, but not when the Incongruent Object-Colour binding was included [$F_{(2, 140)} = 0.517$, $p = 0.598$, $\eta_p^2 = 0.007$, $\beta = 0.134$]. The significant interaction was driven by increased accuracy of older adults in the congruent binding condition.

Finally, the ANCOVA model controlling for the effects of education showed that this covariate had a significant effect [$F_{(1, 69)} = 16.134$, $p < 0.001$, $\eta_p^2 = 0.190$, $\beta = 0.977$]. Nevertheless, education accounted neither for the effect of Group [$F_{(1, 69)} = 9.821$, $p = 0.003$, $\eta_p^2 = 0.125$, $\beta = 0.871$] nor Test Condition [$F_{(3, 207)} = 5.3$, $p = 0.002$, $\eta_p^2 = 0.071$, $\beta = 0.928$]. After controlling for education congruent Object-Colour Binding still yielded better performance (91.62%, SE = 1.07%) than incongruent Object-Colour Binding (87.82%, SE = 1.37%), than Objects Only (86.09%, SE = 1.00%) and Colour Only (83.58%, SE = 1.14%). Education did not interact with Test Condition [$F_{(3, 207)} = 1.071$, $p = 0.362$, $\eta_p^2 = 0.015$, $\beta = 0.287$]. However,

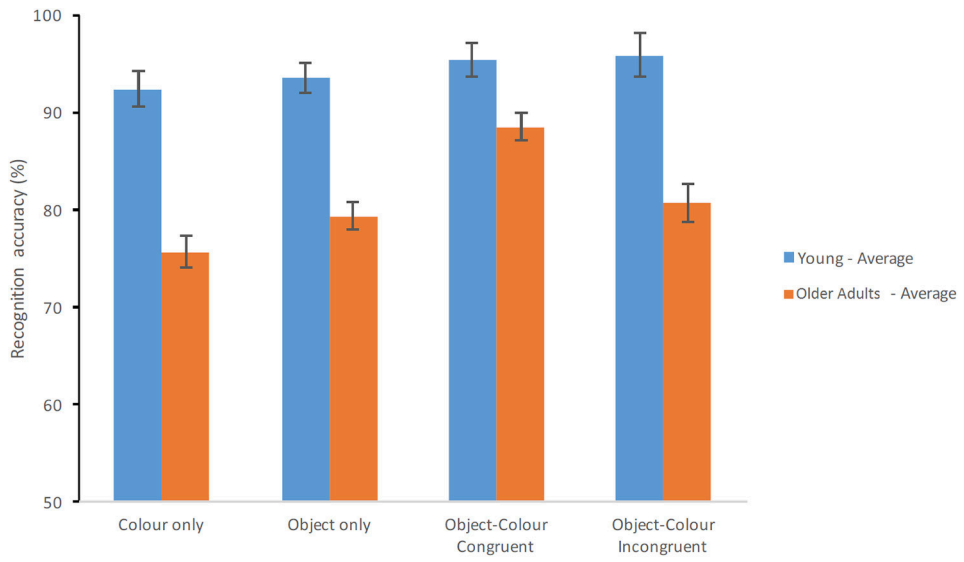


FIGURE 3 | Percentage of correct recognition averaged across test delays. Average scores of young adults and healthy older adults are shown for each test condition (Error bars represent the standard error of the mean).

controlling for Education did remove the Group × Condition interaction observed with the uncontrolled model [$F_{(3, 207)} = 2.389$, $p = 0.07$, $\eta_p^2 = 0.033$, $\beta = 0.592$]. As **Figure 4** shows, overall performance of the younger group dropped after this manipulation which reduced group differences particularly on the Object-Colour Binding conditions. Of note, older adults' performance remained intact (see **Figure 4**). In fact, further contrasts across groups for each condition separately controlling for the effects of Education and type-I error showed that young adults still scored significantly better than healthy elderly on the Colour Only condition [$F_{(1, 69)} = 11.110$, $p = 0.001$, $\eta_p^2 = 0.139$, $\beta = 0.908$] and Objects Only condition [$F_{(1, 69)} = 12.710$, $p = 0.001$, $\eta_p^2 = 0.156$; $\beta = 0.940$] but not on conditions requiring memory binding [Colour-Object Binding for incongruent items: $F_{(1, 69)} = 4.260$, $p = 0.043$, $\eta_p^2 = 0.058$, $\beta = 530$; Colour-object binding for congruent items: $F_{(1, 69)} = 0.671$, $p = 0.415$, $\eta_p^2 = 0.010$, $\beta = 0.127$]. It is worth noting that controlling for Education significantly reduced the power of this analysis. Hence, the lack of interaction under this controlled analysis needs to be interpreted with caution. Nevertheless, it is worth noting that the

TABLE 3 | Results from paired-sample *t*-tests contrasting performance between test conditions across healthy young and older adults.

Performance across groups for each test condition		
Test condition	***t* (p); Effect size (*r*); Power**	
Colours	**7.29 (<0.001); 0.69; 1.00**	
Objects	**7.07 (<0.001); 0.65; 1.00**	
Binding Congruent Objects	**3.30 (0.002); 0.39; 0.91**	
Binding Incontruent Objects	**5.63 (<0.001); 0.61; 1.00**	
Performance across test condition for each group Test condition	**Healthy young adults**	**Healthy older adults**
	t (p); Effect size (*r*); Power (β)	*t* (p); Effect size (*r*); Power (β)
Colours vs. Objects	−1.11 (0.275); 0.20; 0.19	−2.02 (0.05); 0.34; 0.51
Colours vs. Binding Congruent objects	−2.39 (0.023); 0.39; 0.65	**−6.67 (<0.001); 0.77; 1.00**
Colours vs. Binding Incongruent objects	**−3.88 (0.001); 0.57; 0.97**	−2.41 (0.021); 0.40; 0.66
Objects vs. Binding Congruent objects	−1.40 (0.172); 0.24; 0.28	**−4.66 (<0.001); 0.64; 1.00**
Objects vs. Binding Incongruent objects	−2.14 (0.040); 0.36; 0.56	−0.67 (0.508); 0.12; 0.10
Binding Congruent objects vs. Binding Incongruent objects	−0.41 (0.683); 0.07; 0.06	**4.56 (<0.001); 0.63; 0.99**

Remark: The alpha level was set to 0.05 for all mixed-factor ANOVAs. In order to avoid type-I error, we adjusted the alpha level to 0.003, using Bonferroni correction, i.e., 0.05/16 comparisons (6 within-group comparisons per test group, i.e., each comparison is between 2 of the 4 test conditions; 4 between group comparisons for each test condition – see statistical analysis). The values in bold represent statistical significant findings.

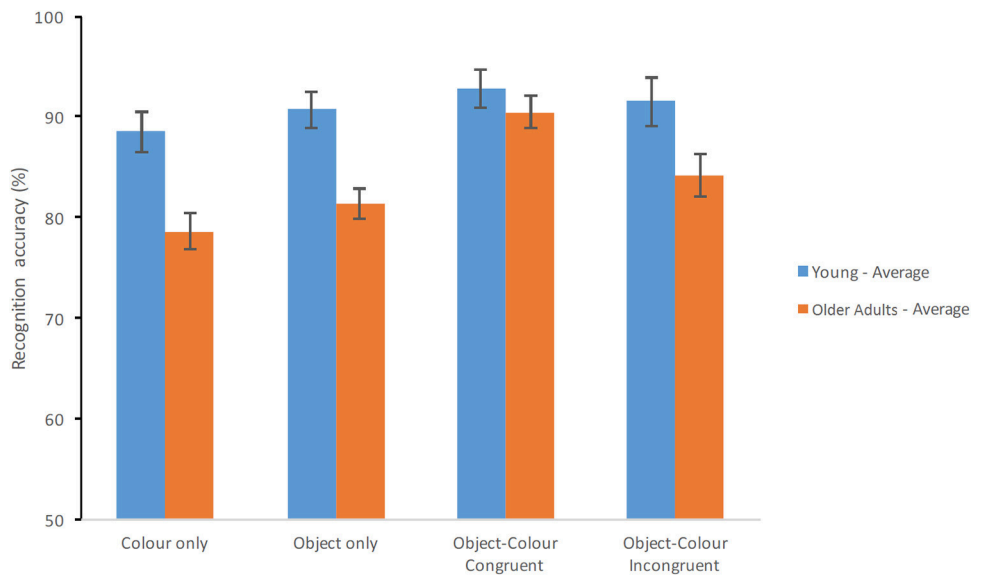


FIGURE 4 | Percentage of correct recognition averaged across test delays when the analysis was controlled for education. Average scores of young adults and healthy older adults are shown for each test condition (Error bars represent the standard error of the mean).

variance controlled for by this analysis, originates, practically in its entirety, from the younger group as older adults' performance was virtually unaffected (if anything it improved).

The effects of education on age and binding congruency were further examined using a 2 (age group) × 2 (binding congruency: Incongruent vs. Congruent Object-Colour Binding) ANOVA and ANCOVA (see Supplementary Table 3). Interestingly, neither Age [$F_{(1, 69)} = 2.808$, $p = 0.098$, $\eta_p^2 = 0.039$, $\beta = 0.379$] nor the Age × Binding Congruency interaction yielded significant effects [$F_{(1, 69)} = 3.258$, $p = 0.075$, $\eta_p^2 = 0.045$, $\beta = 0.429$] once the analysis was controlled for Education. There was a significant main effect of Binding Congruency [$F_{(1, 69)} = 9.347$, $p = 0.003$, $\eta_p^2 = 0.119$, $\beta = 0.854$] with percentage of correct responses higher for the Congruent Object-Colour condition compared to the Incongruent Object-Colour condition (as shown in **Figure 4**). In other words, differences in Education across groups masked a potential benefit for congruent binding memory in young adults (which were highly educated). However, none of these effects suggested that older adults' VSTM binding abilities were poorer than those of younger adults.

In sum, the above data suggest that the ability to bind information in VSTM remains preserved in older adults with a low educational background. Older adults seem to capitalize on the support available from LTM when they perform online tasks (i.e., Congruent Bindings >>> Incongruent Bindings) and in the absent of such a support, they can still form and hold bindings in VWM at a cost no greater than that seen in younger adults.

DISCUSSION

The present study was set out to investigate whether the ability to hold in VSTM congruent and incongruent combinations of features of everyday objects over short and long retention intervals is differentially affected in older adults who have a low educational background. The key findings are: (1) the ability to bind intrinsic features in VWM does not decline with age even when these features belong to everyday items and form novel or well-known associations; (2) the impact of feature congruency on item-recognition appears to be greater in older than in younger adults; (3) preserved VWM binding abilities hold across delays; and (4) education aided performance in younger adults regardless of the memoranda an effect that was not apparent in older adult. We now discuss these findings in turn.

Influences of LTM on VSTM Binding across Age

As suggested earlier, we found an overall impact of age on VWM but not differentially on binding functions carried out within this memory system (Brockmole et al., 2008; Parra et al., 2009b; Rhodes et al., 2015). Earlier studies on ageing and VWM binding used meaningless features or bindings which are unlikely represented in LTM or learn across trials (Colzato et al., 2006; Logie et al., 2009). The question of whether age also spares VWM binding functions than can be supported by LTM had not been explored previously. Moreover, unlike previous studies, in the current study we assessed VWM in conditions where load, as determined by the number of features, was kept constant across conditions. Olson and Jiang (2002) acknowledged that this setting may be appropriate to test the cost of binding. The authors suggested that the hypothesis of an object-based VWM (i.e., features are integrated with object representations at no extra cost) can be upheld if performance on binding conditions is better than that on single feature conditions. This was the case in our study, particularly in our older group.

For younger adults, the percentage of correct responses did not differ significantly between congruent and incongruent colour-object binding conditions. However, these findings should be interpreted cautiously as performance in this group was very high due to possibly a low memory load and the familiarity of the to-be-remembered items. Future work should follow this up where younger adults' and older adults' memory capacity is taken into account and the overall task difficulty is increased. Nevertheless, this finding suggests that holding novel bindings in VWM when memory load is kept within capacity may not add an additional cost. For older adults however, colour-object congruency did play a role. Indeed, the percentage of correct responses improved significantly for congruent combinations of objects and colours compared to when the object-colour combinations were incongruent (see Yang et al., 2013). Thus, our data suggest that LTM does support binding functions carried out in VWM and that such a support would come in handy in old age (e.g., Ruchkin et al., 2003; Postle, 2007). This preserved ability appears to be restricted to VWM as holding bindings of familiar features in LTM seems to be affected by age (Chalfonte and Johnson, 1996). Future studies should investigate the conditions leading to a shortage of this support in old age.

An alternative explanation for the effect of congruency observed in the older group could be that this age-related effect may result from interference (Sapkota et al., 2015). That is, LTM representations of everyday objects would impact either on congruent or incongruent object-colour parings. In the latter condition such existing knowledge may interfere during the reconstruction stage as similar objects had been previously experienced in congruent conjunctions within and outside the task context. As we age, we might be more susceptible to this form of interference as such an effect was not observed in the younger group. However, such an age-related interference seems unlikely as the Group × Condition interaction disappeared when the Congruent Object-Colour binding condition was excluded from the model (see Supplementary Table 2). In contrast, the Group by Condition interaction remained after excluding the Incongruent Object-Colour binding condition from the analysis. Thus, in older adults, LTM does not seem to interfere with incongruent memory binding, but rather support VWM binding of congruent object-colour parings.

Whether or not this benefit is exclusive for older adults remains debatable. In fact, our data suggest that the congruency effect on VWM binding seen in older adults might result, at least in part, from the influence of education (see **Figure 4**). Indeed, once the data was controlled for education, both groups seemed to benefit similarly from congruent Object-Colour parings (see Supplementary Table 3). Therefore, congruent memory binding might be beneficial for people with lower education in general.

As we pointed out in the Results, controlling for Education reduced the power of the analysis and this may have led to a non-significant interaction. Future studies including larger sample sizes or groups with different educational levels will confirm this finding. Crucially, none of these effects indicated that age differently affects VWM binding abilities even when older adults have received fewer years of education than younger adults.

What do these findings tell us about ageing effects and current models of VWM memory (Baddeley, 2000)? When opportunities are created for a greater reliance on the episodic buffer by bridging the content of working memory with that of LTM and such a reliance supports the integration of surface features (i.e., the congruent Object-Colour binding condition), no age-related impairments on binding were observed. One might argue that such representations were more actively kept in a general working memory storage (e.g., the episodic buffer) responsible for linking the content of VWM to that of LTM (Baddeley, 2000). This evidence together with that from previous studies using canonical features of meaningless value, which are thought to be integrated in the visual spatial sketchpath (Baddeley et al., 2011), suggests that integrating features which require the support of the episodic buffer is also spared in ageing (at least for this type of representation).

Influences of Delay on VSTM Binding across Age

Interestingly, the percentage of correct responses improved significantly when the test array was presented 30 s after the second study array compared to when the test array occurred immediately after the initial study array; an effect that was evenly distributed across all test conditions and was independent of age. Age is known to affect memory over longer delays more dramatically than over shorter delays (Poon and Fozard, 1980; Nielsen-Bohlman and Knight, 1995). Older adults' rehearsal abilities are less efficient than that of younger adults (Brown et al., 2012). Taken together these earlier findings one would have predicted poorer performance of older adults over longer delays. It therefore remains questionable whether verbalisation (i.e., rehearsal) significantly influenced performance during the 30 s delay on either group. Indeed, older adults usually do not apply verbal strategies on VWM binding tasks unless they are encouraged to do so (Parra et al., 2009c). It could be that the high familiarity of the features used in this study and their occasional combination into prototypical bindings, drew from semantic memory, a function less affected by age than episodic memory (Nyberg et al., 1996). Future studies should investigate how much verbalisation or semantic memory can support performance on memory binding tasks that promote interactions between VWM and LTM.

In our study we presented participants with the encoding display once more before they entered the delay period. We did not predict that such a repetition would alter representations of the to-be-remembered items (Colzato et al., 2006; Treisman, 2006; see Logie et al., 2009). However, when Logie et al. (2009) probed participants with a recall procedure rather than with a yes/no recognition task, a substantial improvement in recalling colour-shape bindings was observed and small effects of learning on recall for colour-only, but not for shape-only, was also found. In the context of our task, we assessed memory reconstruction, a processes known to rely both on recognition (e.g., for object's parts) and on recollection (e.g., for the binding) (see Brockmole and Logie, 2013). It might be possible that such an effortful process may have resulted in more stable representations which survived the influence of longer delays. This influence appeared to have been strengthened by the availability of LTM which aided VWM performance (due to the familiar nature of features). This together with the availability of rehearsal mechanisms (the delay interval was unfilled) may have led to an improved performance after a longer delay. In terms of contextual demands, this task is less challenging than a pure recall task as it provides cues to retrieve the correct features (Parra et al., 2015; van Geldorp et al., 2015). However, it is more challenging than simple recognition during change detection tasks. Our prediction was that as active recognition/reconstruction processes would be more cognitive demanding than simple change detection, such a methodology would help investigate the actual extent of this age-insensitive function. Taken together these earlier findings and our own data they suggest that such insensitivity holds regardless of the memoranda and the retrieval function used to access it. Within the context of the present study we can claim that when it comes to VWM binding such mechanisms appear to remain available and fully functional as we grow older.

Influences of Education on VSTM Binding across Age

Education has proved one the greatest confounding factors in studies involving neuropsychological populations (Mungas et al., 2011), especially those suffering from dementia (Parra, 2014; Logie et al., 2015; Della Sala et al., 2016). Our group of older adults had significantly fewer years of education than our younger group, yet VWM binding functions were not differentially impaired in the former group (see **Figure 3**). When we controlled for the amount variance accounted for by Education, performance differences across groups decreased at the expense of changes in the younger but not in the older group (see **Figure 4**). From these observations it seems plausible to suggest that low education in old age does not impair those binding functions responsible for holding in VWM integrated features of everyday items whether in familiar or novel combinations. Parra et al. (2011) arrived to the same conclusions when in a *post-hoc* analysis they found that healthy subjects and patients with dementia due to AD from Colombia and the UK did not differ in their VWM binding abilities despite significant differences in the age and education of the samples collected across the two countries. In that earlier study the authors used arbitrary bindings of unfamiliar features, such as random polygons and non-primary colours. These results taken together suggest that is binding the function subserving these conjunctive memory mechanisms that is not affected by variables such as age and education and this seems to be true regardless of the nature of the to-be-bound information.

Towards a Culturally Unbiased Marker of Cognitive Ageing Trajectories

The results presented here have important implications for the selection of culturally unbiased tests which can aid in the assessment of elderly people in countries with low socio-cultural backgrounds. A recent study has stressed on the cultural validity of the VSTM binding test (Della Sala et al., 2016). Here, we have shown that VSTM binding of features of everyday objects is preserved in older adults who have a low educational background. This creates new opportunities to incorporate these tools in the assessment of older adults at risk for dementia minimising the number of false positives and the need of using normative databases which have proved little informative to reveal the links between brain and behaviour across normal and abnormal ageing trajectories.

AUTHOR CONTRIBUTIONS

MP, AG, and AM. designed the study. AG and AM. led the data collection. SH and MP. conducted the statistical analysis. SH, MP, AG, and AM. discussed the data. SH and AM. prepared the first draft. SH, MP, AG, and AM. generated the final draft.

ACKNOWLEDGMENTS

SH Research is supported by Alzheimer's Society (Grant AS-SF-14-008 awarded to MP). We acknowledge the support from the University of Edinburgh's Centre for Cognitive Ageing and Cognitive Epidemiology, part of the UK cross council Lifelong Health and Well-being Initiative (MR/L501530/1). Funding from the Biotechnology and Biological Sciences Research Council (BBSRC) and Medical Research Council (MRC) is gratefully acknowledged. We acknowledge the support from Alzheimer's Scotland Dementia Research Centre also from the University of Edinburgh.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2017.00122/full#supplementary-material

REFERENCES

Aguirre-Acevedo, D. C., Gómez, R. D., Moreno, S., Henao-Arboleda, E., Motta, M., Muñoz, C., et al. (2007). Validity and reliability of the CERAD-Col neuropsychological battery. *Rev. Neurol.* 45, 655–660.

Allen, R. J., Baddeley, A. D., and Hitch, G. J. (2006). Is the binding of visual features in working memory resource-demanding? *J. Exp. Psychol. Gen.* 135, 298–313. doi: 10.1037/0096-3445.135.2.298

Allen, R. J., Brown, L. A., and Niven, E. (2013). "Aging and visual feature binding in working memory," in *Working Memory: Developmental Differences, Component Processes, and Improvement Mechanisms*, ed H. St. Clair-Thompson (New York, NY: Nova Science Publishers, Inc.), 83–96.

Allen, R. J., Hitch, G. J., and Baddeley, A. D. (2009). Cross-modal binding and working memory. *Vis. Cogn.* 17, 83–102. doi: 10.1080/13506280802281386

Baddeley, A. (2000). The episodic buffer: a new component of working memory? *Trends Cogn. Sci.* 4, 417–423. doi: 10.1016/S1364-6613(00)01538-2

Baddeley, A. D., Allen, R. J., and Hitch, G. J. (2011). Binding in visual working memory: the role of the episodic buffer. *Neuropsychologia* 49, 1393–1400. doi: 10.1016/j.neuropsychologia.2010.12.042

Bopp, K. L., and Verhaeghen, P. (2009). Working memory and aging: separating the effects of content and context. *Psychol. Aging* 24, 968–980. doi: 10.1037/a0017731

Brockmole, J. R., and Logie, R. H. (2013). Age-related change in visual working memory: a study of 55,753 participants aged 8-75. *Front. Psychol.* 4:12. doi: 10.3389/fpsyg.2013.00012

Brockmole, J. R., Parra, M. A., Della Sala, S., and Logie, R. H. (2008). Do binding deficits account for age-related decline in visual working memory? *Psychon. Bull. Rev.* 15, 543–547. doi: 10.3758/PBR.15.3.543

Brown, L. A., and Brockmole, J. R. (2010). The role of attention in binding visual features in working memory: evidence from cognitive ageing. *Q. J. Exp. Psychol.* 63, 2067–2079. doi: 10.1080/17470211003721675

Brown, L. A., Brockmole, J. R., Gow, A. J., and Deary, I. J. (2012). Processing speed and visuospatial executive function predict visual working memory ability in older adults. *Exp. Aging Res.* 38, 1–19. doi: 10.1080/0361073X.2012.636722

Brown, L. A., Niven, E. H., Logie, R. H., Rhodes, S., and Allen, R. J. (2017). Visual feature binding in younger and older adults: encoding and suffix interference effects. *Memory* 25, 261–275. doi: 10.1080/09658211.2016.1156705

Chalfonte, B. L., and Johnson, M. K. (1996). Feature memory and binding in young and older adults. *Mem. Cognit.* 24, 403–416. doi: 10.3758/BF03200930

Chen, T., and Naveh-Benjamin, M. (2012). Assessing the associative deficit of older adults in long-term and short-term/working memory. *Psychol. Aging* 27, 666–682. doi: 10.1037/a0026943

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences, 2nd Edn.* Hillsdale, NJ: Lawrence Earlbaum Associates.

Colzato, L. S., Raffone, A., and Hommel, B. (2006). What do we learn from binding features? Evidence for multilevel feature integration. *J. Exp. Psychol. Hum. Percept. Perform.* 32, 705–716. doi: 10.1037/0096-1523.32.3.705

Cowan, N. (2016). "Life-span development of visual working memory": when is feature binding difficult?: Correction to Cowan et al. (2006). *Dev. Psychol.* 52:1544. doi: 10.1037/dev0000222

Cowan, N., Naveh-Benjamin, M., Kilb, A., and Saults, J. S. (2006). Life-span development of visual working memory: when is feature binding difficult? *Dev. Psychol.* 42, 1089–1102. doi: 10.1037/0012-1649.42.6.1089

Della Sala, S., Kozlova, I., Stamate, A., and Parra, M. A. (2016). A transcultural cognitive marker of Alzheimer's Disease. *Int. J. Geriatr. Psychiatry.* doi: 10.1002/gps.4610. [Epub ahead of print].

Della Sala, S., Parra, M. A., Fabi, K., Luzzi, S., and Abrahams, S. (2012). Short-term memory binding is impaired in AD but not in non-AD dementias. *Neuropsychologia* 50, 833–840. doi: 10.1016/j.neuropsychologia.2012.01.018

Gao, Z., Wu, F., Qiu, F., He, K., Yang, Y., and Shen, M. (2017). Bindings in working memory: the role of object-based attention. *Atten. Percept. Psychophys.* 79, 533–552. doi: 10.3758/s13414-016-1227-z

Isella, V., Molteni, F., Mapelli, C., and Ferrarese, C. (2015). Short term memory for single surface features and bindings in ageing: a replication study. *Brain Cogn.* 96, 38–42. doi: 10.1016/j.bandc.2015.02.002

Karlsen, P. J., Allen, R. J., Baddeley, A. D., and Hitch, G. J. (2010). Binding across space and time in visual working memory. *Mem. Cognit.* 38, 292–303. doi: 10.3758/MC.38.3.292

Logie, R. H., Brockmole, J. R., and Vandenbroucke, A. R. E. (2009). Bound feature combinations in visual short-term memory are fragile but influence long-term learning. *Vis. Cogn.* 17, 160–179. doi: 10.1080/13506280802228411

Logie, R. H., Parra, M. A., and Della Sala, S. (2015). From cognitive science to dementia assessment. *Policy Insights Behav. Brain Sci.* 2, 81–91. doi: 10.1177/2372732215601370

Mitchell, K. J., Raye, C. L., Johnson, M. K., and Greene, E. J. (2006). An fMRI investigation of short-term source memory in young and older adults. *Neuroimage* 30, 627–633. doi: 10.1016/j.neuroimage.2005.09.039

Mungas, D., Beckett, L., Harvey, D., Farias, S. T., Reed, B., Carmichael, O., et al. (2011). Heterogeneity of cognitive trajectories in diverse older persons. *Psychol. Aging* 25, 606–619. doi: 10.1037/a0019502

Naveh-Benjamin, M., Brav, T. K., and Levy, O. (2007). The associative memory deficit of older adults: the role of strategy utilization. *Psychol. Aging* 22, 202–208. doi: 10.1037/0882-7974.22.1.202

Naveh-Benjamin, M., Guez, J., and Shulman, S. (2004). Older adults' associative deficit in episodic memory: assessing the role of decline in attentional resources. *Psychon. Bull. Rev.* 11, 1067–1073. doi: 10.3758/BF03196738

Nielsen-Bohlman, L., and Knight, R. T. (1995). Prefrontal alterations during memory processing in aging. *Cereb. Cortex* 5, 541–549. doi: 10.1093/cercor/5.6.541

Nyberg, L., Backman, L., Erngrund, K., Olofsson, U., and Nilsson, L. G. (1996). Age differences in episodic memory, semantic memory, and priming: relationships to demographic, intellectual, and biological factors. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 51, 234–240. doi: 10.1093/geronb/51B.4.P234

Old, S. R., and Naveh-Benjamin, M. (2008). Differential effects of age on item and associative measures of memory: a meta-analysis. *Psychol. Aging* 23, 104–118. doi: 10.1037/0882-7974.23.1.104

Olson, I. R., and Jiang, Y. (2002). Is visual short-term memory object based? Rejection of the "strong-object" hypothesis. *Percept. Psychophys.* 64, 1055–1067. doi: 10.3758/BF03194756

Parra, M. A. (2014). Overcoming barriers in cognitive assessment of Alzheimer's disease. *Dementia Neuropsychol.* 8, 95–98. doi: 10.1590/S1980-57642014DN82000002

Parra, M. A., Abrahams, S., Fabi, K., Logie, R. H., Luzzi, S., and Della Sala, S. (2009a). Short-term memory binding deficits in Alzheimers disease. *Brain* 132, 1057–1066. doi: 10.1093/brain/awp036

Parra, M. A., Abrahams, S., Logie, R. H., and Sala, S., D. (2009b). Age and binding within-dimension features in visual short-term memory. *Neurosci. Lett.* 449, 1–5. doi: 10.1016/j.neulet.2008.10.069

Parra, M. A., Abrahams, S., Logie, R. H., and Della Sala, S. (2010a). Visual short-term memory binding in Alzheimer's disease and depression. *J. Neurol.* 257, 1160–1169. doi: 10.1007/s00415-010-5484-9

Parra, M. A., Abrahams, S., Logie, R. H., Méndez, L. G., Lopera, F., and Della Sala, S. (2010b). Visual short-term memory binding deficits in familial Alzheimer's disease. *Brain* 133, 2702–2713. doi: 10.1093/brain/awq148

Parra, M. A., Fabi, K., Luzzi, S., Cubelli, R., Hernandez, V. M., and Della, S. S. (2015). Relational and conjunctive binding functions dissociate in short-term memory. *Neurocase* 21, 56–66.

Parra, M. A., Sala, S. D., Abrahams, S., Logie, R. H., Méndez, L. G., and Lopera, F. (2011). Specific deficit of colour-colour short-term memory binding in sporadic and familial Alzheimer's disease. *Neuropsychologia* 49, 1943–1952. doi: 10.1016/j.neuropsychologia.2011.03.022

Parra, M. A., Sala, S. D., Logie, R. H., and Abrahams, S. (2009c). Selective impairment in visual short-term memory binding. *Cogn. Neuropsychol.* 26, 583–605. doi: 10.1080/02643290903523286

Pertzov, Y., Heider, M., Liang, Y., and Husain, M. (2015). Effects of healthy ageing on precision and binding of object location in visual short term memory. *Psychol. Aging* 30, 26–35. doi: 10.1037/a0038396

Peterson, D. J., and Naveh-Benjamin, M. (2016). The role of aging in intra-item and item-context binding processes in visual working memory. *J. Exp. Psychol. Learn. Mem. Cogn.* 42, 1713–1730. doi: 10.1037/xlm0000275

Poon, L. W., and Fozard, J. L. (1980). Age and word frequency effects in continuous recognition memory. *J. Gerontol.* 35, 77–86. doi: 10.1093/geronj/35.1.77

Postle, B. R. (2007). "Activated long-term memory? The bases of representation in working memory," in *The Cognitive Neuroscience of Working Memory*, eds N. Osaka, R. H. Logie, and M. D'Esposito (New York, NY: Oxford University Press), 333–349.

Read, C. A., Rogers, J. M., and Wilson, P. H. (2016). Working memory binding of visual object features in older adults. *Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn.* 23, 263–281. doi: 10.1080/13825585.2015.1083937

Reuter-Lorenz, P. A., and Sylvester, C.-Y. C. (2005). "The cognitive neuroscience of working memory and aging," in *Cognitive Neuroscience of Aging: Linking Cognitive and Cerebral Aging*, eds R. Cabeza, L. Nyberg, and D. Park (New York, NY: Oxford University Press), 186–217.

Rhodes, S., Parra, M. A., and Logie, R. H. (2015). Ageing and feature binding in visual working memory: the role of presentation time. *Q. J. Exp. Psychol. (Hove).* 69, 654–668. doi: 10.1080/17470218.2015.1038571

Ruchkin, D. S., Grafman, J., Cameron, K., and Berndt, R. S. (2003). Working memory retention systems: a state of activated long-term memory. *Behav. Brain Sci.* 26, 709–728. doi: 10.1017/S0140525X03000165

Sapkota, R. P., Van Der Linde, I., and Pardhan, S. (2015). How does aging affect the types of error made in a visual short-term memory "object-recall" task? *Front. Aging Neurosci.* 6:346. doi: 10.3389/fnagi.2014.00346

Shen, M., Huang, X., and Gao, Z. (2015). Object-based attention underlies the rehearsal of feature binding in visual working memory. *J. Exp. Psychol. Hum. Percept. Perform.* 41, 479–493. doi: 10.1037/xhp0000018

Shing, Y. L., Werkle-Bergner, M., Brehmer, Y., Müller, V., Li, S. C., and Lindenberger, U. (2010). Episodic memory across the lifespan: the contributions of associative and strategic components. *Neurosci. Biobehav. Rev.* 34, 1080–1091. doi: 10.1016/j.neubiorev.2009.11.002

Stevens, J. P. (2002). *Applied Multivariate Statistics for the Social Sciences*. Hillsdale, NJ: Lawrence Erlbaum.

Treisman, A. (2006). "Object tokens, binding and visual memory," in *Handbook of Binding and Memory: Perspectives From Cognitive Neuroscience*, eds H. Zimmer, A. Mecklinger, and U. Lindenberger (Oxford; New York, NY: CHAP, OUP).

van Geldorp, B., Parra, M. A., and Kessels, R. P. (2015). Cognitive and neuropsychological underpinnings of relational and conjunctive working memory binding across age. *Memory* 23, 1112–1122. doi: 10.1080/09658211.2014.953959

World Medical Association (2001). World medical association declaration of helsinki. ethical principles for medical research involving human subjects. *Bull. World Health Organ.* 79, 373.

Yang, L., Li, J., Spaniol, J., Hasher, L., Wilkinson, A. J., Yu, J., et al. (2013). Aging, culture, and memory for socially meaningful item-context associations: an east-west cross-cultural comparison study. *PLoS ONE* 8:e60703. doi: 10.1371/journal.pone.0060703

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: 16 June 2017
doi: 10.3389/fnagi.2017.00182

Behavioral and Psychological Symptoms Impact Clinical Competence in Alzheimer's Disease

Elodie Bertrand[1], Eelco van Duinkerken[1,2,3], J. Landeira-Fernandez[1], Marcia C. N. Dourado[4], Raquel L. Santos[4], Jerson Laks[4,5] and Daniel C. Mograbi[1,6]*

[1]Department of Psychology, Pontifícia Universidade Católica – Rio (PUC-Rio), Rio de Janeiro, Brazil, [2]Department of Medical Psychology, VU University Medical Center, Amsterdam, Netherlands, [3]Diabetes Center/Department of Internal Medicine, VU University Medical Center, Amsterdam, Netherlands, [4]Institute of Psychiatry—Center for Alzheimer's Disease, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil, [5]Post Graduation Program on Translational Biomedicine, Universidade do Grande Rio (Unigranrio), Caxias, Brazil, [6]Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom

Decision-making is considered a fundamental aspect of personal autonomy and can be affected in psychiatric and neurologic diseases. It has been shown that cognitive deficits in dementia impact negatively on decision-making. Moreover, studies highlighted impaired clinical competence in neuropsychiatric disorders, such as schizophrenia and bipolar disorder. In this context, the current study explored the relationship between behavioral and psychological symptoms of dementia (BPSD) and clinical competence, especially the capacity to consent to treatment, in Alzheimer's disease (AD). Seventy-one patients with mild to moderate AD participated, completing assessments for capacity to consent to treatment, general cognition and neuropsychiatric disturbances. For each neuropsychiatric symptom, patients with and without the particular disturbance were compared on the different subscales of the MacArthur Competence Tool for Treatment (MacCAT-T; Understanding, Appreciation, Reasoning and Expression). The results showed that patients presenting delusions, as well as apathetic patients, had a lower ability to express a clear treatment choice compared to patients without these symptoms. By contrast, patients with dysphoria/depression had higher scores on this variable. Additionally, AD patients with euphoria had more difficulties discussing consequences of treatment alternatives compared to patients without this disturbance. None of the differences were confounded by global cognition. There were no between-group differences in clinical decision-making for patients with hallucinations, agitation/aggression, anxiety, irritability, disinhibition and aberrant motor behavior. These findings highlight the importance of taking BPSD into account when assessing decision-making capacity, especially clinical competence, in AD. Furthermore, reducing BPSD may lead to better clinical competence in patients with AD, as well as to improvements in patients and caregivers' quality of life.

Keywords: BPSD, clinical capacity, decision making, consent to treatment, dementia, Alzheimer's disease

OPEN ACCESS

Edited by:
Agustin Ibanez, Institute of Cognitive and Translational Neuroscience (INCYT), Argentina

Reviewed by:
Olivier Piguet, University of Sydney, Australia
Sandra Baez, Institute of Cognitive and Translational Neuroscience (INCYT), Argentina

****Correspondence:***
Elodie Bertrand
elodie.bertrand1@gmail.com

Received: *26 November 2016*
Accepted: *24 May 2017*
Published: *16 June 2017*

Citation:
Bertrand E, van Duinkerken E, Landeira-Fernandez J, Dourado MCN, Santos RL, Laks J and Mograbi DC (2017) Behavioral and Psychological Symptoms Impact Clinical Competence in Alzheimer's Disease. Front. Aging Neurosci. 9:182. doi: 10.3389/fnagi.2017.00182

INTRODUCTION

In the context of contemporary clinical practice, in which the patients' active participation in medical decisions is valued, the question of decision-making capacity, especially the competence to consent to treatment has become central. Moreover, treatment consent capacity, which refers to the ability to accept an offered treatment, refuse it, or select among alternatives, has important clinical, legal and ethical implications (President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, 1982; Tepper and Elwork, 1984). Indeed, it is considered a fundamental aspect of personal autonomy and its careful assessment is essential to find the balance between autonomy for patients who are able to make their own decisions and protection for those with diminished decisional capacity (Berg et al., 2001; Berghmans and Widdershoven, 2003). In the case of Alzheimer's disease (AD), these concerns seem even more relevant, since this is a condition affecting cognitive abilities critical to healthcare decision-making capacity (Marson, 2001). It has been suggested that offering the possibility to people with dementia (PwD) to participate in healthcare-related decisions may improve their well-being, quality of life and dignity compared to those excluded from taking part in decisions, who tend to be more depressed, frustrated and debilitated (Smebye et al., 2012).

Previous research exploring medical decision-making, also termed clinical competence, has shown that, compared with healthy control individuals, the treatment consent capacity of individuals with AD is reduced (Karlawish et al., 2005; Lui et al., 2012). These findings have been supported by longitudinal studies, which highlighted that clinical competence tends to decrease over time in mild cognitive impairment and AD (Moye et al., 2006; Okonkwo et al., 2008). The decrease of decisional capacity in AD has been related to cognitive decline in this population, with research focusing on cognitive predictors showing that problems with language, memory and executive function impact negatively on decisional capacity (Moye et al., 2006; Stormoen et al., 2014).

In addition to the characteristic cognitive deficits, behavioral and psychological symptoms of dementia (BPSD) are an integral part of AD (Cerejeira et al., 2012; Zhao et al., 2016). BPSD are widespread non-cognitive symptoms, including apathy, aggression, delusions, psychosis, hallucinations, anxiety, irritability, depression and sleep disorders (van der Linde et al., 2014). The presence of BPSD has important negative consequences on AD patients and their caregivers. For instance, BPSD result in premature institutionalization, increased cost of care and diminished quality of life for both patients and caregivers (Black and Almeida, 2004; Scarmeas et al., 2007; Schaller et al., 2015; Feast et al., 2016). Mitoku and Shimanouchi (2014) explored the impact of BPSD on decision making capacity in older adults and highlighted that older adults with dementia presenting BPSD have decreased decisional capacity compared to patients without BPSD. Mograbi et al. (2015) showed in a large community-based study that BPSD correlated with unawareness of memory deficits in dementia, which may negatively affect clinical capacity.

Moreover, it has been shown that decisional capacity is impaired in various neuropsychiatric disorders (for review see Rahman et al., 2001; Candia and Barba, 2011). Looking especially at clinical competence, previous research highlighted that patients with schizophrenia, bipolar mood disorder and major depression have significant impairments in decisional abilities (Grisso and Appelbaum, 1995; Howe et al., 2005; Appelbaum and Redlich, 2006). Additionally, studies demonstrated that for patients with acute psychosis, treatment consent capacity was related to the extent and severity of symptoms, especially positive symptoms such as delusions and hallucinations (Howe et al., 2005; Rutledge et al., 2008). However, for patients with chronic psychosis, Palmer et al. (2004) showed a relationship between cognitive and negative symptoms (unusual thought content, conceptual disorganization) and impaired medical decision-making capacity.

Considering the scarcity of studies in this field, the aim of the present study was to explore the relationship between behavioral disturbance and decision-making in AD by comparing patients with or without BPSD on their capacity to consent to treatment.

MATERIALS AND METHODS

Participants

A consecutive series of 71 patients and caregivers dyads were recruited from an AD outpatient unit. We included participants diagnosed with possible or probable AD according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV-TR). The clinical diagnosis of AD was made by a psychiatrist, based on clinical interviews with the patients and their caregivers, cognitive screening tests, laboratory tests and imaging studies. Inclusion criteria were: mild Clinical Dementia Rating (CDR = 1; n = 50) and moderate (CDR = 2; n = 21) dementia according to the CDR and Mini-Mental State Examination (MMSE) scores of 11–26. We excluded people with head trauma, aphasia, history of alcoholism, psychotic symptoms, epilepsy and uncontrolled medical problems such as hypertension, depression and diabetes.

The primary caregiver was defined as the main person responsible for the care of the patient. All of the caregivers had been previously informed of the AD diagnosis by the psychiatrist. The patients completed assessments about cognition and competence to consent to treatment. The caregivers provided information about the patients' demographics, the ability to perform activities daily living (ADL), neuropsychiatric symptoms, functionality, dementia severity and had depression and burden of care assessments.

Instruments

Competence to Consent to Treatment

The MacArthur Competence Tool for Treatment (MacCAT-T; adapted to Brazilian Portuguese; Santos et al., 2017) was used to assess competence to consent to treatment (Grisso et al., 1997). This scale permits to explore four different abilities:

understanding, appreciation, reasoning and expression. The *Understanding* section assesses the capacity to paraphrase what has been disclosed. The *Appreciation* section assesses whether the individual acknowledges that the disclosed information applies to him or her and whether he or she recognizes the treatment possible benefits. *Reasoning* explores whether the person mentions any consequence of the treatment alternatives, comparison among alternatives and any consequences that were not mentioned in the disclosure. Finally, in the *Expression* section, the individual is supposed to offer a clear expression of a treatment choice and to explain how this choice was made. Patient responses were rated using the following scoring: 2 points for adequate; 1 point for partially sufficient; and 0 points for insufficient. Total scores in the MacCAT-T subscales ranged as follows: Understanding, 0–6; Appreciation, 0–4; Reasoning, 0–8; Expression, 0–2.

Cognition

For a general measure of cognitive level, the MMSE was used (Folstein et al., 1975; Bertolucci et al., 1994). The total score ranges from 0 to 30, with lower scores indicating impaired cognition. The Alzheimer Disease Assessment Scale—Cognitive Subscale (ADAS-Cog; Schultz et al., 2001), which assesses the intensity of cognitive changes, was also applied.

The ADAS-cog is 11-item scale used to assess the severity of selected areas of cognitive impairment (memory, language, orientation, reason and praxis). The maximum score is 70 and higher scores indicate poorer performance. Finally, attention and working memory were assessed with the Wechsler Digit Span Test, Forward and Backward (Wechsler, 1997; Nascimento, 2000). Scores of Digit Span Forward vary from 0 to 16, and scores of Digit Span Backward vary from 0 to 14, with higher scores indicating better performance.

Neuropsychiatric Symptoms

The Neuropsychiatric Inventory (NPI) was used to assess 10 neuropsychiatric disturbances commonly observed in dementia (Cummings et al., 1994; Camozzato et al., 2008). The scale evaluates the presence of delusions, hallucinations, dysphoria, anxiety, agitation/aggression, euphoria, disinhibition, irritability/lability, apathy, aberrant motor activity, night-time behavior disturbances and appetite and eating abnormalities. The NPI is administrated to the patient's caregiver, who rates each item in relation to their frequency (1 [absent] to 4 [frequent]) and to their severity (1 [mild] to 3 [severe]). The total score can range from 0 to 144 points, with higher scores indicating more severe psychopathology. For the purpose of this study, the subscales for each symptom were used, with patients being dichotomized according to the presence of the symptom (see "Statistical Analysis" Section below).

Dementia Severity

To determine dementia severity, the full protocol of the CDR was used (Morris, 1993; Maia et al., 2006), with severity ranging from 0 (no dementia) to 3 (severe dementia). Only patients with CDR 1 and 2 were included in the study.

Ethical Issues

This study was carried out in accordance with the recommendations of the Federal University of Rio de Janeiro (UFRJ)/Institute of Psychiatry Ethics Committee with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Federal University of Rio de Janeiro (UFRJ)/Institute of Psychiatry Ethics Committee (Research Ethics Committee number 536.634).

Statistical Analysis

Demographic and clinical data are presented as means with standard deviation and range for the whole group. Dichotomous variables are presented as absolute numbers with percentages.

First, exploratory Pearsons correlations investigated the relationship between cognitive variables and clinical competence. Second, patients were dichotomized into two groups per subscale of the NPI. The first group included patients that did not report any symptoms on that particular subscale, whereas the second group comprised all the participants that had a score of 1 or more on that specific NPI subscale. Then, for each of the 10 NPI subscales, using Student *t*-tests for independent samples, we compared the mean scores on each scale of the MacCAT-T between the two groups. In case of a significant difference between the groups, the test was repeated now correcting for the ADAS-Cog score, to rule out the effect of global cognition differences. Additionally, to ensure that the significant differences between groups were not due to differences in sex, level of education, age and dementia severity, we tested differences according to these variables for each MacCAT-T section. For this purpose, independent samples *t*-tests were used, with patients being dichotomized according to sex, CDR (1: mild vs. 2: moderate dementia), educational level (1–9 years vs. 10+) and age (61–74 years old vs. 75+).

All tests were performed in SPSS20 (IBM-SPSS, Chicago, IL, USA). A $p < 0.05$ was considered statistically significant.

RESULTS

Participant Characteristics

A total of 71 patients with AD participated in this study. Sample characteristics and scores in the MacCAT-T can be found in **Table 1**. MacCAT-T results are consistent with previous data from patients with dementia, showing lower scores for Understanding and Reasoning when compared to a group of healthy controls (Moye et al., 2004).

The frequency of patients with neuropsychiatric symptoms can be seen in **Table 2**. In addition, no significant differences in the MacCAT-T subscales were found for sex, level of education, age and disease severity.

Correlation Analyses

Correlations between cognitive variables and clinical competence can be seen in **Table 3**. *Understanding* was significantly

TABLE 1 | Participant characteristics and scores on the neuropsychiatric inventory (NPI) and MacArthur Competence Tool for Treatment (MacCAT-T).

	Alzheimer's disease patients (*n* = 71) Mean ± SD (Range)
Demographic variables	
Age (years)	78.2 ± 6.3 (61–93)
Sex*	42/29 (59.2/40.8)
Education (years)	7.5 ± 3.9 (1–15)
MMSE	19.5 ± 4.0 (11–27)
ADAS Cog	24.5 ± 8.9 (8–48)
Digits—Forward	7.6 ± 2.6 (4–15)
Digits—Backward	3.2 ± 1.7 (0–9)
CDR**	50/21 (70.4/29.6)
Disease duration (years)	5.3 ± 3.5 (1–16)
Disease onset age (years)	72.8 ± 7.3 (54–88)
MacCAT-T scales	
Understanding	4.3 ± 0.9 (2.15–6)
Appreciation	3.2 ± 1.0 (0–4)
Reasoning	3.3 ± 1.5 (0–6)
Expression	1.8 ± 0.6 (0–2)

*n female/male, %; **n CDR 1/CDR 2, %.

correlated MMSE ($r = 0.28$, $p < 0.05$), ADAS-Cog ($r = -0.30$, $p < 0.05$) and Digit-backward ($r = 0.24$, $p < 0.05$). There was a significant correlation between *Reasoning* and ADAS-Cog ($r = -0.28$, $p < 0.05$), as well as between *Reasoning* and Digit-backward ($r = 0.25$, $p < 0.05$). There were no significant associations between *Appreciation* score and the cognitive variables, neither between *Expression* and these variables.

NPI Subscales

Patients with delusions had a lower score on the *expression* scale of the MacCAT-T (without: 1.9 ± 0.5 vs. with: 1.4 ± 0.9, $t_{(69)} = 2.91$, $p = 0.005$, **Figure 1B**). This remained statistically significant after correction for the ADAS-Cog score ($p = 0.006$). *Expression* was also affected in patients with dysphoria/depression, who showed higher scores (without: 1.6 ± 0.8 vs. with: 1.9 ± 0.3, $t_{(69)} = -2.10$, $p = 0.039$, **Figure 1D**), which remained significant after correction for the ADAS-Cog score ($p = 0.038$). This variable was also affected in apathetic patients, who had a lower score on *expression* as compared with the patients scoring 0 on this NPI subscale (without 1.9 ± 0.3 vs. with: 1.6 ± 0.7, $t_{(69)} = 2.35$, $p = 0.021$, **Figure 1I**). After correcting for the ADAS-Cog score, this difference remained statistically significant ($p = 0.023$).

AD patients with euphoria had a lower score on the *reasoning* scale of the MacCAT-T (without: 3.4 ± 1.5 vs. with: 1.7 ± 0.5, $t_{(69)} = 2.12$, $p = 0.038$, **Figure 1H**). However, this effect is not statistically significant after correction for the ADAS-Cog scores ($p = 0.082$).

There were no between-group differences in MacCAT-T scores for patients with hallucinations (**Figure 1A**), agitation/aggression (**Figure 1C**), anxiety (**Figure 1E**), irritability (**Figure 1F**), disinhibition (**Figure 1G**) and aberrant motor behavior (**Figure 1J**; in all cases, $p > 0.05$).

TABLE 2 | Frequency of NPI symptoms.

	AD patients (*n* = 71) *n* without/with symptom (%)
NPI symptoms	
Hallucinations	61/10 (85.9/14.1)
Delusions	58/13 (81.7/18.3)
Agitation/aggression	51/20 (71.8/28.2)
Dysphoria/depression	33/38 (46.5/53.5)
Anxiety	36/35 (50.7/49.3)
Irritability	39/32 (54.9/45.1)
Disinhibition	56/15 (78.9/21.1)
Euphoria	67/4 (94.4/5.6)
Apathy	36/35 (50.7/49.3)
Aberrant motor behavior	52/19 (73.2/26.8)

TABLE 3 | Correlations between cognitive variables and clinical competence.

Variable	**Understanding**	**Appreciation**	**Reasoning**	**Expression**
MMSE	**0.28**	0.07	0.21	0.07
ADAS-Cog	**−0.30**	−0.09	**−0.28**	−0.12
Digits—Forward	0.21	−0.02	0.12	−0.11
Digits—Backward	**0.24**	0.14	**0.25**	−0.06

Significant results are presented in bold.

DISCUSSION

The aim of this exploratory study was to investigate the relationship between behavioral symptoms and capacity of clinical decision-making in AD. The results indicate that patients with symptoms of delusions and apathy exhibit impaired *expression* of choice in comparison to patients without these symptoms. By contrast, patients with symptoms of dysphoria and depression have higher scores on this particular subscale relative to patients without symptoms. None of the differences were confounded by global cognition as measured by the ADAS-Cog. Additionally, patients with euphoria score lower on the subscale of *reasoning* compared to patients without these symptoms, however, when correcting for global cognition using the ADAS-Cog, this result is no longer significant.

Worse performances on the *expression* section of the MacCAT-T were seen for patients with both delusion and apathy. The result for delusions may be explained by an error in reality monitoring, which may impair the ability of patients to clearly express and explain treatment choices. For apathy, increasing space has been given to the role of emotional processes in decision making (Kahneman and Tversky, 1979; Damasio, 1994). It is possible that apathetic patients cannot rely on affective information to express their treatment choices. Apathy is also characterized by lack of motivated behavior, including the difficulty to engage in a cognitively demanding task (Marin, 1990) and this may be a potential reason for the lower scores of apathetic patients when asked to express a treatment choice. Indeed, the *expression* section of the MacCAT-T appears as a highly demanding task in term of motivational

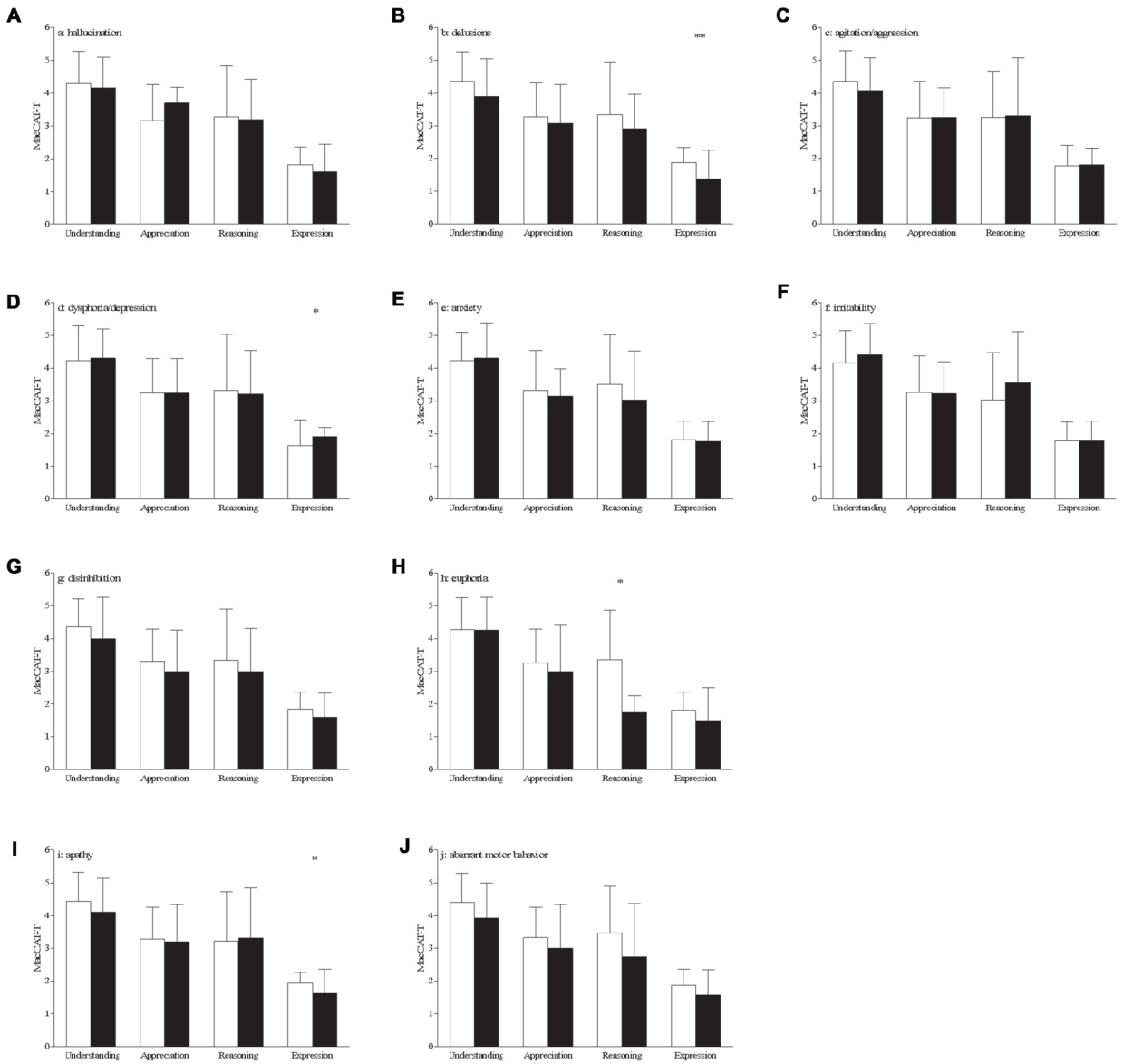


FIGURE 1 | Differences on MacArthur Competence Tool for Treatment (MacCAT-T) subscales according to the presence of behavioral and psychological symptoms of dementia (BPSD; **A–J**) bar graph of the mean with standard deviation of the MacCAT-T subscales per subscale of the neuropsychiatric inventory (NPI). White bars represent the patients with a score of 0 on that domain, black bars those patients with a score of 1 or higher on that domain; $^{*}p < 0.05$, $^{**}p < 0.01$; significant differences can be seen for delusions, dysphoria and apathy (expression of choice) and for euphoria (reasoning).

resources. Additionally, the impaired capacity to clearly express a treatment choice may affect decision making. For example, patients impaired in their ability to express their decisions may be excluded from the decision-making process by caregivers, contributing to malignant social psychology, which is defined by caregivers' behaviors that undermine the personhood and wellbeing of PwD (Kitwood, 1997).

In our study, better expression of treatment choice was shown for individuals presenting depression. This result is in line with the literature highlighting that depressed people may have preserved decision-making capacities (Appelbaum and Grisso, 1995; for review see Hindmarch et al., 2013) and awareness of condition (Mograbi and Morris, 2014; Bertrand et al., 2016). An explanation can be found in the depressive realism theory, which argues that depression contributes to a more realistic judgment, as opposed to the normal positive or optimistic biases that are associated with an euthymic mood state (Dobson and Franche, 1989; Taylor, 1989).

Results showing that the presence of mania decreased the capacity on the *reasoning* section are consistent with findings in the literature showing executive function deficits in

bipolar disorder, especially during mania (Dixon et al., 2004; Mur et al., 2007). Preserved executive functions, including abilities such as cognitive flexibility, problem-solving, planning and inhibition, appear essential in the reasoning section of the MacCAT-T, in which patients have to consider treatment alternatives and compare the consequences of these treatments. Previous studies exploring the relationship between specific neuropsychological abilities and medical decision-making showed that tests evaluating executive functioning correlated with the reasoning component of treatment consent capacity in clinical and healthy populations (for review, see Palmer and Savla, 2007). Additionally, mania has been associated with reduced awareness about the condition (de Assis da Silva et al., 2015a,b; de Assis da Silva et al., 2016), including in patients with AD (Migliorelli et al., 1995). Lack of awareness about the condition may impact negatively reasoning about the treatment.

Some potential limitations of the current study must be considered. First, the absence of a control group may limit the interpretation of the results. Nevertheless, the lack of a control group is a feature of the current study, considering that we are exploring neuropsychiatric symptoms in dementia and their impact on decision making. Additionally, data from previous studies were reported, providing comparison points. Second, additional data from neuropsychological testing would have been useful to support some of the potential cognitive explanations proposed above. Third, because of statistical analysis limitation due to the sample size, the severity and the frequency of the neuropsychiatric symptoms were not taken into account. Fourth, we used DSM-IV-TR diagnosis criteria, which have been criticized by their insufficient diagnostic specificity. Nonetheless, DSM-IV-TR criteria have been used thoroughly in dementia research and this is a limitation that our study shares with a very large number of publications in the field of dementia studies. Finally, the present study did not consider the possible interaction of multiple neuropsychiatric symptoms in relation to decision-making capacity. Specifically in the case of apathy and depression, which have been shown to be both related to expression of a choice in the present study, although there is some overlap between the conditions, recent research has emphasized how these are separate entities (Aalten et al., 2008; Robert et al., 2009; Spalletta et al., 2010; Selbæk and Engedal, 2012). Future studies should explore the impact of multiple neuropsychiatric symptoms on the capacity to consent treatment.

Most studies in the literature focused on the cognitive predictors of decision-making capacity in dementia. However, our study stresses also a relationship between BPSD and treatment consent capacity in AD patients. These findings may have important clinical implications. Indeed, in addition to improving patients and caregivers' quality of life, strategies aiming to reduce behavioral disturbances may lead to better decisional abilities for patient with dementia, which consequently will increase patient's autonomy and well-being. To the best of your knowledge, this is the first study exploring directly the relationship between BPSD and consent to treatment capacity in AD patients. Therefore, future research is needed, especially to distinguish the impact of both cognitive and behavioral deficits on decision-making capacity in AD. Studies using a longitudinal design may be useful in order to understand the direction of the relationship BPSD/decision-making capacity.

AUTHOR CONTRIBUTIONS

MCND, DCM, JL and JL-F conceived the study design. RLS and EB were responsible for data collection. ED and DCM performed the data analysis. EB, DCM and ED drafted the manuscript. All authors revised and approved the final manuscript and are accountable for all aspects of the work.

ACKNOWLEDGMENTS

This study was supported by CNPq—Conselho Nacional de Desenvolvimento Científico e Tecnológico—Brazil and by FAPERJ-Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro-Brazil. The authors also acknowledge financial support from the Capes Foundation, Ministry of Education of Brazil.

REFERENCES

Aalten, P., Verhey, F. R. J., Boziki, M., Brugnolo, A., Bullock, R., Byrne, E. J., et al. (2008). Consistency of neuropsychiatric syndromes across dementias: results from the European Alzheimer Disease Consortium. Part II. *Dement. Geriatr. Cogn. Disord.* 25, 1–8. doi: 10.1159/000111082

Appelbaum, P. S., and Grisso, T. (1995). The macarthur treatment competence study. I: mental illness and competence to consent to treatment. *Law Hum. Behav.* 19, 105–126. doi: 10.1007/bf01499321

Appelbaum, P. S., and Redlich, A. (2006). Impact of decisional capacity on the use of leverage to encourage treatment adherence. *Community Ment. Health J.* 42, 121–130. doi: 10.1007/s10597-005-9015-6

Berg, J. W., Appelbaum, P. S., , Lidz, C. W., Parker, L. S. (2001). *Informed Consent: Legal Theory and Clinical Practice.* Fair Lawn, NJ: Oxford University Press.

Berghmans, R. L., and Widdershoven, G. A. (2003). Ethical perspectives on decision-making capacity and consent for treatment and research. *Med. Law* 22, 391–400.

Bertolucci, P. H., Brucki, S. M., Campacci, S. R., and Juliano, Y. (1994). The mini-mental state Examination in a general population: impact of educational status. *Arq. Neuropsiquiatr.* 52, 1–7. doi: 10.1590/S0004-282X1994000100001

Bertrand, E., Dourado, M. C. N., Laks, J., Morris, R. G., Landeira-Fernandez, J., and Mograbi, D. C. (2016). Mood-congruent recollection and anosognosia in Alzheimer's disease. *Cortex* 84, 55–62. doi: 10.1016/j.cortex.2016.09.001

Black, W., and Almeida, O. P. (2004). A systematic review of the association between the Behavioral and Psychological Symptoms of Dementia and burden of care. *Int. Psychogeriatr.* 16, 295–315. doi: 10.1017/s1041610204000468

Camozzato, A. L., Kochhann, R., Simeoni, C., Konrath, C. A., Pedro Franz, A., Carvalho, A., et al. (2008). Reliability of the Brazilian Portuguese version of the Neuropsychiatric Inventory (NPI) for patients with Alzheimer's disease and their caregivers. *Int. Psychogeriatr.* 20, 383–393. doi: 10.1017/S1041610207006254

Candia, P. C., and Barba, A. C. (2011). Mental capacity and consent to treatment in psychiatric patients: the state of the research. *Curr. Opin. Psychiatry* 24, 442–446. doi: 10.1097/YCO.0b013e328349bba5

Cerejeira, J., Lagarto, L., and Mukaetova-Ladinska, E. B. (2012). Behavioral and psychological symptoms of dementia. *Front. Neurol.* 3:73. doi: 10.3389/fneur.2012.00073

Cummings, J. L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D. A., and Gornbein, J. (1994). The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 44, 2308–2314. doi: 10.1212/WNL.44.12.2308

Damasio, A. R. (1994). *Descartes' Error: Emotion, Reason and The Human Brain.* New York, NY: Putnam and Sons.

de Assis da Silva, R., Mograbi, D. C., Bifano, J., Santana, C. M. T., and Cheniaux, E. (2016). Insight in bipolar mania: evaluation of its heterogeneity and correlation with clinical symptoms. *J. Affect. Disord.* 199, 95–98. doi: 10.1016/j.jad.2016.04.019

de Assis da Silva, R., Mograbi, D. C., Camelo, E. V. M., Bifano, J., Wainstok, M., Silveira, L. A. S., et al. (2015a). Insight in bipolar disorder: a comparison between mania, depression and euthymia using the Insight Scale for Affective Disorders. *Trends Psychiatry Psychother.* 37, 152–156. doi: 10.1590/2237-6089-2015-0014

de Assis da Silva, R., Mograbi, D. C., Silveira, L. A. S., Nunes, A. L. S., Novis, F. D., Landeira-Fernandez, J., et al. (2015b). Insight across the different mood states of bipolar disorder. *Psychiatr. Q.* 86, 395–405. doi: 10.1007/s11126-015-9340-z

Dixon, T., Kravariti, E., Frith, C., Murray, R. M., and McGuire, P. K. (2004). Effect of symptoms on executive function in bipolar illness. *Psychol. Med.* 34, 811–821. doi: 10.1017/s0033291703001570

Dobson, K., and Franche, R.-L. (1989). A conceptual and empirical review of the depressive realism hypothesis. *Can. J. Behav. Sci.* 21, 419–433. doi: 10.1037/h0079839

Feast, A., Moniz-Cook, E., Stoner, C., Charlesworth, G., and Orrell, M. (2016). A systematic review of the relationship between behavioral and psychological symptoms (BPSD) and caregiver well-being. *Int. Psychogeriatr.* 28, 1761–1774. doi: 10.1017/s1041610216000922

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

Grisso, T., and Appelbaum, P. S. (1995). The macarthur treatment competence study. III: abilities of patients to consent to psychiatric and medical treatments. *Law Hum. Behav.* 19, 149–174. doi: 10.1007/bf01499323

Grisso, T., Appelbaum, P. S., and Hill-Fotouhi, C. (1997). The MacCAT-T: a clinical tool to assess patients' capacities to make treatment decisions. *Psychiatr. Serv.* 48, 1415–1419. doi: 10.1176/ps.48.11.1415

Hindmarch, T., Hotopf, M., and Owen, G. S. (2013). Depression and decision-making capacity for treatment or research: a systematic review. *BMC Med. Ethics* 14:54. doi: 10.1186/1472-6939-14-54

Howe, V., Foister, K., Jenkins, K., Skene, L., Copolov, D., and Keks, N. (2005). Competence to give informed consent in acute psychosis is associated with symptoms rather than diagnosis. *Schizophr. Res.* 77, 211–214. doi: 10.1016/j.schres.2005.03.005

Kahneman, D., and Tversky, A. (1979). Prospect theory: an analysis of decision under risk. *Econometrica* 47, 263–292. doi: 10.2307/1914185

Karlawish, J. H. T., Casarett, D. J., James, B. D., Xie, S. X., and Kim, S. Y. H. (2005). The ability of persons with Alzheimer disease (AD) to make a decision about taking an AD treatment. *Neurology* 64, 1514–1519. doi: 10.1212/01.wnl.0000160000.01742.9d

Kitwood, T. M. (1997). *Dementia Reconsidered: The Person Comes First.* Buckingham: Open University Press.

Lui, V. W.-C., Lam, L. C.-W., Chau, R. C.-M., Fung, A. W.-T., Wong, B. M.-L., Leung, G. T.-Y., et al. (2012). Capacity to make decisions on medication management in Chinese older persons with mild cognitive impairment and mild Alzheimer's disease. *Int. Psychogeriatri.* 24, 1103–1111. doi: 10.1017/s1041610212000129

Maia, A. L. G., Godinho, C., Ferreira, E. D., Almeida, V., Schuh, A., Kaye, J., et al. (2006). Application of the Brazilian version of the CDR scale in samples of dementia patients. *Arq. Neuropsiquiatr.* 64, 485–489. doi: 10.1590/S0004-282X2006000300025

Marin, R. S. (1990). Differential diagnosis and classification of apathy. *Am. J. Psychiatry* 147, 22–30. doi: 10.1176/ajp.147.1.22

Marson, D. C. (2001). Loss of competency in Alzheimer's disease: conceptual and psychometric approaches. *Int. J. Law Psychiatry* 24, 267–283. doi: 10.1016/s0160-2527(01)00064-4

Migliorelli, R., Tesón, A., Sabe, L., Petracca, G., Petracchi, M., Leiguarda, R., et al. (1995). Anosognosia in Alzheimer's disease: a study of associated factors. *J. Neuropsychiatry Clin. Neurosci.* 7, 338–344. doi: 10.1176/jnp.7.3.338

Mitoku, K., and Shimanouchi, S. (2014). The decision-making and communication capacities of older adults with dementia: a population-based study. *Open Nurs. J.* 8, 17–24. doi: 10.2174/1874434620140512001

Mograbi, D. C., Ferri, C. P., Stewart, R., Sosa, A. L., Brown, R. G., Laks, J., et al. (2015). Neuropsychological and behavioral disturbance correlates of unawareness of memory impairment in dementia: a population-based study. *J. Geriatr. Psychiatry Neurol.* 28, 3–11. doi: 10.1177/0891988714541868

Mograbi, D. C., and Morris, R. G. (2014). On the relation among mood, apathy, and anosognosia in Alzheimer's disease. *J. Int. Neuropsychol. Soc.* 20, 2–7. doi: 10.1017/S1355617713001276

Morris, J. C. (1993). The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* 43, 2412–2414. doi: 10.1212/WNL.43.11.2412-a

Moye, J., Karel, M. J., Azar, A. R., and Gurrera, R. J. (2004). Capacity to consent to treatment: empirical comparison of three instruments in older adults with and without dementia. *Gerontologist* 44, 166–175. doi: 10.1093/geront/44.2.166

Moye, J., Karel, M. J., Gurrera, R. J., and Azar, A. R. (2006). Neuropsychological predictors of decision-making capacity over 9 months in mild-to-moderate dementia. *J. Gen. Intern. Med.* 21, 78–83. doi: 10.1111/j.1525-1497.2005.00288.x

Mur, M., Portella, M. J., Martínez-Arán, A., Pifarré, J., and Vieta, E. (2007). Persistent neuropsychological deficit in euthymic bipolar patients: executive function as a core deficit. *J. Clin. Psychiatry* 68, 1078–1086. doi: 10.4088/jcp.v68n0715

Nascimento, E. (2000). *Adaptation and Validation of Test Wais-III for a Brazilian Context*. Doctoral thesis. Brasilia: Universidade de Brasília. Available online at: http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=LILACS&lang=p&nextAction=lnk&exprSearch=317037&indexSearch=ID

Okonkwo, O. C., Griffith, H. R., Copeland, J. N., Belue, K., Lanza, S., Zamrini, E. Y., et al. (2008). Medical decision-making capacity in mild cognitive impairment: a 3-year longitudinal study. *Neurology* 71, 1474–1480. doi: 10.1212/01.WNL.0000334301.32358.48

Palmer, B. W., Dunn, L. B., Appelbaum, P. S., and Jeste, D. V. (2004). Correlates of treatment-related decision-making capacity among middle-agedand older patients with schizophrenia. *Arch. Gen. Psychiatry* 61, 230–236. doi: 10.1001/archpsyc.61.3.230

Palmer, B. W., and Savla, G. N. (2007). The association of specific neuropsychological deficits with capacity to consent to research or treatment. *J. Int. Neuropsychol. Soc.* 13, 1047–1059. doi: 10.1017/s1355617707071299

President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. (1982). *Making Health Care Decisions: A Report on the Ethical and Legal Implications of Informed Consent in the Patient-Practitioner Relationship.* (Vol. 1) Washington, DC: Government Printing Office.

Rahman, S., Sahakian, B. J., Cardinal, R. N., Rogers, R. D., and Robbins, T. W. (2001). Decision making and neuropsychiatry. *Trends Cogn. Sci.* 5, 271–277. doi: 10.1016/S1364-6613(00)01650-8

Robert, P., Onyike, C. U., Leentjens, A. F. G., Dujardin, K., Aalten, P., Starkstein, S., et al. (2009). Proposed diagnostic criteria for apathy in Alzheimer's disease and other neuropsychiatric disorders. *Eur. Psychiatry* 24, 98–104. doi: 10.1016/j.eurpsy.2008.09.001

Rutledge, E., Kennedy, M., O'Neill, H., and Kennedy, H. G. (2008). Functional mental capacity is not independent of the severity of psychosis. *Int. J. Law Psychiatry* 31, 9–18. doi: 10.1016/j.ijlp.2007.11.002

Santos, R. L., de Sousa, M. F. B., Simões Neto, J. P., Bertrand, E., Mograbi, D. C., Landeira-Fernandez, J., et al. (2017). MacArthur competence assessment tool for treatment in Alzheimer disease: cross-cultural adaptation. *Arq. Neuropsiquiatr.* 75, 36–43. doi: 10.1590/0004-282x20160181

Scarmeas, N., Brandt, J., Blacker, D., Albert, M., Hadjigeorgiou, G., Dubois, B., et al. (2007). Disruptive behavior as a predictor in Alzheimer disease. *Arch. Neurol.* 64, 1755–1761. doi: 10.1001/archneur.64.12.1755

Schaller, S., Mauskopf, J., Kriza, C., Wahlster, P., and Kolominsky-Rabas, P. L. (2015). The main cost drivers in dementia: a systematic review. *Int. J. Geriatr. Psychiatry* 30, 111–129. doi: 10.1002/gps.4198

Schultz, R. R., Siviero, M. O., and Bertolucci, P. H. F. (2001). The cognitive subscale of the andquot "Alzheimer's disease assessment Scaleandquot" in a brazilian sample. *Braz. J. Med. Biol. Res.* 34, 1295–1302. doi: 10.1590/s0100-879x2001001000009

Selbæk, G., and Engedal, K. (2012). Stability of the factor structure of the neuropsychiatric inventory in a 31-month follow-up study of a large sample of nursing-home patients with dementia. *Int. Psychogeriatr.* 24, 62–73. doi: 10.1017/S104161021100086X

Smebye, K. L., Kirkevold, M., and Engedal, K. (2012). How do persons with dementia participate in decision making related to health and daily care? A multi-case study. *BMC Health Serv. Res.* 12:241. doi: 10.1186/1472-6963-12-241

Spalletta, G., Musicco, M., Padovani, A., Perri, R., Fadda, L., Canonico, V., et al. (2010). Neuropsychiatric symptoms and syndromes in a large cohort of newly diagnosed, untreated patients with alzheimer disease. *Am. J. Geriatr. Psychiatry* 18, 1026–1035. doi: 10.1097/JGP.0b013e3181d6b68d

Stormoen, S., Almkvist, O., Eriksdotter, M., Sundström, E., and Tallberg, I.-M. (2014). Cognitive predictors of medical decision-making capacity in mild cognitive impairment and Alzheimer's disease. *Int. J. Geriatr. Psychiatry* 29, 1304–1311. doi: 10.1002/gps.4114

Taylor, S. E. (1989). *Positive Illusions: Creative Self-Deception and The Healthy Mind*. New York, NY: Basic Books.

Tepper, A. M., and Elwork, A. (1984). Competence to consent to treatment as a psycholegal construct. *Law Hum. Behav.* 8, 205–223. doi: 10.1007/bf01044693

van der Linde, R. M., Dening, T., Matthews, F. E., and Brayne, C. (2014). Grouping of behavioural and psychological symptoms of dementia. *Int. J. Geriatr. Psychiatry* 29, 562–568. doi: 10.1002/gps.4037

Wechsler, D. (1997). Wechsler Adult Intelligence Scale® -3rd Edn. (WAIS® -III). San Antonio, TX: Harcourt Assessment.

Zhao, Q.-F., Tan, L., Wang, H.-F., Jiang, T., Tan, M.-S., Tan, L., et al. (2016). The prevalence of neuropsychiatric symptoms in Alzheimer's disease: systematic review and meta-analysis. *J. Affect. Disord.* 190, 264–271. doi: 10.1016/j.jad.2015.09.069

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 SB 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.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 10 March 2017
doi: 10.3389/fnagi.2017.00046

Adding Recognition Discriminability Index to the Delayed Recall Is Useful to Predict Conversion from Mild Cognitive Impairment to Alzheimer's Disease in the Alzheimer's Disease Neuroimaging Initiative

*María J. Russo *, Jorge Campos, Silvia Vázquez, Gustavo Sevlever, Ricardo F. Allegri and the Alzheimer's Disease Neuroimaging Initiative †*

Aging and Memory Center, Instituto de Investigaciones Neurológicas Raúl Carrea, Fundación Para la Lucha Contra las Enfermedades Neurológicas de la Infancia (FLENI), Buenos Aires, Argentina

OPEN ACCESS

Edited by:
Agustin Ibanez, Institute of Cognitive and Translational Neuroscience, Argentina

Reviewed by:
Jessica Peter, University of Bern, Switzerland
Guido Gainotti, Policlinico Universitario Agostino Gemelli, Italy

***Correspondence:**
María J. Russo
jrusso@fleni.org.ar; mariajulietarusso@gmail.com

† Data used in preparation of this article were obtained from the Alzheimer's disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

Received: *17 December 2016*
Accepted: *20 February 2017*
Published: *10 March 2017*

Citation:
Russo MJ, Campos J, Vázquez S, Sevlever G, Allegri RF and the Alzheimer's Disease Neuroimaging Initiative (2017) Adding Recognition Discriminability Index to the Delayed Recall Is Useful to Predict Conversion from Mild Cognitive Impairment to Alzheimer's Disease in the Alzheimer's Disease Neuroimaging Initiative. Front. Aging Neurosci. 9:46. doi: 10.3389/fnagi.2017.00046

Background: Ongoing research is focusing on the identification of those individuals with mild cognitive impairment (MCI) who are most likely to convert to Alzheimer's disease (AD). We investigated whether recognition memory tasks in combination with delayed recall measure of episodic memory and CSF biomarkers can predict MCI to AD conversion at 24-month follow-up.

Methods: A total of 397 amnestic-MCI subjects from Alzheimer's disease Neuroimaging Initiative were included. Logistic regression modeling was done to assess the predictive value of all RAVLT measures, risk factors such as age, sex, education, APOE genotype, and CSF biomarkers for progression to AD. Estimating adjusted odds ratios was used to determine which variables would produce an optimal predictive model, and whether adding tests of interaction between the RAVLT Delayed Recall and recognition measures (traditional score and d-prime) would improve prediction of the conversion from a-MCI to AD.

Results: 112 (28.2%) subjects developed dementia and 285 (71.8%) subjects did not. Of the all included variables, CSF Aβ1-42 levels, RAVLT Delayed Recall, and the combination of RAVLT Delayed Recall and d-prime were predictive of progression to AD ($\chi^2 = 38.23$, $df = 14$, $p < 0.001$).

Conclusions: The combination of RAVLT Delayed Recall and d-prime measures may be predictor of conversion from MCI to AD in the ADNI cohort, especially in combination with amyloid biomarkers. A predictive model to help identify individuals at-risk for dementia should include not only traditional episodic memory measures (delayed recall or recognition), but also additional variables (d-prime) that allow the homogenization of the assessment procedures in the diagnosis of MCI.

Keywords: disease progression, memory, recognition discriminability, mild cognitive impairment, Alzheimer's disease, signal detection theory

INTRODUCTION

Mild Cognitive Impairment (MCI) is a common condition defined as transitional state between normal cognition and dementia (Petersen et al., 2001). In general, subjects with MCI convert to dementia at an annual rate in the range of 10–15% (Farias et al., 2009). Predicting who among a group of MCI patients are more likely to further decline in cognition would be essential to ensure an early intervention and appropriate treatment as well as future preventive and treatment trials.

Of the MCI subtypes, patients with amnestic MCI (a-MCI) are at greatest risk. Poor delayed recall and recognition memory is a well-established pattern in the Alzheimer's disease (AD) literature, which is considered to reflect deficits in storage caused by deficient consolidation of new memory traces (Weintraub et al., 2012). Abundant evidence indicates delayed recall scores in word-list learning tasks such as the Rey Auditory Verbal Learning Test (RAVLT) (Rey, 1941, 1964) are perhaps the most challenging and accurate measures of episodic memory when used to accurately predict diagnostic conversion to AD (Estévez-González et al., 2003; Maruff et al., 2004; Griffith et al., 2006). It should be noted that other memory tests have shown predictive validity for clinical progression from MCI to AD, including Free and Cued Selective Reminding Test (FCSRT-FR) (Derby et al., 2013), delayed recall of story memory and verbal paired associates (Guarch et al., 2008) and visuospatial paired associate learning (Ahmed et al., 2008). Some authors (Dubois and Albert, 2004; Dubois et al., 2007) proposed using memory tests that provide encoding and retrieval facilities, such as the free and cued recall test (Grober and Buschke, 1987; Gainotti et al., 2014) to improve the prediction of MCI-AD conversion. However, a recently review showed inconclusive results (Grober and Buschke, 1987; Carlesimo et al., 2011; Gainotti et al., 2014). It is mandatory to have adequate and well-controlled studies to determine whether that test paradigms can improve the prediction of conversion from MCI to AD.

Thus, most clinical studies assume that memory is a single entity and extrapolate the results of a single memory measure (e.g., delayed recall or recognition task, verbal, or visual memory test) to the global memory functioning. Episodic memory can be subdivided according to the different stages on the reproduction process (e.g., free recall, cued recall, or recognition) and this multicomponent analysis may be used to describe the real risk of MCI to AD conversion. To our knowledge, it is not clear whether measurement of recognition task adds benefit for measuring conversion over time in a clinical cohort.

In the present study, we investigated whether recognition memory tasks in combination with delayed recall measure of episodic memory and CSF biomarkers can predict MCI to AD conversion at 24-month follow-up. We chose to examine this issue using ADNI, a public data set with a large sample and prospective nature. Delayed recall is the most important predictor for MCI to AD conversion. In addition, AD's patient deficits are evident in recognition tasks, with the presence of many false alarms errors. Most studies simplify recognition task by only registering the number of correct identifications (hits), without the inclusion of false alarms in their interpretation. Based on the signal detection theory, a discriminability recognition index (d-prime) can be calculated including both hits and false alarms (Russo et al., 2016). In addition, d-prime has been shown to distinguish healthy older adults from those with a-MCI or mild AD. We hypothesized that the addition of the d-prime measure to the delayed recall task would be good predictor of conversion from a-MCI to AD at 24-month follow-up in combination with CSF biomarkers related to AD, which were significant discriminators in other studies (Tabert et al., 2006; Lanari and Parnetti, 2009; Gomar et al., 2011) and were independently associated with future cognitive decline compared to other surrogate of neurodegeneration as MRI (Vemuri et al., 2009). We also thought that the addition of the traditional recognition score would be not a good predictor in combination with the same markers.

With the concepts explained in these paragraphs, we measured:

(i) The main effect of the cognitive measures: RAVLT delayed recall, traditional RAVLT recognition, and d-prime.
(ii) The main effect of the known risk factors such as age, sex, education, and APOE genotype.
(iii) The main effect of the CSF biomarkers related to AD: Aβ1-42, P-tau181, and total tau.
(iv) The interaction between RAVLT delayed recall and recognition measures (traditional score and d-prime), which represents the influence of the delayed recall on the magnitude of the recognition task.

Many papers have looked at specific clinical markers and biomarkers in isolation but never set up a study to compare the added effect to predict conversion to dementia. We believed that this analysis would help to interpret the MCI-AD transition in this sample and to propose the d-prime as an early useful cognitive marker to predict AD conversion.

MATERIALS AND METHODS

Subjects

Data used in the preparation of this article were obtained from the Alzheimer's disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center, and University of

California—San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 subjects but ADNI has been followed by ADNI-GO and ADNI-2. To date these three protocols have recruited over 1,500 adults, ages 55–90, to participate in the research, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. The follow up duration of each group is specified in the protocols for ADNI-1, ADNI-2, and ADNI-GO. Subjects originally recruited for ADNI-1 and ADNI-GO had the option to be followed in ADNI-2. For up-to-date information, see http://www.adni-info.org (Mueller et al., 2005; Petersen et al., 2010).

A total of 397 subjects with a-MCI from the ADNI study were included in the current analysis. MCI subjects fulfilled criteria for a-MCI (Petersen, 2004): nondemented subjects with memory complaint (global CDR score = 0.5, with a Memory Box score $\geq$ 0.5), MMSE score of 24–30, a Modified Hachinski Ischemic Score (Rosen et al., 1980) $\leq$4, a Geriatric Depression Score short form (Sheikh and Yesavage, 1986) <6, and preserved instrumental activities of daily living. Subjects performed at an objective cut-off of 1.5 standard deviations (SD) below education-adjusted cut-off scores on the Logical Memory IIa of the Weschler Memory Scale-Revised (Wechsler, 1987). The summary of the baseline characteristics of the ADNI subjects indicated that the subjects with MCI presented memory-only deficits (Petersen et al., 2010).

The subjects with a-MCI were divided into two groups for the comparison between those who convert to AD dementia during 24-month follow-up and those who did not.

Psychometric Testing

The baseline characteristics of the subjects from ADNI cohort relevant to describe their neuropsychological profile were the Mini Mental State Examination (MMSE) (Folstein et al., 1975); Logical memory test of immediate and delayed recognition, Wechsler Memory Scale III (Wechsler, 1987); Boston Naming Test (Kaplan et al., 1983); Categorical and Phonological Verbal Fluency test (Morris et al., 1989); Digit Span Forward and Backward (Wechsler, 1997); Trail Making Test A and B (Reitan, 1958); GDS short form (Sheikh and Yesavage, 1986); and RAVLT (Rey, 1941, 1964).

The RAVLT consists of five learning trials in which a list of 15 words is read and the subject is asked to immediately recall orally as many items as possible. After an interference list of 15 novel words is read and recalled, subjects are then asked to recall words from the initial list (5-min delayed recall). A 30-min delayed recall trial and recognition test follow. For the recognition test, subjects are presented with a list of the 15 studied words and 15 non-studied foils and are asked to circle all words previously learned.

Discriminability refers to the ability to distinguish target words from distractor words, and is widely considered the best measure of recognition memory accuracy. Discriminability index (d-prime = Z hits rate – Z false alarms rate) is adapted from signal detection theory (Snodgrass and Corwin, 1988) and is analogous to a contrast z score, reflecting the absolute difference in standard deviation units between subject hit rate and false alarm rate (Donaldson, 1992).

CSF Analysis

CSF was available from 198 (49.80%) patients. Methods for cerebrospinal fluid (CSF) acquisition and biomarker measurement using the ADNI cohort has been reported previously (Shaw et al., 2009). In brief, CSF was collected and stored at −80°C at the University of Pennsylvania ADNI Biomarker Core Laboratory. Amyloid-β from peptides 1-42 (Aβ1-42), tau phosphorylated at threonine 181 (P-tau181), and total tau was measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin TX) with Innogenetics (INNOBIA AlzBio3, Ghent, Belgium) immunoassay kit–based reagents.

APOE ε4 Status

APOE ε4 status was considered as a binary variable (i.e., at least one ε4 allele vs. none).

Statistical Analyses

Statistical analysis was performed with SPSS∗ Version 19.0 software for Windows. Independent sample *t*-tests were used to assess differences in clinical, cognitive and biomarkers CSF variables. The χ^2-test was used for the analysis of gender and APOEε4 carrier status differences between groups. The Pearson's test of correlation was used to measure the degree of relationship between conversion status at 24-month follow-up and the predictive factors. Logistic regression modeling was done to assess the predictive value of all RAVLT measures (Delayed Recall, Recognition, and d-prime), risk factors such as age (in years), sex (male/female), education (in years), APOEε4 carrier status (at least 1 allele vs. none allele, MMSE score, and CSF biomarkers levels for progression to AD. Estimating adjusted odds ratios was used to determine which variables would produce an optimal predictive model, and whether adding tests of interaction between the RAVLT Delayed Recall and RAVLT recognition measures (traditional score and d-prime) would improve prediction of the conversion from a-MCI to AD by including the main effects and two interaction terms (RAVLT Delayed Recall∗RAVLT Recognition; RAVLT Delayed Recall∗d-prime).

RESULTS

The clinical and demographic characteristics of the subjects as well as CSF biomarkers are listed by conversion status in **Table 1**. At the 24-month follow-up, 112 (28.2%) subjects developed AD dementia (converters) and 285 (71.8%) subjects did not (nonconverters). Those who developed AD dementia had worse performance on baseline MMSE ($t = 3.35$, $p = 0.001$), Delayed Recall Logical Memory ($t = 5.17$, $p < 0.001$), RAVLT Delayed Recall ($t = 5.28$, $p < 0.001$), RAVLT Recognition ($t = 3.98$, $p < 0.001$), and d-prime ($t = 3.53$, $p = 0.001$), but were not significantly different with respect to sex, age,

TABLE 1 | Demographic characteristics, cognitive tests scores, and CSF biomarker profile on the MCI sample at baseline, split according to progression status at 24-month follow-up.

Variable	a-MCI Non-converters ($n = 285$)	a-MCI converters ($n = 112$)	Statistical Test t/χ^2	p-value
Age, y	74.12 ± 7.1	74.3 ± 6.8	−0.96	0.339
Female, n (%)	97 (34)	44 (39)	0.97	0.352
Education, y	15.7 ± 3.1	15.5 ± 2.9	0.50	0.614
GDS	1.6 ± 1.4	1.4 ± 1.1	1.21	0.226
MMSE	27.2 ± 1.8	26.6 ± 1.6	3.35	0.001
WMS—Immediate Recall	7.5 ± 3.1	6.2 ± 3.1	3.56	0.000
WMS—Delayed Recall	4.2 ± 2.7	2.7 ± 2.2	5.52	0.000
Boston Naming Test	25.7 ± 4.0	24.9 ± 4.2	1.72	0.087
Forward Digit Span	8.2 ± 2.0	8.2 ± 2.0	−0.05	0.961
Backward Digit Span	6.3 ± 2.1	5.9 ± 1.8	1.41	0.159
Category VFT	16.1 ± 5.1	15.1 ± 4.4	1.86	0.064
TMT—A (seconds)	42.9 ± 20.9	49.9 ± 26.3	−2.83	0.395
TMT—B (seconds)	122.6 ± 69.9	152.0 ± 77.7	−3.63	0.000
RAVLT trial 1–5	32.3 ± 9.5	26.7 ± 6.1	5.76	0.000
RAVLT Delayed Recall	3.4 ± 3.5	1.5 ± 2.0	5.28	0.000
RAVLT Delayed-Intrusions	1.4 ± 1.57	1.5 ± 1.9	−0.36	0.721
RAVLT Recognition	10.1 ± 3.4	8.5 ± 3.8	3.98	0.000
RAVLT Recognition-False Alarms	1.9 ± 2.1	2.1 ± 2.6	−0.69	0.487
d-prime	1.7 ± 1.0	1.3 ± 0.9	3.42	0.001
APOEε4 carriers, n (%)			11.81	0.003
Noncarriers	147 (51.6)	38 (33.9)		
Carriers 1 allele	111 (38.9)	54 (48.2)		
Carriers 2 alleles	27 (9.5)	20 (17.9)		
CSF biomarkers, pg/ml, n	140	58		
Tau	102.3 ± 66.5	106.6 ± 45.5	−0.45	0.653
A-β1-42	172.7 ± 58.9	141.8 ± 35.2	3.72	0.000
p-Tau 181P	34.2 ± 18.8	38.6 ± 15.7	−1.57	0.117
Tau/ A-β 1-42	0.7 ± 0.7	0.8 ± 0.3	−0.67	0.506
p-Tau 181P/A-β 1-42	0.2 ± 0.2	0.3 ± 0.1	−1.62	0.106
CSF AD profile	0.5 ± 0.2	0.4 ± 0.1	3.78	0.000

Values are numbers (percentages) or mean ± SD. a-MCI, amnestic mild cognitive impairment; MMSE, Mini-Mental State Examination Test; WMS III, Wechsler Memory Scale III, Logical memory test of immediate and delayed recall; TMT, Trail Making Test A and B; RAVLT, Rey Auditory Verbal Learning Test; CSF, cerebrospinal fluid; AD, Alzheimer's disease.

education, and other neuropsychological tests. The proportion of the individuals with APOEε4 was also significantly higher in those who developed AD dementia than those who did not ($\chi^2 = 11.81$, $p = 0.003$). CSF Aβ 11-42, tau, and P-tau181 levels were available in 198 subjects from two groups (58 vs. 140) for those who developed AD dementia and those who did not respectively. The group of a-MCI patients who progressed to AD dementia had lower levels of CSF Aβ42 ($t = 3.71$, $p < 0.001$) and CSF AD profile ($t = 3.78$, $p < 0.001$) than the group of non-progressive a-MCI patients.

Results of logistic regression analyses are shown in **Table 2**. Of the variables entered into the first step, only RAVLT Delayed Recall and CSF Aβ42 levels reached significance with the odds of 0.79 (95% CI 0.66–0.94) and 0.99 (95% CI 0.98–0.99), respectively. The overall model was statistically significant ($\chi^2 = 32.03$, $df = 12$, $p = 0.001$). When both interactions (RAVLT Delayed Recall∗RAVLT Recognition; RAVLT Delayed Recall∗d-prime) were entered to evaluate the impact of both recognition measures in predicting conversion, only CSF Aβ42 levels and RAVLT Delayed Recall∗d-prime interaction remained significant in the model with the odds of 0.98 (95% CI 0.97–0.99) and 0.75 (95% CI 0.58–0.97), respectively. Also, the overall model was statistically significant ($\chi^2 = 38.23$, $df = 14$, $p < 0.001$). The area under the curve was.75, and the percentage of cases classified correctly was 77.4% at $c = 0.50$. Sensitivity was 0.41 and specificity was 0.93 at c = 0.50. The decrease in deviance was the largest for the second step including CSF Aβ42 levels and RAVLT Delayed Recall∗d-prime interaction, indicating that adding d-prime to delayed recall increased the predictive accuracy compared to delayed recall alone (205.354 – 199.149 = 6.205). The drop in deviance was significant ($t = -52.79$, $df = 196$, $p < 0.001$). CSF markers by each potential effect modifier (age, gender, MMSE, RAVLT delayed recall, RAVLT recognition memory, d-prime, or APOE ε4)

TABLE 2 | Results of logistic regression analysis.

Variable	Beta	*SE*	Wald	Df	*p*-value	OR (95% CI)
Age	0.022	0.025	0.777	1	0.38	1.02 (0.97–1.07)
Gender (Male)	0.013	0.389	0.001	1	0.97	1.01 (0.47–2.17)
Education	−0.113	0.059	3.631	1	0.06	0.89 (0.79–1.00)
MMSE	−0.150	0.101	2.212	1	0.14	0.86 (0.71–1.05)
RAVLT Delayed Recall	−0.239	0.092	6.764	1	**0.01**	0.79 (0.66–0.94)
RAVLT Recognition	−0.093	0.281	0.110	1	0.74	0.91 (0.52–1.58)
d-prime	0.379	0.705	0.288	1	0.59	1.46 (0.37–5.82)
APOEε4	−0.066	0.378	0.030	1	0.86	0.94 (0.45–1.96)
Tau	−0.009	0.005	3.524	1	0.06	0.99 (0.98–1.00)
$A\beta_{1-42}$	−0.014	0.005	9.302	1	**0.00**	0.99 (0.98–0.99)
P-tau_{181}	0.012	0.016	0.636	1	0.42	1.01 (0.98–1.04)
*RAVLT Delayed Recall*RAVLT Recognition*	0.036	0.035	1.058	1	0.30	1.04 (0.97–1.11)
*RAVLT Delayed Recall*d-prime*	−0.283	0.132	4.596	1	**0.03**	0.75 (0.58–0.98)

MMSE, Mini-Mental State Examination Test; RAVLT, Rey Auditory Verbal Learning Test; APOE, Apolipoprotein E; Aβ1-42, Amyloid-β from peptides 1-42; P-tau181, tau phosphorylated at threonine 181; Standard error; DF, Degrees of freedom; OR, Odds ratio; CI, Confidence interval.

interaction term was added to model; there was no change in the results.

There were weak positive correlations between conversion ratio to AD dementia and MMSE, RAVLT Delayed Recall, RAVLT Recognition, and d-prime ($r = 0.207$, 0.299, −0.215, 0.254, respectively, $n = 397$, $p < 0.05$) (**Table 3**). There was also a significant but negative correlation between conversion ratio to AD dementia and CSF Aβ42 levels ($r = -0.659$, $n = 198$, $p < 0.001$). There were also a significant but positive correlation between conversion ratio to AD dementia and APOEε4 carrier status, ($r = 0.355$, $n = 397$, $p < 0.001$), and CSF P-tau181 levels ($r = 0.429$, $n = 198$, $p < 0.001$).

TABLE 3 | Correlations between conversion status at 24-month follow-up and the predictive factors.

	Conversion ratio	MMSE	RAVLT Delayed Recall	RAVLT Recognition	*d*-prime
Conversion ratio	1.000	0.207*	0.299*	0.215*	0.254*
MMSE	0.207*	1.000	0.515*	0.457*	0.517*
RAVLT Delayed Recall	0.299*	0.515*	1.000	0.622*	0.715*
RAVLT Recognition	0.215*	0.457*	0.622*	1.000	0.876*
d-prime	0.254*	0.517*	0.715*	0.876*	1.000

Values are Pearson's Correlation Coefficient (r). MMSE, Mini-Mental State Examination Test; RAVLT, Rey Auditory Verbal Learning Test.
**p < 0.05.*

DISCUSSION

In this study, we demonstrated that baseline CSF Aβ1-42 levels, RAVLT Delayed Recall, and the combination of RAVLT Delayed Recall and Recognition Discriminability Index (d-prime) were the strongest predictors of conversion from a-MCI to AD at 24-month follow-up in patients in the ADNI sample. These results were independent of other factors known to increase the risk of developing dementia, such as age, gender, education level, and APOEε4 status (Tabert et al., 2006; Lanari and Parnetti, 2009; Gomar et al., 2011). We also showed that the combination of RAVLT Delayed Recall and traditional recognition measure may not predict MCI-AD conversion.

One might argue that the use of verbal episodic memory markers in this context is redundant. However, this may not be correct because the RAVLT memory measures examined as a predictor in this paper was not part of the study inclusion criteria in ADNI cohort (WMS-R logical memory was used). Nevertheless, evaluation of different components of verbal episodic memory deficit as a predictor in patients with a-MCI may be an advantage to interpret our patients in clinical practice or to improve their recruitment in clinical trials. Other important issue is that the diagnostic conversions over the 24-month period should be viewed with some caution. A 24-month change period is not a sufficient amount of time to draw conclusions regarding the likelihood of clinical change. However, we preferred not include the 36- or 48-month period due to partially missing baseline data.

Numerous studies that characterized the episodic memory deficit in AD have used word list learning tasks such as those from the Consortium to Establish a Registry for Alzheimer Disease (CERAD) (Welsh et al., 1992), the RAVLT (Rey, 1941, 1964), and the California Verbal Learning Test (CVLT) (Delis et al., 1991). These studies consistently showed that AD patients are equally impaired (relative to age-matched controls) on recognition and free recall components of the tasks. This pattern of performance is consistent with impaired consolidation rather than ineffective retrieval of new information (Delis et al., 1991). This memory profile is a useful measure for a clinical diagnosis and classification MCI subjects.

However, MCI in general and a-MCI in particular are heterogeneous clinical constructs, and the reported literature

that investigated the role of different verbal episodic memory task as a predictor of cognitive decline in MCI is variable, with inhomogeneous results. Explicit measures of delayed recall have been reported the best predictors of the development of AD in MCI subjects (Gainotti et al., 2014). With respect to the recognition memory task, we found few studies that assessed their predictive value. Rabin et al. (2009) revealed that logical memory recognition best predicted the progression to AD followed closely by the delayed recall condition of CVLT-II. Yang et al. (2012) subdivided a-MCI sample from ADNI cohort into two subtypes, encoding failure (a-MCI-E) vs. retrieval deficit (a-MCI-R) but did not find that a-MCI-E was an independent prognostic factor to predict the progression to AD. In addition to the neuropsychological predictors, current criteria and recommendations (Dubois et al., 2007; Albert et al., 2011) proposed that incorporation of distinctive and reliable biomarkers of underlying AD to predict MCI-AD conversion. Most prior studies of CSF biomarkers have showed only modest prognostic accuracy for prediction of conversion from MCI to AD (Vemuri et al., 2009). On the other hand, most of these studies aimed to identify the predictive value of a set of commonly measured variables, with classification accuracies reaching up to 80%. However, none of these prior studies have investigated the predictive value when combining different related variables to homogenize the group of subjects with higher risk of conversion.

We have found that delayed recall can make important influence to recognition task in patients who convert to dementia, when the hits and false alarms are considered. Considering that memory is not a unitary concept, the strategy of combining recall and recognition memory measures might provide a more accurate assessment of MCI individuals who perform poorly on list learning tasks or allow to develop an empirical perspective of subtyping within a-MCI that may identify more homogenous subgroups reflecting common etiology and better predictors of decline. We considered that the simultaneous analysis of CSF biomarkers or the combination of different assessment yielded a better prediction of conversion from MCI to AD.

Future research should more closely examine which memory processes are tapped by specific tasks and the nature and extent of decline in these processes during the insidious transition from healthy aging to MCI to AD. This suggested subdivision within a-MCI based on recall and recognition performance need to be assessed using traditional delayed recall and recognition measures, but with inclusion of additional variables (e.g. d-prime). We also expect that future prediction studies consider the co-occurrence effects of different variables (interactions), and not only traditional additive (main effects) models.

ETHICS STATEMENT

The study was approved by the Institute of Neurological Research FLENI Ethics and Research Committee. The paper was reviewed and was acceptable for submission by the Alzheimer's Disease Neuroimaging Initiative Data and Publications Committee (ADNI DPC).

AUTHOR CONTRIBUTIONS

All authors contributed extensively to the work presented in this paper. MR designed the study. JC and SV helped to evaluate and edit the manuscript. GS helped in the interpretation of data related to biomarkers. RA supervised development or research work and helped in data interpretation and manuscript evaluation.

ACKNOWLEDGMENTS

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Alzheimer's Association; Alzheimer's Drug Discovery Foundation; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; GE Healthcare; Innogenetics, N.V.; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California. The other authors report no conflicts of interest and have nothing to disclose.

REFERENCES

Ahmed, S., Mitchell, J., Arnold, R., Nestor, P. J., and Hodges, J. R. (2008). Predicting rapid clinical progression in amnestic mild cognitive impairment. *Dement. Geriatr. Cogn. Disord.* 25, 170–177. doi: 10.1159/000113014

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., et al. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the national institute on aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 270–279. doi: 10.1016/j.jalz.2011.03.008

Carlesimo, G. A., Perri, R., and Caltagirone, C. (2011). Category cued recall following controlled encoding as a neuropsychological tool in the diagnosis of Alzheimer's disease: a review of the evidence. *Neuropsychol. Rev.* 21, 54–65. doi: 10.1007/s11065-010-9153-7

Delis, D. C., Massman, P. J., Butters, N., Salmon, D. P., Cermak, L. S., and Kramer, J. H. (1991). Profiles of demented and amnesic patients on the California verbal learning test: implications for the assessment of memory disorders. *Psychol. Assess.* 3, 19–26. doi: 10.1037/1040-3590.3.1.19

Derby, C. A., Burns, L. C., Wang, C., Katz, M. J., Zimmerman, M. E., L'italien, G., et al. (2013). Screening for predementia AD: time-dependent operating characteristics of episodic memory tests. *Neurology* 80, 1307–1314. doi: 10.1212/WNL.0b013e31828ab2c9

Donaldson, W. (1992). Measuring recognition memory. *J. Exp. Psychol. Gen.* 121, 275–277. doi: 10.1037/0096-3445.121.3.275

Dubois, B., and Albert, M. L. (2004). Amnestic MCI or prodromal Alzheimer's disease? *Lancet Neurol.* 3, 246–248. doi: 10.1016/S1474-4422(04)00710-0

Dubois, B., Feldman, H. H., Jacova, C., Dekosky, S. T., Barberger-Gateau, P., Cummings, J., et al. (2007). Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol.* 6, 734–746. doi: 10.1016/S1474-4422(07)70178-3

Estévez-González, A., Kulisevsky, J., Boltes, A., Otermín, P., and García-Sánchez, C. (2003). Rey verbal learning test is a useful tool for differential diagnosis in the preclinical phase of Alzheimer's disease: comparison with mild cognitive impairment and normal aging. *Int. J. Geriatr. Psychiatry* 18, 1021–1028. doi: 10.1002/gps.1010

Farias, S. T., Mungas, D., Reed, B. R., Harvey, D., and DeCarli, C. (2009). Progression of mild cognitive impairment to dementia in clinic- vs community-based cohorts. *Arch. Neurol.* 66, 1151–1157. doi: 10.1001/archneurol.2009.106

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

Gainotti, G., Quaranta, D., Vita, M. G., and Marra, C. (2014). Neuropsychological predictors of conversion from mild cognitive impairment to Alzheimer's disease. *J. Alzheimers Dis.* 38, 481–495. doi: 10.3233/JAD-130881

Gomar, J. J., Bobes-Bascaran, M. T., Conejero-Goldberg, C., Davies, P., and Goldberg, T. E. (2011). Utility of combinations of biomarkers, cognitive markers, and risk factors to predict conversion from mild cognitive impairment to Alzheimer disease in patients in the Alzheimer's disease neuroimaging initiative. *Arch. Gen. Psychiatry* 68, 961–969. doi: 10.1001/archgenpsychiatry.2011.96

Griffith, H. R., Netson, K. L., Harrell, L. E., Zamrini, E. Y., Brockington, J. C., and Marson, D. C. (2006). Amnestic mild cognitive impairment: diagnostic outcomes and clinical prediction over a two-year time period. *J. Int. Neuropsychol. Soc.* 12, 166–175. doi: 10.1017/S1355617706060267

Grober, E., and Buschke, H. (1987). Genuine memory deficits in dementia. *Dev. Neuropsychol.* 3, 13–36. doi: 10.1080/87565648709540361

Guarch, J., Marcos, T., Salamero, M., Gastó, C., and Blesa, R. (2008). Mild cognitive impairment: a risk indicator of later dementia, or a preclinical phase of the disease? *Int. J. Geriatr. Psychiatry* 23, 257–65. doi: 10.1002/gps.1871

Kaplan, E., Goodglass, H., and Weintraub, S. (1983). *The Boston Naming Test*. Philadelphia: Lea & Febiger.

Lanari, A., and Parnetti, L. (2009). Cerebrospinal fluid biomarkers and prediction of conversion in patients with mild cognitive impairment: 4-year follow-up in a routine clinical setting. *Sci. World J.* 9, 961–966. doi: 10.1100/tsw.2009.106

Maruff, P., Collie, A., Darby, D., Weaver-Cargin, J., Masters, C., and Currie, J. (2004). Subtle memory decline over 12 months in mild cognitive impairment. *Dement Geriatr. Cogn. Disord.* 18, 342–348. doi: 10.1159/000080229

Morris, J. C., Heyman, A., Mohs, R. C., Hughes, J. P., van Belle, G., Fillenbaum, G., et al. (1989). The consortium to establish a registry for Alzheimer's disease (CERAD). Part, I. clinical and neuropsychological assessment of Alzheimer's disease. *Neurology* 39, 1159–1165. doi: 10.1212/WNL.39.9.1159

Mueller, S. G., Weiner, M. W., Thal, L. J., Petersen, R. C., Jack, C. R., Jagust, W., et al. (2005). Ways toward an early diagnosis in Alzheimer's disease: the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Alzheimers Dement.* 1, 55–66. doi: 10.1016/j.jalz.2005.06.003

Petersen, R. C. (2004). Mild cognitive impairment as a diagnostic entity. *J. Intern. Med.* 256, 183–194. doi: 10.1111/j.1365-2796.2004.01388.x

Petersen, R. C., Aisen, P. S., Beckett, L. A., Donohue, M. C., Gamst, A. C., Harvey, D. J., et al. (2010). Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. *Neurology* 74, 201–209. doi: 10.1212/WNL.0b013e3181cb3e25

Petersen, R. C., Stevens, J. C., Ganguli, M., Tangalos, E. G., Cummings, J. L., and DeKosky, S. T. (2001). Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 56, 1133–1142. doi: 10.1212/WNL.56.9.1133

Rabin, L. A., Paré, N., Saykin, A. J., Brown, M. J., Wishart, H. A., Flashman, L. A., et al. (2009). Differential memory test sensitivity for diagnosing amnestic mild cognitive impairment and predicting conversion to Alzheimer's disease. *Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn.* 16, 357–376. doi: 10.1080/13825580902825220

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

Rey, A. (1941). Psychological examination of traumatic encephalopathy. *Arch. Psychol.* 28, 286–340.

Rey, A. (1964). *L'examen Clinique En Psychologie*. Paris: Presses Universitaires de France.

Rosen, W. G., Terry, R. D., Fuld, P. A., Katzman, R., and Peck, A. (1980). Pathological verification of ischemic score in differentiation of dementias. *Ann. Neurol.* 7, 486–488. doi: 10.1002/ana.410070516

Russo, M. J., Cohen, G., Campos, J., Martin, M. E., Clarens, M. F., Sabe, L., et al. (2016). Usefulness of discriminability and response bias indices for the evaluation of recognition memory in mild cognitive impairment and Alzheimer disease. *Dement. Geriatr. Cogn. Disord.* 43, 1–14. doi: 10.1159/000452255

Shaw, L. M., Vanderstichele, H., Knapik-Czajka, M., Clark, C. M., Aisen, P. S., Petersen, R. C., et al. (2009). Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann. Neurol.* 65, 403–413. doi: 10.1002/ana.21610

Sheikh, J., and Yesavage, J. (1986). "Recent evidence and development of a shorter version," in *Clinical Gerontology: A Guide to Assessment and Intervention,* ed T. L. Brink (New York, NY: The Haworth Press), 165–173.

Snodgrass, J. G., and Corwin, J. (1988). Pragmatics of measuring recognition memory: applications to dementia and amnesia. *J. Exp. Psychol. Gen.* 117, 34–50. doi: 10.1037/0096-3445.117.1.34

Tabert, M. H., Manly, J. J., Liu, X., Pelton, G. H., Rosenblum, S., Jacobs, M., et al. (2006). Neuropsychological prediction of conversion to Alzheimer disease in patients with mild cognitive impairment. *Arch. Gen. Psychiatry* 63, 916–924. doi: 10.1001/archpsyc.63.8.916

Vemuri, P., Wiste, H. J., Weigand, S. D., Shaw, L. M., Trojanowski, J. Q., Weiner, M. W., et al. (2009). MRI and CSF biomarkers in normal, MCI, and AD subjects: diagnostic discrimination and cognitive correlations. *Neurology* 73, 287–293. doi: 10.1212/wnl.0b013e3181af79e5

Wechsler, D. (1987). *WMS-R Wechsler Memory Scale - Revised Manual*. New York; NY: The Psychological Corporation, Harcourt Brace Jovanovich, Inc.

Wechsler, D. (1997). *Wechsler Memory Scale, 3rd Edn*. San Antonio, TX: The Psychological Corporation.

Weintraub, S., Wicklund, A. H., and Salmon, D. P. (2012). The neuropsychological profile of Alzheimer disease. *Cold Spring Harb. Perspect. Med.* 2:a006171. doi: 10.1101/cshperspect.a006171

Welsh, K. A., Butters, N., Hughes, J. P., Mohs, R. C., and Heyman, A. (1992). Detection and staging of dementia in Alzheimer's disease. Use of the neuropsychological measures developed for the consortium to establish a registry for Alzheimer's Disease. *Arch. Neurol.* 49, 448–452. doi: 10.1001/archneur.1992.00530290030008

Yang, H. D., Yang, Y. S., Legesse, B. T., and Kim, S. Y. (2012). Subtypes of amnestic mild cognitive impairment based on memory impairment pattern and its potential clinical significance. *Dement. Neurocognitive Disord.* 11, 59–66. doi: 10.12779/dnd.2012.11.2.59

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: 13 September 2016
doi: 10.3389/fnagi.2016.00218

Whole-Brain Atrophy Differences between Progressive Supranuclear Palsy and Idiopathic Parkinson's Disease

Carlos Guevara[1], Katherina Bulatova[1], Gareth J. Barker[2], Guido Gonzalez[1], Nicolas A. Crossley[2] and Matthew J. Kempton[2]*

[1] Facultad de Medicina, Universidad de Chile, Santiago, Chile, [2] Department of Neuroimaging, Institute of Psychiatry, King's College London, London, UK

Background: The absence of markers for ante-mortem diagnosis of progressive supranuclear palsy (PSP), results in this disorder being commonly mistaken for other conditions, such as idiopathic Parkinson's disease (IPD). Such mistakes occur particularly in the initial stages, when "plus syndrome" has not yet clinically emerged.

Objective: To investigate the global brain volume and tissue loss in patients with PSP relative to patients with IPD and healthy controls and correlations between clinical parameters and magnetic resonance imaging (MRI)-derived brain volume estimates.

Methods: T1-weighted images were obtained from three groups of Chilean Latin American adults: 21 patients with IPD, 18 patients with PSP and 14 healthy controls. We used Structural Imaging Evaluation with Normalization of Atrophy (SIENAX) to assess white matter, gray matter and whole-brain volumes (normalized to cranial volume). Imaging data were used to analyze putative correlations with the clinical status of PSP and IPD patients using the Unified Parkinson's Disease Rating Scale Part III (UPDRS III), Hoehn and Yahr (H&Y), the Clinical Global Impression for Disease Severity Scale (CGI-S) and the Frontal Assessment Battery (FAB).

Results: PSP patients had significantly lower whole brain volume than both IPD patients and controls. Whole brain volume reduction in PSP patients was primarily attributable to gray matter volume reduction. We found a significant correlation between brain volume reduction and clinical status in the PSP group.

Conclusions: At the group level, the whole brain and gray matter volumes differentiated patients with PSP from patients with IPD. There was also significant clinical-imaging correlations with motor disturbances in PSP.

Keywords: progressive supranuclear palsy, idiopathic Parkinson's disease, whole brain atrophy state, SIENA, SIENAX

OPEN ACCESS

Edited by:
Patricia Cogram, Fraunhofer Research, Chile

Reviewed by:
Federica Agosta, Vita-Salute San Raffaele University, Italy
Josselin Houenou, Institut National de la Santé et de la Recherche Médicale, France

****Correspondence:***
Carlos Guevara
neurocrs@hotmail.com

Received: *10 December 2015*
Accepted: *02 September 2016*
Published: *13 September 2016*

Citation:
Guevara C, Bulatova K, Barker GJ, Gonzalez G, Crossley NA and Kempton MJ (2016) Whole-Brain Atrophy Differences between Progressive Supranuclear Palsy and Idiopathic Parkinson's Disease. Front. Aging Neurosci. 8:218. doi: 10.3389/fnagi.2016.00218

INTRODUCTION

Progressive supranuclear palsy (PSP) is the second most common neurodegenerative movement disorder after idiopathic Parkinson's disease (IPD). PSP is a debilitating disease characterized by early postural instability, ophthalmoplegia, pseudobulbar palsy, dysarthria, axial rigidity, frontal lobe dysfunction and dementia. PSP exhibits inexorable

progression, with a median survival time of between 5 and 10 years (Bower et al., 1997). No disease-modifying treatments have been developed since Steele et al. (1964) described PSP in 1963. The definitive diagnosis of PSP is based on the presence of intracellular deposits of neurofibrillary tangle inclusions composed of abnormally phosphorylated microtubules associated with protein-tau (Verny et al., 1996). It is suspected that abnormal inclusions are causally related to cell death because widespread neuronal loss is generally observed in those areas with tau pathology (Williams et al., 2007).

In contrast, IPD is a synucleinopathy restricted to the substantia nigra and other subcortical brain nuclei, and it is characterized by neural depletion, replacement gliosis and intraneural formation of Lewy bodies. The main clinical manifestations are resting tremor, rigidity, impaired postural reflexes and sustained response to levodopa (L-DOPA) treatment.

Despite these differences, both disorders share some common clinical features, such as akinetic rigidity, making the diagnosis, which is initially based on clinical presentation only, rather difficult, especially in early stages. For the approach to the diagnosis and differential diagnosis of parkinsonian syndromes no quantitative magnetic resonance imaging (qMRI) technique is specifically recommended for routine use in clinical practice (Politis, 2014).

In vivo qMRI studies have largely used voxel based morphometry (VBM) to study tissue loss independently of prior (regional) assumptions throughout brains. A recent meta-analysis of gray matter loss in IPD suggests that these patients have reduced volume in the inferior frontal gyrus and precentral gyrus (Shao et al., 2014), but another meta-analysis of gray matter loss reports that IPD does not show any significant distinguished area of atrophy (Yu et al., 2015). By contrary, in PSP, qMRI studies have been more consistent to reflect the ongoing neuronal loss in the lateral orbitofrontal and dorsolateral prefrontal cortices (Brenneis et al., 2004; Cordato et al., 2005; Padovani et al., 2006) and in the thalamus and caudate nucleus (Price et al., 2004; Boxer et al., 2006). Some VBM studies have reported being able to differentiate PSP from IPD at the group level (Price et al., 2004). However, there is no clinical application for VBM. This technique only allows between groups analyses or within group correlations to explore tissue loss independently of prior assumptions throughout the brains.

Here, we are interested in a qMRI technique suitable to be applied at the individual level with a potential clinical utility in the differential diagnosis in parkinsonian syndromes. In this study, we have chosen the Structural Imaging Evaluation with Normalization of Atrophy (SIENAX). SIENAX is an MRI-based algorithm that quantifies loss of brain tissue volume by normalizing the brain volume to the cranial volume (a proxy for premorbid brain volume). The method is fully automated with 0.5–1% brain volume accuracy for single time point (cross-sectional) designs (Smith et al., 2001, 2004). SIENA will be used in patients with IPD and PSP and in healthy controls. Our hypothesis is that IPD at early stages may not have atrophy outside the substantia nigra and that PSP does have widespread tissue loss. Thus global measurement of brain volume and tissue type-specific volumes (i.e., gray and white matter volumes) could be a useful tool to improve the differentiation between IPD and PSP patients.

MATERIALS AND METHODS

This project was approved by the local Research Ethics Committees of San Juan de Dios Hospital, Santiago, Chile. Written informed consent was provided by all of the subjects prior to participation in the study.

Subjects and Clinical Assessments

Three groups of Chilean Latin American adults, including PSP patients ($n = 18$), IPD patients ($n = 21$) and healthy controls ($n = 14$), were selected. The patients were recruited from the Movement Disorders Clinic at Hospital San Juan de Dios, Santiago, Chile. Internationally established operational criteria were used to assess the diagnoses of PSP and IPD (Hughes et al., 1992; Litvan et al., 2003). The clinical characteristics and demographics of each group are shown in **Table 1**. Every patient was examined by the same clinician (CG) within 1 week of the MRI scan acquisition. None of the patient was treated with cognition enhancing agents. Fourteen IPD patients had the tremor dominant phenotype and seven had the postural instability gait disorder phenotype. All IPD subjects were treated with dopaminergic medication and were examined first on one morning in their "best on" state. Of the 18 PSP patients, 16 had the typical features of classic PSP (Richardson's syndrome) and two had an atypical profile with tremor and moderate L-DOPA responsiveness (PSP-Parkinsonism variant).

Clinical assessments of both disease groups included the following instruments:

- Hoehn and Yahr Scale (H&Y). This scale was originally designed for the assessment of the general severity of IPD patients. However, it is also widely used for PSP. This simple tool estimates both the transition from unilateral to bilateral motor involvement and the impairment of balance and gait (Hoehn and Yahr, 2001).
- Unified Parkinson's disease Rating Scale Part III (UPDRS III). This scale is also commonly used in PSP evaluations. Factor analysis has revealed that some of the items of the UPDRS III can be applied in assessing both conditions regarding axial and limb bradykinesia, tremor and rigidity, and face and speech disturbances (Cubo et al., 2000).
- Clinical Global Impression for Disease Severity (CGI-S). This is a short scale that relies upon the clinician's appraisal of the severity of the disorder. It rates patient's status on a 1–7 scale, where 1 = "normal, not at all ill", and 7 = "extremely ill". It has been suggested that this scale is particularly influenced by motor signs, general disability and cognitive impairment in IPD (Martínez-Martín et al., 2006).
- The Frontal Assessment Battery (FAB). This scale was designed to detect the dysexecutive syndrome at the bedside of

TABLE 1 | Demographics, clinical and volumetric data in idiopathic Parkinson's disease (IPD), progressive supranuclear palsy (PSP) and control groups.

	IPD group $n = 21$	PSP group $n = 18$	Control group $n = 14$	Group comparisons	Signifcant pair wise comparisons
Age at examination (years ± SD)	62.6 ± 11.1	67.7 ± 9.0	65.2 ± 6.4	$F = 1.9$; df = 2; $p = 0.14$	
Gender Female (*n*): Male (*n*)	12:9	9:9	9:5	$\chi^2 = 0.65$; df = 1; $p = 0.71$	
Disease duration (years ± SD)	3.3 ± 3.5	2.8 ± 2.1	–	$p = 0.26$; $t = 0.54$; df = 37; $F = 5.36$	
UPDRS III (mean ± SD)	23.0 ± 12.0	44.0 ± 16.0	–	$p < 0.001$; $z = -3.75$	
H & Y (mean ± SD)	1.9 ± 0.7	2.8 ± 0.9	–	$p < 0.001$; $z = -3.25$	
CGI-S (mean ± SD)	3.5 ± 0.7	4.5 ± 0.7	–	$p < 0.001$; $z = -3.64$	
FAB (mean ± SD)	14.5 ± 3.3	10.8 ± 4.8	–	$p = 0.015$; $z = -2.42$	
Normalized brain volumes					
Whole brain volume (ml ± SD)	1547.0 ± 67.0	1470.0 ± 104.0	1548.0 ± 76.0	$F = 5.08$; df = 2; $p = 0.01$	PSP vs.Controls = 0.031 PSP vs. IPD = 0.016
Gray matter volume (ml ± SD)	775.0 ± 41.0	723.0 ± 85.0	757.0 ± 41.0	$F = 5.5$; df = 2; $p = 0.007$	PSP vs. Controls = 0.01 PSP vs. IPD = 0.031
White matter volume (ml ± SD)	772.0 ± 40.0	731.0 ± 79.0	757.0 ± 40.0	$F = 2.6$; df = 2; $p = 0.08$	

patients with extrapyramidal disorders, including PSP (Dubois et al., 2000). It consists of six sub-tests intended to explore individual executive functions: conceptualization, mental flexibility, motor programming, sensitivity to interference of inhibitory control and environmental autonomy. The scale has a maximum of 18 points, with higher scores indicating better performance.

MRI Acquisition

MRI images were acquired on a 3.0 T Philips Medical System. Axial T1-weighted images, covering the whole brain, were obtained using a 3D inversion recovery prepared spoiled gradient echo (IR-SPGR) sequence. The following parameters were used: repetition time (TR) of 8.1 ms; echo time (TE) of 3.7 ms; inversion time (TI) of 450 ms; voxel size of 0.699 × 0.699 × 1 mm; excitation flip angle of 8°; matrix size of 248 × 226; field of view (FOV) of 24 cm; 198 axial 1-mm slices. MRI scans of every patient were assessed by an experienced neuroradiologist (GG) to exclude gross anatomical abnormalities.

Imaging Processing

All of the data were made anonymous by removing any references to the patient's name from the image headers and replacing these data with a unique ID. Whole brain volume and brain tissue volumes were estimated using SIENAX (Smith et al., 2001, 2002, 2004). Briefly, SIENAX extracts brain and skull images from the acquired MRI data. The brain image is then affine-registered to Montreal Neurological Institute (MNI) 152 space, using the skull image to determine the registration scaling. The registration scaling is then used to obtain a volumetric scaling factor, which is employed to normalize tissue volume estimates. Segmentation with partial volume estimation is subsequently performed to calculate total volume of brain tissue, including separate estimates of volumes of gray matter and white matter (Smith et al., 2004).

Statistical Analyses

Analyses of the clinical data and clinical-imaging correlations were performed using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL, USA, version 22). The results are presented as the mean ± standard deviation (SD). In all cases, a two-sided p value of <0.05 was considered as significant. Visual inspection of the data, using histograms and QQ-plots, was undertaken to check for violations of the assumption of normal distribution. Levene's test of equal variances was used to verify the assumption of homogeneity of variances. Based on these assessments, parametric or non-parametric statistical tests were then used, as appropriate. Disease duration was compared using a two-tailed t-test. Disease severity and cognitive estimations, which were not distributed normally, were assessed using the Mann-Whitney test. The chi-square test for homogeneity was used to compare the distribution of men and women across groups. The associations between MRI-derived measurements and clinical scores were assessed with bivariate correlations. One way analysis of variance (ANOVA) was performed for normally distributed data (age at examination and MRI-derived measures). Tukey's test was used to control for multiple comparisons.

RESULTS

Demographics, Clinical and MRI Variables

The PSP and IPD patients exhibited clinical features typical for their respective diagnoses (**Table 1**). All 18 PSP cases had progressive symmetric parkinsonism accompanied by postural instability, and 14 had supranuclear ophthalmoplegia. All 21 IPD patients had L-DOPA-responsive akinetic-rigid syndrome. There were no significant differences in age or sex between the groups. The IPD patients had longer disease durations than the PSP patients, while the PSP patients showed greater impairment in the UPDRS III, H&Y, CGI-S and FAB assessments.

Normalized Brain Tissue Volumes (Table 1, Figure 1)

The mean whole-brain volume in PSP was significantly lower, compared to the IPD group ($p = 0.016$). The gray matter volume in PSP was also significantly lower than in IPD ($p = 0.031$). The white matter volume did not differ among the study groups. The IPD patients did not differ from the controls in any of these parameters.

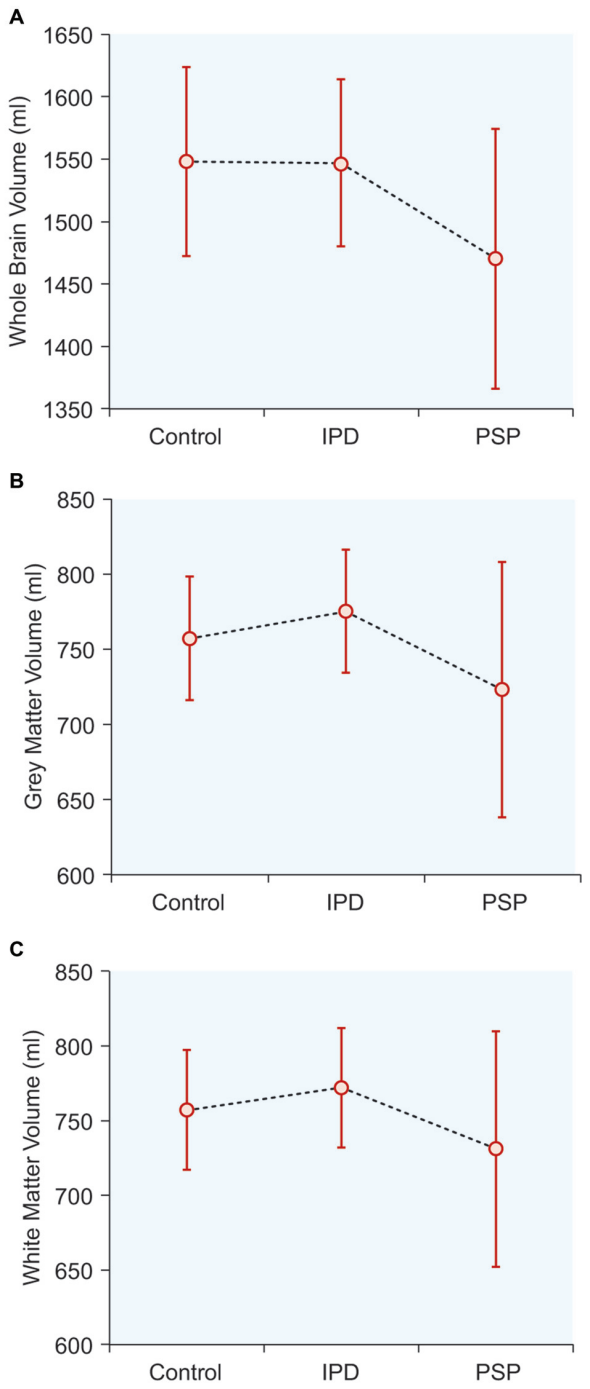


FIGURE 1 | Normalized brain tissue volumes in controls, idiopathic Parkinson's disease (IPD) and progressive supranuclear palsy (PSP): (A) Whole brain volume; (B) Gray matter volume; (C) White matter volume.

Exploratory Correlations Between Clinical Evaluations and Brain Tissue Volumes (Table 2, Figure 2)

Large ($r > 0.5$) and significant clinical-imaging associations were identified in PSP between the whole-brain volume and the UPDRS III ($r = -0.645$; $p = 0.004$), H&Y ($r = -0.660$; $p = 0.002$) and CGI-S scales ($r = -0.650$; $p = 0.003$). Additionally, the PSP patients exhibited significant correlations between gray matter volume and UPDRS III ($r = -0.510$; $p = 0.030$), H&Y ($r = -0.660$; $p = 0.003$), and CGI-S scores ($r = -0.610$; $p = 0.007$). In PSP, white matter volume was correlated with the UPDRS III ($r = -0.55$; $p = 0.017$) and CGI-S ($r = -0.65$; $p = 0.003$).

No significant correlations were found between whole-brain, gray and white matter volumes with clinical parameters in IPD (**Table 2**). In both disease groups, neither disease duration nor FAB was correlated with any MRI-derived measurements.

DISCUSSION

The aim of this study was to explore global brain volume loss in patients with PSP compared to patients with IPD and healthy controls and to evaluate the correlations between clinical parameters and MRI-derived brain volume estimation. Our results showed that patients with PSP had reduced total brain volumes, compared to both IPD patients and controls. Volume

TABLE 2 | Correlations between normalized brain volumes and clinical scores in PSP and IPD.

Group	PSP		IPD	
	r	*p*	*R*	*p*
Gray matter				
UPDRS III[a]	**−0.510**	**0.030**	−0.39	0.08
H & Y[b]	**−0.660**	**0.003**	−0.30	0.17
CGI-S[c]	**−0.610**	**0.007**	−0.18	0.41
FAB[d]	0.480	0.090	0.27	0.23
Disease duration	−0.340	0.100	0.12	0.57
White matter				
UPDRS III[a]	**−0.550**	**0.017**	0.14	0.54
H & Y[b]	−0.420	0.080	0.11	0.62
CGI-S[c]	**−0.650**	**0.030**	−0.20	0.91
FAB[d]	0.360	0.200	0.14	0.54
Disease duration	0.060	0.800	0.11	0.60
Whole brain volume				
UPDRS III[a]	**−0.645**	**0.004**	−0.12	0.60
H & Y[b]	**−0.660**	**0.002**	−0.12	0.60
CGI-S[c]	**−0.650**	**0.003**	−0.13	0.57
FAB[d]	0.480	0.080	0.39	0.08
Disease duration	−0.290	0.240	0.14	0.53

Bold text indicates significant correlations. [a]Unified Parkinson's Disease Rating Scale Part III; [b]Hoehn & Yahr Scale; [c]Clinical Global Impression for Disease Severity; [d]The Frontal Assessment Battery.

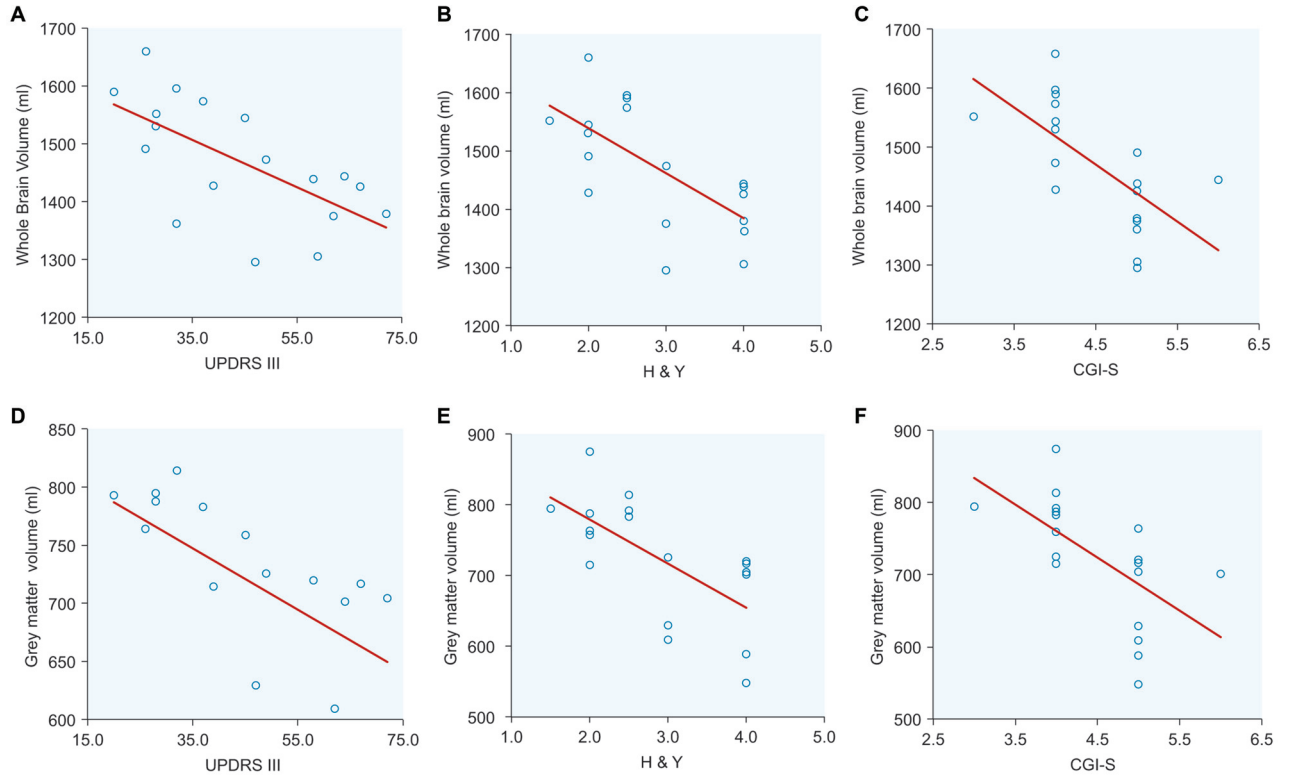


FIGURE 2 | Correlations between normalized whole brain and gray matter volumes with clinical scores in PSP patients: (A) Whole brain volume and Unified Parkinsons Disease Rating Scale Part III (UPDRS III); (B) Whole brain volume and Hoehn and Yahr (H&Y); (C) Whole brain volume and Clinical Global Impression for Disease Severity (CGI-S); (D) Gray matter volume and UPDRS III; (E) Gray matter volume and H&Y; (F) Gray matter volume and CGI-S.

loss was mainly due to reductions in gray matter. Furthermore, these changes were significantly related to the clinical findings in PSP. These results suggested that whole-brain quantitative MRI studies could be helpful in differentiating these patients.

Patients with IPD did not show global-brain atrophy. This could be due to a genuine lack of neuronal loss in the early and middle stage, or it could simply reflect that the magnitude of atrophy is not detectable when quantified *in vivo* using this qMRI technique. Furthermore, neither whole brain volume nor gray matter volume showed any association with clinical deterioration in the IPD group in our study. There have been two previous works using SIENAX in IPD patients, and our results are consistent with these studies as no brain volume changes were found when compared with healthy controls (Tessa et al., 2008; Dalaker et al., 2009). Thus, the mean global brain volume in IPD supports the idea that motor deficits in L-DOPA-responsive IPD patients are related predominantly to localized loss of selective dopaminergic neurons in the substantia nigra.

Conversely, the results in the PSP group are according to the pathological data and with previous studies using qMRI techniques. Gray matter and whole-brain volume loss in PSP were correlated with motor disability, as quantified using the UPDRS III, H&Y and CGI-S. Previous studies have reported that UPSRS III score was correlated with atrophy in the caudate and motor cingulate cortices (Cordato et al., 2005). Our qMRI findings were also consistent with studies in PSP patients in which degeneration in both subcortical and cortical sites was correlated with clinical disability (Cordato et al., 2005; Padovani et al., 2006; Tessa et al., 2008; Lagarde et al., 2013).

Patients with PSP scored lower on the FAB test. However, FAB scores were not significantly correlated with gray matter loss. Previous studies have found correlations between FAB scores and localized changes in the frontal lobe, orbitofrontal cortex, midbrain and cerebellum (Cordato et al., 2005; Giordano et al., 2013). Others have found no significant correlations between localized gray matter reductions and cognitive measurements suggesting that reductions are more related to global cortical reductions. Using VBM, Lagarde et al. (2013) recently reported that PSP patients, compared to healthy controls, showed significantly lower gray matter volumes in the left inferior temporal gyrus, right precentral gyrus, right central gyrus, middle temporal gyrus and the anterior nucleus of the right thalamus. However, none of these areas showed a correlation with FAB or with other neuropsychological evaluations, such as

the Mattis Dementia Rating Scale (MDRS) and the Mini-Mental State Examination (MMSE). These authors suggested that widespread atrophy of subcortical and cortical gray matter might have prevented them from detecting significant correlations.

In relation with other qMRI techniques, SIENAX estimates global tissue measures and is suitable to be applied at the individual level. This may provide a *potential* utility on the clinical ground, but it is not suitable for detecting localized gray matter atrophy. Regional-specific method (e.g., VBM) have different purposes. VBM has largely been used in an unbiased fashion in these disorders and many sites of neuronal vulnerability have been reported in IPD and PSP. These findings are useful for generating biological hypotheses or suggesting regions of interest for clinical and radiological works.

We think that global tissue-specific measures (e.g., SIENAX) and regional-specific method (f.e VBM) are complementary approaches in neurodegeneration.

A problem with using brain volume as a disease outcome is that it may not reflect physiologic or synaptic health. Furthermore, we do not know if the loss of brain volume might be influenced by causes that are common in people with chronic brain disorders, but only indirectly related to the disease itself, such as minor head trauma, nutritional deficiency or dehydration. Although, these sources of variance are certainly less than those for the clinical measure. Given the actual state of the art in neuroimaging, SIENAX may be among the simplest MRI tools, but complex methodologies do not necessarily lead to robust and coherent results. SIENAX offers several advantages over other quantitative MRI techniques, including high reproducibility of results and the capability to provide a robust measurement of the global changes associated with disease conditions. Moreover, a particular advantage of SIENAX is its relative insensitivity to differences in scanning parameters, making this tool suitable for multicenter studies (Smith et al., 2004).

Although we presented a relatively small number of patients in each group, our findings suggested that SIENAX can be used to quantify *in vivo* brain volume loss in IPD and PSP at the group level. Before the technique can be used diagnostically; however, a greater number of patients and longer prospective follow-up are needed to establish discriminatory cut-off points of these measurements and to estimate sensitivity, specificity and positive predictive values.

KEY CONCEPTS

- SIENAX is an MRI-based algorithm that quantifies brain tissue volume by normalizing the brain volume to the cranial volume. SIENAX extracts the brain and skull images from the acquired MRI data. Segmentation with partial volume estimation is subsequently performed to calculate total volume of brain tissue, including separate estimates of volumes of gray matter and white matter.
- PSP is the second most common neurodegenerative movement disorder after IPD. PSP is a debilitating disease characterized by early postural instability, ophthalmoplegia, pseudobulbar palsy, dysarthria, axial rigidity, frontal lobe dysfunction and dementia. PSP exhibits inexorable progression, with a median survival time of between 5 and 10 years.
- PSP and PD share some common clinical features, such as akinetic rigidity, making the diagnosis rather difficult which is initially based on clinical presentation only, especially in the early stages. Some imaging studies have reported being able to differentiate PSP from IPD, although standard MRI assessment of images is rather insensitive for the estimation of neurodegeneration.
- At the group level, whole brain and gray matter volumes differentiated patients with PSP from patients with IPD.

AUTHOR CONTRIBUTIONS

CG, KB, GJB, GG, NAC, MJK have made substantial contribution to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published; Agreement 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.

ACKNOWLEDGMENTS

This study was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT), a non-profit organization, from the Chilean government, grant 11121212.

REFERENCES

Bower, J. H., Maraganore, D. M., McDonnell, S. K., and Rocca, W. A. (1997). Incidence of progressive supranuclear palsy and multiple system atrophy in Olmsted county, Minnesota, 1976 to 1990. *Neurology* 49, 1284–1288. doi: 10.1212/wnl.49.5.1284

Boxer, A. L., Geschwind, M. D., Belfor, N., Gorno-Tempini, M. L., Schauer, G. F., Miller, B. L., et al. (2006). Patterns of brain atrophy that differentiate corticobasal degeneration syndrome from progressive supranuclear palsy. *Arch. Neurol.* 63, 81–86. doi: 10.1001/archneur.63.1.81

Brenneis, C., Seppi, K., Schocke, M., Benke, T., Wenning, G. K., and Poewe, W. (2004). Voxel based morphometry reveals a distinct pattern of frontal atrophy in progressive supranuclear palsy. *J. Neurol. Neurosurg. Psychiatry* 75, 246–249. doi: 10.1136/jnnp.2003.015297

Cordato, N. J., Duggins, A. J., Halliday, G. M., Morris, J. G., and Pantelis, C. (2005). Clinical deficits correlate with regional cerebral atrophy in progressive supranuclear palsy. *Brain* 128, 1259–1266. doi: 10.1093/brain/awh508

Cubo, E., Stebbins, G. T., Golbe, L. I., Nieves, A., Leurgans, S., Goetz, C. G., et al. (2000). Application of the unified Parkinson's disease rating scale in progressive supranuclear palsy: factor analysis of the motor scale. *Mov. Disord.* 15, 276–279. doi: 10.1002/1531-8257(200003)15:2<276::AID-MDS1010>3.0.CO;2-Q

Dalaker, T. O., Larsen, J. P., Bergsland, N., Beyer, M. K., Alves, G., Dwyer, M. G., et al. (2009). Brain atrophy and white matter hyperintensities in early

Parkinson's disease(a). *Mov. Disord.* 24, 2233–2241. doi: 10.1002/mds.22754

Dubois, B., Slachevsky, A., Litvan, I., and Pillon, B. (2000). The FAB: a frontal assessment battery at bedside. *Neurology* 55, 1621–1626. doi: 10.1212/wnl.55.11.1621

Giordano, A., Tessitore, A., Corbo, D., Cirillo, G., de Micco, R., Russo, A., et al. (2013). Clinical and cognitive correlations of regional gray matter atrophy in progressive supranuclear palsy. *Parkinsonism Relat. Disord.* 19, 590–594. doi: 10.1016/j.parkreldis.2013.02.005

Hoehn, M. M., and Yahr, M. D. (2001). Parkinsonism: onset, progression and mortality 1967. *Neurology* 57, S11–S26. doi: 10.1212/WNL.17.5.427

Hughes, A. J., Daniel, S. E., Kilford, L., and Lees, A. J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J. Neurol. Neurosurg. Psychiatry* 55, 181–184. doi: 10.1136/jnnp.55.3.181

Lagarde, J., Valabrègue, R., Corvol, J. C., Pineau, F., Le Ber, I., Vidailhet, M., et al. (2013). Are frontal cognitive and atrophy patterns different in PSP and bvFTD? A comparative neuropsychological and VBM study. *PLoS One* 8:e80353. doi: 10.1371/journal.pone.0080353

Litvan, I., Bhatia, K. P., Burn, D. J., Goetz, C. G., Lang, A. E., McKeith, I., et al. (2003). Movement disorders society scientific issues committee report: SIC task force appraisal of clinical diagnostic criteria for Parkinsonian disorders. *Mov. Disord.* 18, 467–486. doi: 10.1002/mds.10459

Martínez-Martín, P., Forjaz, M. J., Cubo, E., Frades, B., de Pedro Cuesta, J., and ELEP Project Members. (2006). Global versus factor-related impression of severity in Parkinson's disease: a new clinimetric index (CISI-PD). *Mov. Disord.* 21, 208–214. doi: 10.1002/mds.20697

Padovani, A., Borroni, B., Brambati, S. M., Agosti, C., Broli, M., Alonso, R., et al. (2006). Diffusion tensor imaging and voxel based morphometry study in early progressive supranuclear palsy. *J. Neurol. Neurosurg. Psychiatry* 77, 457–463. doi: 10.1136/jnnp.2005.075713

Politis, M. (2014). Neuroimaging in Parkinson disease: from research setting to clinical practice. *Nat. Rev. Neurol.* 10, 708–722. doi: 10.1038/nrneurol.2014.205

Price, S., Paviour, D., Scahill, R., Stevens, J., Rossor, M., Lees, A., et al. (2004). Voxel-based morphometry detects patterns of atrophy that help differentiate progressive supranuclear palsy and Parkinson's disease. *Neuroimage* 23, 663–669. doi: 10.1016/j.neuroimage.2004.06.013

Shao, N., Yang, J., Li, J., and Shang, H. F. (2014). Voxelwise meta-analysis of gray matter anomalies in progressive supranuclear palsy and Parkinson's disease using anatomic likelihood estimation. *Front. Hum. Neurosci.* 8:63. doi: 10.3389/fnhum.2014.00063

Smith, S. M., De Stefano, N., Jenkinson, M., and Matthews, P. M. (2001). Normalized accurate measurement of longitudinal brain change. *J. Comput. Assist. Tomogr.* 25, 466–475. doi: 10.1097/00004728-200105000-00022

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E., Johansen-Berg, H., et al. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23, S208–S219. doi: 10.1016/j.neuroimage.2004.07.051

Smith, S. M., Zhang, Y., Jenkinson, M., Chen, J., Matthews, P. M., Federico, A., et al. (2002). Accurate, robust and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 17, 479–489. doi: 10.1006/nimg.2002.1040

Steele, J. C., Richardson, J. C., and Olszewski, J. (1964). Progressive supranuclear palsy a heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia. *Arch. Neurol.* 10, 333–359. doi: 10.1001/archneur.1964.00460160003001

Tessa, C., Giannelli, M., Della Nave, R., Lucetti, C., Berti, C., Ginestroni, A., et al. (2008). A whole-brain analysis in *de novo* Parkinson disease. *Am. J. Neuroradiol.* 29, 674–680. doi: 10.3174/ajnr.a0900

Verny, M., Duyckaerts, C., Agid, Y., and Hauw, J. J. (1996). The significance of cortical pathology in progressive supranuclear palsy. Clinico-pathological data in 10 cases. *Brain* 119, 1123–1136. doi: 10.1093/brain/119.4.1123

Williams, D. R., Holton, J. L., Strand, C., Pittman, A., de Silva, R., Lees, A. J., et al. (2007). Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from Richardson's syndrome. *Brain* 130, 1566–1576. doi: 10.1093/brain/awm104

Yu, F., Barron, D. S., Tantiwongkosi, B., and Fox, P. (2015). Patterns of gray matter atrophy in atypical parkinsonism syndromes: a VBM meta-analysis. *Brain Behav.* 5:e00329. doi: 10.1002/brb3.329

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: 20 June 2017
doi: 10.3389/fnagi.2017.00198

Variants in SNCA Gene Are Associated with Parkinson's Disease Risk and Cognitive Symptoms in a Brazilian Sample

*Clarissa L. C. Campêlo[1], Fernanda C. Cagni[1], Diego de Siqueira Figueredo[2], Luiz G. Oliveira Jr.[3], Antônio B. Silva-Neto[3], Priscila T. Macêdo[1], José R. Santos[4], Geison S. Izídio[5], Alessandra M. Ribeiro[6], Tiago G. de Andrade[2,7], Clécio de Oliveira Godeiro Jr.[3] and Regina H. Silva[8]**

[1] Memory Studies Laboratory, Physiology Department, Universidade Federal do Rio Grande do Norte, Natal, Brazil, [2] Molecular Biology and Gene Expression Laboratory, Universidade Federal de Alagoas, Arapiraca, Brazil, [3] Medicine Department, Universidade Federal do Rio Grande do Norte, Natal, Brazil, [4] Bioscience Department, Universidade Federal de Sergipe, Itabaiana, Brazil, [5] Department of Cell Biology, Embryology and Genetics, Universidade Federal de Santa Catarina, Florianópolis, Brazil, [6] Biosciences Department, Universidade Federal de São Paulo, Santos, Brazil, [7] Faculty of Medicine, Universidade Federal de Alagoas, Maceió, Brazil, [8] Behavioral Neuroscience Laboratory, Pharmacology Department, Universidade Federal de São Paulo, São Paulo, Brazil*

OPEN ACCESS

Edited by:
Lucas Sedeño, Institute of Cognitive Neurology (INECO), Argentina

Reviewed by:
Arianna Bellucci, University of Brescia, Italy
Yamile Bocanegra, University of Antioquia, Colombia

***Correspondence:**
Regina H. Silva
reginahsilva@gmail.com

Received: *22 November 2016*
Accepted: *02 June 2017*
Published: *20 June 2017*

Citation:
Campêlo CLC, Cagni FC, de Siqueira Figueredo D, Oliveira LG Jr., Silva-Neto AB, Macêdo PT, Santos JR, Izídio GS, Ribeiro AM, de Andrade TG, de Oliveira Godeiro C Jr. and Silva RH (2017) Variants in SNCA Gene Are Associated with Parkinson's Disease Risk and Cognitive Symptoms in a Brazilian Sample. Front. Aging Neurosci. 9:198. doi: 10.3389/fnagi.2017.00198

Genetic susceptibility contributes to the etiology of sporadic Parkinson's Disease (PD) and worldwide studies have found positive associations of polymorphisms in the alpha-synuclein gene (SNCA) with the risk for PD. However, little is known about the influence of variants of SNCA in individual traits or phenotypical aspects of PD. Further, there is a lack of studies with Latin-American samples. We evaluated the association between SNCA single nucleotide polymorphisms (single nucleotide polymorphisms, SNPs – rs2583988, rs356219, rs2736990, and rs11931074) and PD risk in a Brazilians sample. In addition, we investigated their potential interactions with environmental factors and specific clinical outcomes (motor and cognitive impairments, depression, and anxiety). A total of 105 PD patients and 101 controls participated in the study. Single locus analysis showed that the risk allele of all SNPs were more frequent in PD patients ($p < 0.05$), and the associations of SNPs rs2583988, rs356219, and rs2736990 with increased PD risk were confirmed. Further, the G-rs356219 and C-rs2736990 alleles were associated with early onset PD. T-rs2583988, G-rs356219 and C-2736990 alleles were significantly more frequent in PD patients with cognitive impairments than controls in this condition. In addition, in a logistic regression model, we found an association of cognitive impairment with PD, and the practice of cognitive activity and smoking habits had a protective effect. This study shows for the first time an association of SNCA polymorphism and PD in a South-American sample. In addition, we found an interaction between SNP rs356219 and a specific clinical outcome, i.e., the increased risk for cognitive impairment in PD patients.

Keywords: Parkinson's disease, alpha-synuclein, SNCA gene, polymorphism, cognitive impairment, clinical assessment, Brazil

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease. This condition mainly affects motor function, but also causes non-motor symptoms (Fahn, 2003; Wirdefeldt et al., 2011; Pihlstrøm et al., 2013). Among other neurological sings, the cardinal features of this disorder are bradykinesia, rigidity and resting tremor. In parallel, impairment of executive functions and the presence of apathy, anxiety, and depression are the main neuropsychiatric manifestations in PD patients (Rodriguez-Oroz et al., 2009). The onset of PD is usually after 50 years old, and a sharp increase of the incidence is seen after the age of 60 (1% of the population; Lau and Breteler, 2006). Although PD's etiology remains unclear, the interaction between genetic and environmental substrates has been associated with the development of the disease (Lau and Breteler, 2006; Wirdefeldt et al., 2011). Among those environmental factors, several studies pointed the inverse correlation between cigarette smoking and PD risk (Allam et al., 2004; Li et al., 2015). On the other hand, history of professional pesticide exposure, rural living or well water drinking were reported to increase PD risk (Semchuk et al., 1991; Firestone et al., 2005). In addition, physical activity (Paillard et al., 2015; Shih et al., 2016), cognitive reserve (Hindle et al., 2014, 2015) and caffeine intake (Costa et al., 2010) are suggested as protective factors, but with insufficiently consistent results.

Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNP) in many candidate genes that contribute to PD susceptibility such as microtubule-associated protein tau (MAPT), leucine-rich repeat kinase (LRRK2) and alpha-synuclein (SNCA) (Mata et al., 2011; Satake et al., 2009; Simón-Sánchez et al., 2009; Sharma et al., 2012). The relevance of SNCA variations for PD risk is already well established through linkage and GWAS studies. Moreover, certain polymorphisms of SNCA are among the major risk factors for sporadic PD (Simón-Sánchez et al., 2009) and have been correlated with increased plasmatic levels of alpha-synuclein (Mata et al., 2010).

The presynaptic protein alpha-synuclein is the major component of the Lewy body, which is the pathological hallmark of PD (Spillantini et al., 1997, 1998; Trojanowski and Lee, 1998; Xu et al., 2015). The physiological function of alpha-synuclein implicates molecular mechanisms of dopaminergic neurotransmission such as regulation of oxidative stress, maintenance of synaptic function and neuronal trafficking (Schapira, 2007; Bendor et al., 2013; Eisbach and Outeiro, 2013). The overexpression of alpha-synuclein reduces tyrosine hydroxylase activity and dopamine release (Perez et al., 2002; Ozansoy and Basak, 2012), disrupts microtubule-dependent trafficking (Lee et al., 2006), increases oxidative by complex I mitochondrial dysfunction (Mullin and Schapira, 2013; Wu-Chou et al., 2013) and impairs neurotransmitter storage which leads to cytoplasmic accumulation (Lotharius and Brundin, 2002). Mutant protein can result in increased dopamine intracytoplasmic concentration, which contributes to raise the sensitivity to dopamine toxicity by reactive oxygen species generation (Tabrizi et al., 2000).

Case-control studies in different populations have also found associations between several SNCA polymorphisms and increased risk of PD. For example, the dinucleotide repeat REP1 located in SNCA promoter (SNCA-Rep1) and the 3′ untranslated region (UTR) variants have been broadly investigated (Pals et al., 2004; Hu et al., 2010; Ritz et al., 2012). Variations in these regions may confer susceptibility to PD by altering transcription factor binding sites (Chiba-Falek and Nussbaum, 2001; Chiba-Falek et al., 2003) and generating or destroying microRNA target sites, which in turn modifies gene expression (Wang et al., 2008; Sotiriou et al., 2009; Mccarthy et al., 2011). Several investigations had focused on the association between SNCA SNPs and PD in ethnic groups, most of them performed in Caucasian and Asian populations. Hence, the results may be applicable only for these groups (Han et al., 2015). To our knowledge, no studies have investigated associations of SNCA polymorphisms in South American populations.

Currently, there is much interest in the search for clinical predictors of motor and non-motor symptoms in PD. Notwithstanding, most of the gene association studies are limited to genetic risk factor data. Importantly, the consequences of genetic variability on clinicalphenotypes, as well the interaction between genetic and environmental substrates, are poorly elucidated. Few studies pointed weak or absence of associations with clinical outcomes such as motor impairment (Ritz et al., 2012; Markopoulou et al., 2014), anxiety, or depression (Verbaan et al., 2008; Guo et al., 2014; Chen W. et al., 2015; Cheng et al., 2016), sleep, and autonomic disorders (Verbaan et al., 2008; Chen W. et al., 2015), and cognitive impairments (Verbaan et al., 2008; Guo et al., 2014; Chen W. et al., 2015; Chen Y.P. et al., 2015; Cheng et al., 2016; Wang et al., 2016). Thus, there is little information concerning motor and non-motor symptoms assessment, lifestyle and environmental expositions. Gene-environmental studies investigate whether environmental factors such as smoking habits and coffee consumption could modify genetic associations with PD (Gao et al., 2012; Miyake et al., 2012; Ritz et al., 2012; Trotta et al., 2012). However, studies that investigate possible SNCA polymorphisms associations with specific clinical aspects of PD are inconclusive. Therefore, the elucidation of the genetic contribution to clinical phenotypes remains a challenge. In this respect, the description of genetic predictors and their relationship with other etiological factors and clinical outcomes is determinant to improve the knowledge of pathophysiological pathways and help to target the best therapeutic program.

In the present study, we investigated possible interactions between polymorphisms in the SNCA gene and PD in a Brazilian sample, and examined potential associations between these polymorphisms and environmental factors and specific clinical outcomes.

SUBJECTS AND METHODS

Patients and Controls

The unrelated sample consisted of 105 PD patients and 101 control subjects recruited from Onofre Lopes University

Hospital, in Rio Grande do Norte (Northeastern – Brazil) from June, 2013 to November, 2014. PD was diagnosed by a neurologist according to UK Parkinson's Disease Society Brain Bank Clinical Criteria (Hughes, 2004). Recruitment of control subjects was conducted in the same hospital at other departments than the neurology department. Controls were subjects from the general population without neurological disease and family history of PD. The groups were matched by age and sex. This study was approved by the ethical committee of Onofre Lopes University Hospital (protocol number 04261012.5.1001.5292). All the patients and controls were requested to sign the written informed consent.

Clinical Assessment

Case and control subjects filled out a set of eight questionnaires. Information of demographic variables and medical history, such as sex, age, education level, age at PD onset and disease duration were obtained in the baseline interview. Family history was considered positive until second-degree relatives. A structured questionnaire of environmental factors delivered information about risk (pesticide exposition, living in rural areas and well-water consumption) and protective (smoking habits, coffee intake, physical exercises and cognitive activities) factors. History of smoking was defined on basis of self-report as never vs. ever having smoked at least once a day for at least one year (Miyake et al., 2012). Coffee consumption was assessed on basis of self-report and consumption was defined as more than two coffee cups per day (Trotta et al., 2012). Similarly, self-reported physical exercises or cognitive actives (i.e., reading, crossword puzzles, card games, chess, and others) were considered when carried out at least once a week.

The evaluation of different clinical aspects comprised the application of the following inventories: (1) PD motor symptoms were assessed by Unified Parkinson's Disease Rating Scale (UPDRS I, II, and III) (Fahn and Elton, 1987), Hoehn & Yahr scale (HY) (Hoehn and Yahr, 1967) and Schwab and England Activities Daily Living Scale (SE); (2) emotional status was assessed by Beck Depression Inventory (BDI) (Beck et al., 1988b) with a cut-off score of 10 to detect depression (Tröster et al., 1995) and Beck Anxiety Inventory (BAI) (Beck et al., 1988a) with a cut-off score of 10 to detect anxiety (Julian, 2011). The severities of depression and anxiety were determined, according to the following scores, respectively: moderate (BDI: 19–29 points; BAI: 20–30 points) and severe (BDI: higher than 29 points; BAI: higher than 30 points); (3) Mini Mental State Examination (Folstein et al., 1975) and Frontal Assessment Battery (FAB) (Beato et al., 2007) assessed cognitive functions. Cognitive impairment was defined by the application of the cut- off MMSE scores taking the educational level into consideration: 20 for illiterate, 25 for lower education (1 to 4 years), 27 for middle education (5 to 8 years), and 28 for high education (greater than 8 years) (Brucki et al., 2003). All clinical evaluations were conducted during the "on" state of levodopa treatment.

DNA Extraction and Genotyping

Genomic DNA was extracted from EDTA-containing peripheral blood samples (commercial kit FlexiGene® DNA kit, Qiagen, Germany). Genotyping of SNPs rs2583988 (C > T), rs356219 (A > G), rs2736990 (T > C), and rs11931074 (G > T) in SNCA gene was performed using real-time TaqMan® polymerase chain reaction assay according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, United States). Four samples were lost due to poor quality of DNA. 104 PD patients and 98 controls were successfully genotyped.

Statistical Analysis

Non-parametric data were presented as median [minimum value; maximum value]. Inventories scores were compared between groups with Mann-Whitney and Kruskal-Wallis given the nonparametric nature of the data. X^2 statistics (Fisher exact test) was used to compare categorical data, calculation of frequency significance and odds ratio (OR). Parametric data were presented as mean and standard deviation. *T*-student independent test was used to assess differences in mean age at interview between PD and control groups. Calculations of Hardy–Weinberg equilibrium, linkage disequilibrium (LD), estimation of haplotypes and haplotype frequency were performed by the software Snpstat[1]. Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate risk size for the heterozygotes and homozygotes for the risk alleles by binary logistic regression analysis, adjusted for age and gender. The statistical power calculation was performed using G * Power statistical program. Statistical significance was defined when p-values < 0.05.

RESULTS

Study Population

Two hundred and six subjects participated in the study. In PD group ($n = 105$, 73 men and 32 women) the mean age was 64.42 years (range: 37–89 years) and in control group ($n = 101$, 68 men and 33 women) it was 62.98 years (range: 38–88 years), without significant difference ($U = 4632.00$, $p = 0.260$) (**Table 1**). The majority of PD and control subjects were married, living in urban area and had basic education (35.7% of cases and 35.3% of controls had less than four years of education). There were no differences in percent of subjects in each level of education between groups ($p = 0.486$). Most of PD cases (81.4%) were retired while 49% of controls subjects had a work activity.

PD Group's Profile

The mean age of PD onset was 55.7 ± 11.9 years (range 30–87), with 37 patients (35.2%) classified as early onset (≤50 years). The mean of disease duration was 8.80 ± 5.78 years (range 1–32) and of treatment duration was 5.6 ± 4.5 years (range 0–21). Seventy patients (69.3%) describe their first symptom as involuntary tremors. A positive family history was reported by 45 patients (42.9%). Those cases were categorized as "familial", the remaining subjects as "sporadic" (**Table 1**).

[1]http://bioinfo.iconcologia.net/SNPstats

TABLE 1 | Profile and frequencies of exposition to environmental factors.

	Cases ($n = 105$)	Control ($n = 101$)	Unadjusted OR (95% CI)	p
Mean age at interview (years)[a]	64.42 ± 11.69	62.98 ± 10.04	–	0.259
Mean onset age (years)[a]	55.7 ± 11.9	–	–	–
Mean disease duration	8.80 ± 5.78	–	–	–
Familial history (%)	42	–	–	–
Early onset (%)[b]	35.2	–	–	–
Education (%)				
Illiterate	11.9	17.2	–	0.486
Low[c]	35.7	35.3		
Midlle[d]	15.8	11.1		
Higher[e]	36.6	36.4		
Risk Factors				
Pesticides (%)				
No	77.2	73.0	0.79 (0.42–1.51)	0.511
Yes	22.8	27.0		
Countryside (%)				
No	37.3	40.0	1.12 (0.63–1.97)	0.773
Yes	62.7	60.0		
Well-water consumption				
No	27.5	25.0	0.88 (0.47–1.65)	0.750
Yes	72.5	75.0		
Protective Factors				
Coffee consumption (%)				
No	71.7	81.4	0.58 (0.29–1.27)	0.106
Yes	28.3	18.6		
History of Smoking (%)				
No	55.4	51.5	0.85 (0.49–1.48)	0.671
Yes	11.9	20.2		
Abstinent	32.7	28.3		
Physical Activity (%)				
No	54.4	68.0	1.64 (0.92–2.91)	0.110
Yes	43.6	32.0		
Cognitive Activity (%)				
No	45.5	17.3	**0.25 (0.13–0.48)**	**<0.001**
Yes	54.5	82.7		

[a]*Data presented as mean ± standard deviation. Groups compared by independent sample t-test.*
[b] *≤50 years*
[c]*Less than 4 years of education*
[d]*Between 4 and 8 years of education*
[e] *9 years of education or more*
p-value, odds ratios and confidence intervals (CI) calculated by Fisher's test for frequencies of environmental factors. Chi-square test evaluated frequencies of educational level. Significant p-values are indicated in bold.

Frequencies of exposition to environmental factors are shown in **Table 1**. We found a similar frequency of exposure to risk factors (pesticide contact, living in countryside and well-water consumption) and protective factors (coffee consumption, smoking habits, and physical activities) between groups, without significant associations with PD risk. Although most of the subjects reported living in countryside (62.7% in cases and 60% in controls), there were few reports of pesticide use in agriculture (22.8% in cases and 27% in controls). There was a low frequency of reported coffee consumption (28.3% in cases and 18.6% in controls) and current smokers (11.9% in cases and 20.2% in controls) in our sample. We found a higher frequency of practice of cognitive activities in the control group, and this factor showed a strong protective effect against PD (OR = 0.25; 95% CI = 0.13–0.48).

Clinical Assessment

Performances of PD and control group in the clinical assessment are described in **Table 2**. Median of disease progression measured by Hoehn & Yahr scale was 2.5 [1–5], and 53% of the patients were in stages III to V. Assessment of motor activity measured by UDPRS III indicated a significant motor impairment in PD group [19.0 [0–50] versus 0.0 [0–19]; $U = 181.0$, $p < 0.001$]. PD patients also showed greater difficulty to perform daily living activities (20.0 [3–45 versus 0.0 [0–12]) when evaluated by UDPRS II ($U = 82.5$, $p < 0.001$).

TABLE 2 | Clinical assessment scores of Pakinson's disease (PD) patients and Controls.

	PD	Control	*p*
Hoehn & Yahr	2.5 [1;5]	0.0	–
UPDRS I	1.0 [0;4]	2.0 [0;11]	**0.006**
UPDRS II	20.0 [3;45]	0.0 [0;12]	**<0.001**
UPDRS III	19.0 [0;50]	0.0 [0;19]	**<0.001**
SE	70.0 [10; 90]	100.0 [60;100]	**<0.001**
MMSE score	23.0 [10;30]	25.0 [8.;30]	0.097
Cognitive impairment (%)[a]	61.0	57.9	0.663
MMSE			
Illiterate	21.0 [10;25]	22.0 [8;29]	0.187
Lower	21.0 [11;27]	25.0 [19;30]	**0.003**
Middle	26.0 [12;29]	25.0 [15;30]	0.904
Higher	26.0 [11;30]	25.0 [13;30]	0.667
FAB	11.0 [2;16]	11.0 [2;18]	0.063
Illiterate	6.0 [2;11]	8.0 [4;16]	**0.011**
Lower	7.0 [2;16]	9.0 [4;18]	**0.027**
Middle	13.0 [2;16]	12.0 [2;18]	0.790
Higher	13.0 [2;16]	14.0 [3;18]	0.279
BDI score	14.0 [2;47]	5.0 [0;47]	**<0.001**
Depression (%)[b]	74.7	29.0	**<0.001**
BAI score	16.0 [0;47]	7.0 [0;37]	**<0.001**
Anxiety (%)[c]	72.6	36.0	**<0.001**

PD, Parkinson's Disease; UPDRS, Unified Parkinson's Disease Rating Scale; SE, Schwab & England Activities Daily Living Scale; MMSE, Mini Mental State Examination; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory

[a]*Applied cut-off point for education level to MMSE scores (illiterate: 20, lower education:25, middle education: 27, high education: 28).*

[b,c]*Presence of depression and anxiety after applied cut-off points.*

Data are reported as median, minimum and maximum values; p-value by Mann–Whitney test or chi-square for frequencies. Significant p-values are indicated in bold.

In addition, patients presented a reduction of functional independence (70.0 [10–90] versus 100.0 [60–100]; $U = 296.0$, $p < 0001$).

Total score of MMSE (PD: 23[10-30] versus Control: 25.0 [8–30]) and FAB PD: 11.0 [2–16] versus control: 11.0 [2–18]) (**Table 2**), as well as the presence of cognitive impairment (61% in PD and 57.9% in control group) did not differ between groups [$\chi^2(1) = 0.243$; $p = 0.663$]. However, we observed a significant lower score of MMSE in PD group after the stratification of data by educational levels, which indicates a larger cognitive impairment in the subgroup of PD patients with lower education compared to controls with the same level of education (21.0 [11–27] versus 25.0 [19–30]) ($U = 336.50$, $p = 0.003$). Similar effect of education level was found after the stratification of FAB scores. Regarding emotional evaluation, PD group had significantly higher scores in BDI (11.0 [2–47] versus 5.0 [4–74]) and BAI (16.0 [0–47] versus 7.0 [0–37]) than the control group. Furthermore, levels of depression and anxiety were significantly more severe in the PD group (BDI: 24.2% moderate and 10.5% severe; BAI: 26.3% moderate and 9.5% severe, respectively), which resulted in a higher frequency of depression [74.7% versus 2%; $\chi^2(1) = 40.971$, $p < 0.001$] and anxiety symptoms [72.6% versus 36.0%; $\chi^2(1) = 26.305$, $p < 0.001$] in the PD group.

SNP Assessment

Deviation from Hardy-Weinberg equilibrium was not observed for any of the SNPs. 104 participants in PD group and 98 in control group were genotyped for all SNPs. Linkage disequilibrium (LD) structure of SNCA indicates a significant pairwise value of LD between rs356219 and rs2736990 ($r^2 = 0.888$, $D' = 0.988$, $p < 0.001$). Allele and genotype distributions of SNPs in patients and controls are summarized in **Table 3**.

Single locus analysis of SNPs rs2583988, rs356219, rs2736990, and rs11931074 showed that the risk alleles of each SNP, as well as homozygotes for these alleles, were more frequent in PD patients compared to controls. Logistic regression analysis confirmed a significant association between risk genotypes and PD for rs2583988 (OR = 12.20, 95%IC: 1.52–97.58, $p = 0.018$), rs356219 (OR = 2.94, 95%IC: 1.29–6.67, $p = 0.010$), rs2736990 (OR = 2.65, 95%IC: 1.16–2.60, $p = 0.024$), that remained significant after correction for the covariates age and sex. There was no significant difference in genotype distribution for rs11931074 between patients and controls (OR = 1.55, 95% IC: 0.58–1.14, $p = 0.379$). Statistical power calculation applied to the sample size used in this research indicate a detection of a gene-disease association for SNPs rs2583988, rs356219, rs2736990 with values of OR higher than 2.60, with an accuracy between 68 and 85% under the recessive model.

Age at disease onset was not different between the three genotypes for the SNPs evaluated (data not shown). However, frequency analyses of risk allele in patients with early disease onset (EOPD) indicated a significant higher frequency of G-rs356219 and C-rs2736990 in patients when compared to controls ($p = 0.040$; $p = 0.028$, respectively), with OR indicating an increased risk of 1.82 (95%IC: 1.05–3.14) and 1.88 (95%IC: 1.07–3.29), respectively (**Table 4**).

The frequencies of risk alleles in cases and control groups considering only the subjects that presented cognitive impairment, anxiety and depression by clinical assessment were described in **Table 4**. There were no differences in the frequency of risks alleles between groups considering presence of depression and anxiety. However, the risk alleles T-rs2583988, G-rs356219, and C-2736990 had higher frequencies in patients (31, 67, and 70%, respectively) than controls (16, 47, and 52%) with cognitive impairments (ORs = 2.21 to 2.39). When patients were stratified by genotypes, Kruskal-Wallis test detected no differences in the scores of disease progression (HY stage), daily living activities (UPDRS-II score) or motor assessment (UPDRS-III score) between genotypes in each SNP and assessment (data not shown).

Analyses restricted to patients were performed to investigate whether risk genotypes influenced clinical outcomes (cognitive impairments, anxiety and depression). **Table 5** shows the significant and marginal associations with each SNP in SNCA by binary logistic regression. There was no significant association with motor impairment. Both rs356219 heterozygotes (OR = 4.74, 95% CI: 1.27–17.75, $p < 0.05$) and homozygotes (OR = 5.74, 95% CI: 1.42-23.21, $p < 0.05$) had significantly increased risk for cognitive impairment, while the CT-rs2736990 (OR = 3.87, 95% IC: 0.97–15.36, $p = 0.054$) and CC-rs2736990

TABLE 3 | Genotypic and allelic frequencies of single nucleotide polymorphisms (SNPs) in alpha-synuclein gene (SNCA) gene in PD cases ($n = 104$) and controls ($n = 98$).

Genotype	PD *n*(%)	Control *n*(%)	Unadjusted analysis		Adjusted analysis	
			OR (95% CI)	*p*	OR (95% CI)	*p*
rs2583988						
C/C	55(53)	61 (62)	1.00 (reference)		1.00 (reference)	
C/T	38 (37)	36 (37)	1.17 (0.65–2.09)	0.597	1.19 (0.65–2.15)	0.562
T/T	11 (11)	1 (1)	12.20 (1.52–97.58)	**0.018**	12.35 (1.52–99.88)	**0.018**
C allele	148 (71.1)	158 (80.6)	1.68 (1.06–2.68)	**0.026**		
T allele	60 (28.8)	38 (19.3)				
rs356219						
A/A	16 (15)	26 (27)	1.00 (reference)		1.00 (reference)	
G/A	50 (48)	51 (52)	1.59 (0.76–3.32)	0.214	1.60 (0.76–3.35)	0.209
G/G	38 (37)	21 (21)	2.94 (1.29–6.67)	**0.010**	2.94 (1.29–6.72)	**0.010**
A allele	82 (39.4)	103 (52.5)	1.70 (1.14–2.52)	**0.008**		
G allele	126 (60.5)	93 (47.4)				
rs2736990						
T/T	14 (13)	19 (19)	1.00 (reference)		1.00 (reference)	
T/C	43 (41)	55 (56)	1.06 (0.47–2.35)	0.884	1.18 (0.52–2.66)	0.687
C/C	47 (45)	24 (24)	2.65 (1.13–6.20)	**0.024**	2.73 (1.15–6.48)	**0.022**
T allele	71 (34.1)	93 (47.4)	1.74 (1.16–2.60)	**0.006**		
C allele	137 (65.8)	103 (52.5)				
rs11931074						
G/G	54 (52)	61 (62)	1.00 (reference)		1.00 (reference)	
G/T	39 (38)	29 (30)	1.51 (0.83–2.78)	0.175	1.52 (0.83–2.80)	1.70
T/T	11 (11)	8 (8)	1.55 (0.58–1.14)	0.379	1.56 (0.58–4.18)	0.377
G allele	146 (70.1)	151 (77.0)	1.40 (0.89–2.19)	0.138		
T allele	61 (29.3)	45 (22.9)				

PD, Parkinson's disease; OR, odds ratio; CI, confidence interval; p, significance level.
Significant p-values are indicated in bold.

(OR = 3.84, 95% IC: 0.96–15.30, $p = 0.056$) genotypes presented a trend toward to increased risk of the same outcome. No association of rs2583988 genotypes were found for this outcome. Conversely, TT-rs2583988 genotype significantly reduced the risk of depression (OR = 0.21, 95% CI: 0.04–0.97, $p = 0.046$) and had a marginal significance for reduced risk of anxiety (OR = 0.24, 95% CI: 0.05–1.07, $p = 0.061$). There was no significant association for rs2583988 heterozygote genotype and anxiety or depression. The SNPs rs356219 and rs2736990 had no association with these outcomes.

TABLE 4 | Comparisons of risk allele frequencies between case and control with non-motor clinical outcomes.

Outcomes	rs2583988			rs356219			rs2736990		
	T allele (%)	OR (95%CI)	*p*	G allele (%)	OR (95%CI)	*p*	C allele (%)	OR (95%CI)	*p*
Age at onset									
EOPD	17 (23.0)	1.24 (0.64–2.36)	0.503	46 (62.0)	1.82 (1.05–3.14)	**0.040**	50 (68.0)	1.88 (1.07–3.29)	**0.028**
Controls	38 (19.3)			93 (47.4)			79 (80.6)		
Cognitive impairment									
PD	34 (31.0)	2.39 (1.25–4.58)	**0.010**	72 (67.0)	2.22 (1.29–3.84)	**0.004**	76 (70.0)	2.21(1.26–3.85)	**0.005**
Controls	18 (16.0)			53 (47.0)			58 (52.0)		
Anxiety									
PD	31 (23.0)	1.29 (0.62–2.66)	0.591	81 (60.0)	1.47 (0.82–2.63)	0.235	89 (65.0)	1.63 (0.55–4.85)	0.398
Controls	13 (19.0)			55 (40.0)			38 (54.0)		
Depression									
PD	34 (24.0)	1.31 (0.61–2.81)	0.574	80 (57.0)	1.24 (0.66–2.31)	0.527	89 (64.0)	1.59 (0.88–2.87)	0.132
Controls	11 (20.0)			29 (52.0)			32 (57.0)		

OR, odds ratio; CI, confidence interval; p, significance level; EOPD, early onset Parkinson's disease; PD, Parkinson's disease.

TABLE 5 | Binary logistic regression for risk genotypes predicting cognitive, anxiety, and depression in PD patients.

	B	*SE*	Wald	*df*	OR (95%CI)	*p*
Cognitive impairment						
rs2736990						
CT	1.355	0.703	3.714	1	3.87 (0.97–15.36)	0.054
CC	1.346	0.705	3.641	1	3.84 (0.96–15.30)	0.056
Constant	–2.451	1.409	3.025	1		0.089
rs356219						
GA	1.558	0.673	5.363	1	4.74 (1.27–17.75)	**0.021**
GG	1.748	0.713	6.016	1	5.74 (1.42–23.21)	**0.014**
Constant	–2.668	1.407	3.598	1		0.058
Anxiety						
rs2583988						
CT	–0.490	0.515	0.905	1	0.61 (0.22–1.68)	0.341
TT	–1.427	0.763	3.501	1	0.24 (0.05–1.07)	0.061
Constant	3.364	1.474	5.212	1		0.022
Depression						
rs2583988						
CT	0.106	0.540	0.039	1	1.12 (0.38–3.20)	0.844
TT	–1.520	0.762	3.983	1	0.21 (0.04–0.97)	**0.046**
Constant	0.162	1.363	0.014	1		0.905

B, regression coefficient; SE, standard error; df, degrees of freedom; OR, odds ratio; CI, confidence interval; p, significance level. Significant p-values are indicated in bold. Only significant and marginally significant associations are shown.

The best logistic regression models (with case or control as dependent variable) performed to investigate genotypes, clinical aspects and environmental factors in prediction of disease are represented in **Table 6**. TT-rs2583988 (OR = 16.25, 95%IC: 1.72–152.97, $p = 0.015$) and CC-rs2736990 (OR = 2.37, 95%IC: 1.10–5.03, $p = 0.026$) risk genotypes were associated with increased PD risk. There was no significant improvement in the fit of the model by addition of rs356219 and rs11931074. Higher scores in BDI (OR = 1.16, 95%IC: 1.10–1.22, $p < 0.001$) and presence of cognitive impairment (OR 2.27, 95%IC: 1.03–4.98, $p = 0.040$) were directly associated with increased risk of disease. Conversely, practice of cognitive activity (OR = 0.37, 95%IC: 0.16–0.84, $p = 0.019$) and smoking habits (OR = 0.45, 95%IC: 0.21–0.97, $p = 0.042$) had a protective effect against PD.

Logistic regression analysis revealed an association with the number of risk alleles and the presence of disease (OR = 1.27, 95% CI = 1.06–1.53, $p = 0.009$), indicating a cumulative effect. Finally, haplotype analysis result in two blocks with significant association with PD. **Table 7** shows the estimated frequency of each haplotype for cases and controls when all four markers are considered. Estimated odds ratios and associated 95% CIs indicate that haplotype T-rs2583988 + G-rs356219 + C-rs2736990 + T-rs11931074 had a greater risk for PD (OR = 2.51, 95%IC: 1.37–4.58, $p = 0.003$) than CGCT haplotype (OR = 1.85, 95%IC: 1.12–3.05, $p = 0.017$).

DISCUSSION

The present study investigated the association between four variants of the SNCA gene and clinical outcomes in Parkinson's disease patients compared to controls in a Brazilian sample. The risk alleles and the risk genotypes were significantly more frequent in cases than in controls and the PD risk associations for SNPs rs2583988, rs356219, and rs2736990 were confirmed for the homozygote genotype. Furthermore, rs356219 and rs2736990 demonstrated a similar frequency in cases, corroborating the positive correlation for the pairs rs356219 and rs2736990. Carriers of risk allele T-rs2583988, G-rs356219, and C-rs2736990 were significantly more frequent in cases than in controls with cognitive impairments. Regression analysis suggested associations between risk alleles and clinical outcomes and the contribution of environmental factors for PD risk.

Parkinson's disease is a neurodegenerative, chronic, and progressive disease characterized by the presence of motor (tremor, bradykinesia, rigidity, and postural instability) and non-motor (mood disturbs, cognitive alterations, sleep, and autonomic dysfunctions) symptoms (Lauterbach, 2004; Wirdefeldt et al., 2011). Studies show higher prevalence of cognitive deficits and depression in PD (Reijnders et al., 2009; Kehagia et al., 2010) and there is a clinical association between the two kinds of symptoms (Chagas et al., 2014). Our PD sample presented moderate motor impairment, higher prevalence of anxiety and depression symptoms and a similar frequency of cognitive impairment compared to control group. Cognitive impairment is a usual finding in elderly population (Ward et al., 2012) which can explain the higher frequency of this outcome

TABLE 6 | Binary logistic regression model for SNPs genotypes, clinical variables, and environmental factors predicting PD.

Variables	*B*	*SE*	Wald	*df*	OR (95% CI)	*p*
TT-rs2583988	2.788	1.144	5.942	1	16.25 (1.72 – 152.97)	**0.015**
CC-rs2736990	0.866	0.389	4.955	1	2.37 (1.10 – 5.03)	**0.026**
BDI	0.148	0.027	29.499	1	1.16 (1.10 – 1.22)	**<0.001**
Cognitive Impairment	0.943	0.400	5.558	1	2.27 (1.03 – 4.98)	**0.040**
Cognitive Activity	– 0.983	0.417	5.547	1	0.37 (0.16 – 0.84)	**0.019**
Smoking	– 0.784	0.385	4.147	1	0.45 (0.21 – 0.97)	**0.042**
Constant	– 0.780	1.173	0.0442	1		0.334

B, regression coefficient; SE, standard error; df, degrees of freedom; OR, odds ratio; CI, confidence interval; p, significance level; BDI, Beck Depression Inventory score. Data adjusted for sex and age. Significant p-values are indicated in bold.

in controls, and also highlight a difficulty in identifying typical cognitive alterations of PD. Furthermore, we observed an effect of educational levels on our results. The patients with lower education presented a larger degree of cognitive impairment than controls with this same educational level. In a longitudinal study, Hindle et al. (2015) demonstrated that PD patients with a higher cognitive reserve had a better performance in cognitive tests. In addition, educational experiences are essential to attenuate age-related cognitive decline, and a major protective factor in dementia (Stern, 2009). Similarly, the practice of cognitive activities was associated with decreased risk for PD in our regression model.

Environmental factors have been related with PD in epidemiologic researches (Lau and Breteler, 2006; Wirdefeldt et al., 2011). Toxins as MPTP, herbicide paraquat and pesticide rotenone are selective complex I inhibitor and induce neuronal degeneration demonstrated *in vivo* (Betarbet et al., 2000; Lima et al., 2012) and *in vitro* (Chun et al., 2001; Uversky et al., 2002; Giordano et al., 2012) studies. Studies on the association between environmental toxins and the risk for PD present inconsistent results when rural living, pesticide use and well-water consumption are assessed (Allam et al., 2005; Firestone et al., 2005). In contrast, habits as cigarette smoking (Allam et al., 2004; Li et al., 2015) and caffeine intake (Costa et al., 2010) have been linked to protective effects, even though the mechanisms underlying these protective effects remain to be clarified. In our study, the frequencies of exposure to environmental factors were similar in cases and controls, which prevented significant associations with the disease. However, cognitive activities as reading, playing cards, board games and crossword puzzles were more frequent in controls, which suggests a protective role. To our knowledge, history of cognitive stimulation through leisure cognitive activities as a protective factor to PD has not been previously described in the literature. In this respect, the association of cognitive activities and a possible decreased risk of PD described in the present study is a new finding. However, this conclusion is limited because our protocol did not allow a precise description of type, frequency, intensity, and other aspects of the self-reported cognitive activities. It is worth mention that a recent experimental study showed that environmental stimulation facilitated motor recovery and prevented cognitive impairment in a mice model of PD (Campêlo et al., 2017). Thus, although not conclusive, the present finding encourages investigations regarding the protective role of cognitive activities in PD, possibly by prospective clinical studies.

Despite the consistent importance of environmental factors in PD etiology, most genetic association studies have not incorporated gene-environmental interactions in the researches. The interaction of smoking habits and coffee consumption and SNCA variations have been investigated, but had provided inconsistent results (De Palma et al., 1998; Gao et al., 2012; Miyake et al., 2012; Ritz et al., 2012; Trotta et al., 2012). SNPs rs2583988 and rs356219 had no association with coffee drinking and cigarette smoking when investigated in an Italian sample (Trotta et al., 2012). Similarly, the SNPs rs2736990 and rs11931074 did not demonstrate significant results in a North American study (Gao et al., 2012). However, a study in a Japanese sample reported addictive interactions between SNP rs356219 and smoking for increased risk of PD in subjects with GG- rs356219 genotype who had never smoked (Miyake et al., 2012). One of the biological mechanisms proposed to explain the protective effect of smoking is that nicotine inhibits alpha-synuclein fibrillation and stabilizes soluble oligomeric forms (Hong et al., 2009). In our sample, the logistic regression model including cognitive activities and smoking habits within TT-rs2583988 and CC-rs2736990 genotypes revealed a protective effect for PD. Taking together, these results suggest that nicotine might neutralize the detrimental effect of the risk-associated genotypes of rs2583988 and rs27366990, and a higher cognitive stimulation might be protective against PD in individuals with the risk genotypes.

Genetic data supports the role of alpha-synuclein in the pathogenic process of PD. Duplications and triplications of SNCA and a higher production of alpha-synuclein correlate with disease severity (Chartier-Harlin et al., 2004; Ibáñez et al., 2004). However, these mutations of SNCA are rare and the role of common variants are investigated as modifiers of PD susceptibility. Case-control studies have linked SNCA to sporadic PD susceptibility using SNPs analysis. Two major linkage disequilibrium blocks in SNCA gene had been proposed (Mueller et al., 2005; Myhre et al., 2008): a 5′ block that extends to promoter-enhancer region to exon 4 and a 3′ block that comprises intron, 3′ untranslated region, and the 3′ end region of the gene. Associations between SNPs rs2583988 in 5′ region (Pals et al., 2004; Winkler et al., 2007; Heckman et al., 2012; Trotta et al., 2012), rs2736990 in intron 4 (Mata et al., 2011; Heckman et al., 2012; Miyake et al., 2012; Alieva et al., 2013; Guo et al., 2014; Davila-Ortiz de Montellano et al., 2016), rs356219 (Lazzarini et al., 1994; Mata et al., 2010, 2011; Botta-Orfila et al., 2011; Wider et al., 2011; Trotta et al., 2012; Brockmann et al., 2013; Emelyanov et al., 2013), and rs11931074 (Gao et al., 2012; Wu-Chou et al., 2013) in 3′ end were demonstrated by recent studies. A meta-analysis confirmed the risk association of rs2583988, rs356219, and rs11931074 variants and PD susceptibility, performed in dominant and recessive genetic models (Han et al., 2015).

Of notice, the majority of these studies were performed in Caucasian and Asian populations. Data from Latin American populations are quite recent (Davila-Ortiz de Montellano et al., 2016; García et al., 2016), and show associations for rs3857059, rs356220, rs356203, rs7684318, and rs2736990 variants and

TABLE 7 | Estimated haplotype frequencies for SNPs rs2583988, rs356219, rs2736990, and rs11931074 in SNCA gene.

Haplotype[a]	Frequency in cases (%)	Frequency in controls (%)	OR (95% CI)	*p*
CATG	32.1	45.7	(reference)	–
CGCT	29.3	22.9	1.85 (1.12 – 3.05)	0.017
TGCG	26.3	17.5	2.51 (1.37 – 4.58)	0.003

[a]*Haplotype is defined by rs2583988– rs356219– rs2736990– rs11931074. Adjusted for sex and age.*

OR, odds ratio; CI, confidence interval; p, significance level. p-value uncorrected for multiple testing.

PD in Mexican samples. Further, no prior study investigated these associations in Brazilians, or even in a South American population. Our findings were in agreement with the literature, indicating a higher PD risk for homozygote genotypes in our sample for all SNPs studied, except for rs11931074 that had no significant association with the disease. The Brazilian population is one of the most heterogeneous populations in the world and such ethnical heterogeneity creates a particular background. Variations in our findings in comparison to other studies can be explained by differences in the genetic backgrounds (Dahodwala et al., 2009). Although this feature can be a limitation due to the lack of a straight biological category, several countries in Latin America and worldwide have a multiethnic profile, which reinforces the need of genetic association studies in heterogeneous populations.

Genetic polymorphisms may contribute to specific disease characteristics and play an important role in phenotypic diversity of PD. Age at onset of PD is a predictor of progression and mortality (Wirdefeldt et al., 2011) and has been associated with multiple SNPs in several studies (Rajput et al., 2009; Yu et al., 2010; Botta-Orfila et al., 2012; Brockmann et al., 2013; Pan et al., 2013; Cardo et al., 2014; Huang et al., 2015). We found a significantly higher frequency of the G-rs356219 and C-rs2736990 risk alleles in patients with earlier onset compared to controls. Similarly, case-control studies in Germanic (Brockmann et al., 2013) and Chinese (Pan et al., 2012) samples showed that G-rs356219 and C-rs2736990 alleles significantly contributed to earlier age onset. In contrast, a lack of association was observed in Italians (Trotta et al., 2012) and North Americans (Mata et al., 2011; Heckman et al., 2012), which suggest the participation of other modifying factors in age onset across different populations. Therefore, the contributions of these polymorphisms to age onset remain unclear. We speculate that increased expression of alpha-synuclein protein may result in an early manifestation of PD symptoms.

Studies have investigated the functional effects of the different SNCA SNPs on gene expression in brain tissues and protein levels in blood samples, but the results were inconsistent (Fuchs et al., 2008; Linnertz et al., 2009; Mccarthy et al., 2011; Alieva et al., 2013; Cardo et al., 2014). For example, a study with a transgenic mouse model demonstrated that REP1 variants in 5′ region affect the regulation of transcriptional activity (Cronin et al., 2009). Human studies demonstrated reduction of SNCA-mRNA levels in brain tissues and protein levels in blood in the absence of REP1 risk allele (Fuchs et al., 2008; Linnertz et al., 2009). Despite some evidence of correlations between rs2583988 and REP1 variants (Pals et al., 2004; Winkler et al., 2007; Myhre et al., 2008), no significant associations of rs2583988 were found for assessment of blood protein levels (Fuchs et al., 2008) or SNCA-mRNA (Fuchs et al., 2008; Linnertz et al., 2009; Alieva et al., 2013).

For the intronic SNP rs2736990, an investigation of gene expression revealed a trend toward lower levels of SNCA-mRNA in blood samples of PD patients (Alieva et al., 2013), but in healthy subjects the T allele was correlated with higher levels of the isoform SNCA112-mRNA in frontal cortex tissue samples.

SNPs rs356219 and rs11931074 are part of 3′ block and variants in this region may affect post-transcriptional regulation factors, such as biding sites of mRNA, and impair RNA processing or stability and gene or protein expression (Linnertz et al., 2009; Venda et al., 2010; Elbaz et al., 2011; Mccarthy et al., 2011). For rs356219, the heterozygote genotype was correlated with higher levels of mRNA in substantia nigra of PD cases (Fuchs et al., 2008). Further, G allele carriers presented higher levels of the SNCA112-mRNA isoform in frontal cortex (Mccarthy et al., 2011). However, a divergent result was found by Linnertz et al. (2009) that showed a correlation between higher levels of SNCA-mRNA and the protective allele A.

As mentioned, literature supports that polymorphisms in the SNCA gene increase genetic susceptibility to sporadic PD and suggests an increased expression of alpha-synuclein. Despite disease severity (rapid cognitive decline, severe non-motor symptoms, more widespread neurodegeneration and faster disease progression) is related to increased alpha-synuclein expression in familial PD (Venda et al., 2010), there is a lack of evidence regarding the SNCA variants' influence on specific clinical outcomes. Previous studies that assessed associations between polymorphisms and motor impairment and disease progression (Ritz et al., 2012; Markopoulou et al., 2014; Cheng et al., 2016; Davis et al., 2016; Wang et al., 2016), cognitive functions (Goris et al., 2007; Guo et al., 2014; Markopoulou et al., 2014; Chen W. et al., 2015; Wang et al., 2016) and presence or absence of anxiety and depression (Guo et al., 2014; Chen W. et al., 2015; Cheng et al., 2016; Dan et al., 2016) present poorly consistent results.

In our study, no associations were observed regarding the distribution of risk allele and presence of depression or anxiety. Nevertheless, here we indicate that the TT- rs2583988 genotype has a protective effect against these outcomes in patients. In contrast, a study with a Chinese sample found a decreased risk of depression in carriers of REP1 risk homozygote genotype and a correlation with UPDRS part II score, motor fluctuation and female sex in prediction of PD depression (Dan et al., 2016).

Concerning cognitive aspects, T-rs2583988, C-rs2736990, and G-rs356219 risk alleles were more frequent in cases than controls with cognitive impairment, indicating a greater risk of this outcome in PD. Furthermore, we found an association of cognitive impairment with risk of PD in the logistic regression model. An unexpected found for REP1 genotypes was described in a longitudinal North American study: PD cases with higher REP1 scores were associated with better motor function and reduced risk of cognitive impairments (Markopoulou et al., 2014). These data highlight a possible dual effect or time-dependent role for SNCA variants.

Regarding motor aspects, a cohort study with American patients (Ritz et al., 2012) demonstrated an increased risk of faster decline of motor function in carriers of the REP1 263bp promoter variant and G-rs356165 allele. In our transversal study, we did not found correlations between genotypes and severity of motor symptoms in the patients.

Our study had some limitations. Environmental data were self-reported, which could provide a misclassification. Further, the MMSE used to assess cognitive impairment may not be

the limited number of patients, the power analysis indicated a moderate statistical power. Irrespective, it would be interesting to expand the sample to reinforce the results. Indeed, the few conflicting results may be explained by the small sample size and differences in genetic background among different ethnic populations, as mentioned.

CONCLUSION

This study confirms the association between PD and SNCA SNPs and haplotypes in a Brazilian population. Further, the data provide evidence that the SNCA variants are associated with increased risk of cognitive impairment in PD patients. Therefore, our results encourage the investigation of associations between genetic variants in SNCA and specific clinical outcomes. Phenotypic studies and functional assays, with a large sample and different ethnicities should be performed to confirm and specify the nature of the associations.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Comissão Nacional de Ética em Pesquisa (CONEP), Brazil, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the ethical committee of Onofre Lopes University Hospital (protocol number 04261012.5.1001.5292).

AUTHOR CONTRIBUTIONS

CC and RS designed the research and wrote the paper. CC, FC, LO, AS-N, and PT collected the data. CC, DdS, and TdA conducted molecular assays. CC. analyzed the data. JS, GI, AR, and CdO contributed with theoretical support and data analysis.

FUNDING

The research was supported by fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (grant # 402054/2010-5), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Apoio à Pesquisa do Estado do Rio Grande do Norte (FAPERN/PRONEX) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (grant #2015/12308-5).

ACKNOWLEDGMENT

The authors would like to thank Ywilliane Meurer for helpful suggestions and Valter Andrade Neto for technical support.

REFERENCES

Alieva, A. K., Shadrina, M. I., Filatova, E. V., Ustinova, V. V., Fedotova, E. Y., Karabanov, A., et al. (2013). Polymorphisms in the SNCA gene: association with the risk of development of the sporadic form of Parkinson's disease and the level of SNCA gene expression in peripheral blood of patients from Russia. *Neurosci. Med.* 4, 208–214. doi: 10.4236/nm.2013.44032

Allam, M., Del Castillo, A., and Navajas, R. (2005). Parkinson's disease risk factors: genetic, environmental, or both? *Neurol. Res.* 27, 206–208. doi: 10.1179/016164105x22057

Allam, M. F., Campbell, M. J., Hofman, A., Del Castillo, A. S., and Fernandez-Crehuet Navajas, R. (2004). Smoking and Parkinson's disease: systematic review of prospective studies. *Mov. Disord.* 19, 614–621. doi: 10.1002/mds.20029

Beato, R., Nitrini, R., Formigoni, A., and Caramelli, P. (2007). Brazilian version of the Frontal Assessment Battery (FAB): preliminary data on administration to healthy elderly. *Dement. Neuropsychol.* 1, 59–65.

Beck, A. T., Epstein, N., Brown, G., and Steer, R. A. (1988a). An inventory for measuring clinical anxiety: psychometric properties. *J. Consult. Clin. Psychol.* 56, 893–897. doi: 10.1037/0022-006X.56.6.893

Beck, A. T., Steer, R. A., and Garbin, M. G. (1988b). Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. *Clin. Psychol. Rev.* 8, 77–100. doi: 10.1016/0272-7358(88)90050-5

Bendor, J., Logan, T., and Edwards, R. H. (2013). The function of α-synuclein. *Neuron* 79, 1044–1066. doi: 10.1016/j.neuron.2013.09.004

Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., and Greenamyre, J. T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* 3, 1301–1306. doi: 10.1038/81834

Botta-Orfila, T., Ezquerra, M., Pastor, P., Fernández-Santiago, R., Pont-Sunyer, C., Compta, Y., et al. (2012). Age at onset in LRRK2-associated PD is modified by SNCA variants. *J. Mol. Neurosci.* 48, 245–247. doi: 10.1007/s12031-012-9820-7

Botta-Orfila, T., Ezquerra, M., Ríos, J., Fernández-Santiago, R., Cervantes, S., Samaranch, L., et al. (2011). Lack of interaction of SNCA and MAPT genotypes in Parkinson's disease. *Eur. J. Neurol.* 18:e32. doi: 10.1111/j.1468-1331.2010.03245.x

Brockmann, K., Schulte, C., Hauser, A., Lichtner, P., Huber, H., Maetzler, W., et al. (2013). SNCA: major genetic modifier of age at onset of Parkinson's disease. *Mov. Disord.* 28, 1217–1221. doi: 10.1002/mds.25469

Brucki, S. M. D., Nitrin, R., Caramelli, P., Bertolucci, P. H. F., and Okamoto, I. H. (2003). Sugestões para o uso do mini-exame do estado mental no Brasil. *Arq. Neuropsiquiatr.* 61, 777–781. doi: 10.1590/S0004-282X2003000500014

Campêlo, C. L. C., Santos, J. R., Silva, A. F., Dierschnabel, A. L., Pontes, A., Cavalcante, J. S., et al. (2017). Exposure to an enriched environment facilitates motor recovery and prevents short-term memory impairment and reduction of striatal BDNF in a progressive pharmacological model of parkinsonism in mice. *Behav. Brain Res.* 328, 138–148. doi: 10.1016/j.bbr.2017.04.028

Cardo, L. F., Coto, E., Mena, L., Ribacoba, R., Mata, I. F., Menéndez, M., et al. (2014). Alpha-synuclein transcript isoforms in three different brain regions from Parkinson's disease and healthy subjects in relation to the SNCA rs356165/rs11931074 polymorphisms. *Neurosci. Lett.* 562, 45–49. doi: 10.1016/j.neulet.2014.01.009

Chagas, M. H. N., Moriyama, T. S., Felício, A. C., Sosa, A. L., Bressan, R. A., and Ferri, C. P. (2014). Depression increases in patients with Parkinson' s disease according to the increasing severity of the cognitive impairment. *Arq. Neuropsiquiatr.* 72, 426–429. doi: 10.1590/0004-282X20140049

Chartier-Harlin, M. C., Kachergus, J., Roumier, C., Mouroux, V., and Douay, X. (2004). Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* 364, 1167–1169. doi: 10.1016/S0140-6736(04)17103-1

Chen, W., Kang, W. Y., Chen, S., Wang, Y., Xiao, Q., Wang, G., et al. (2015). Hyposmia correlates with SNCA variant and non-motor symptoms in Chinese patients with Parkinson's disease. *Park. Relat. Disord.* 21, 610–614. doi: 10.1016/j.parkreldis.2015.03.021

Chen, Y. P., Wei, Q. Q., Ou, R. W., Cao, B., Chen, X. P., Zhao, B., et al. (2015). Genetic variants of SNCA are associated with susceptibility to Parkinson's disease but not amyotrophic lateral sclerosis or multiple system atrophy in

a Chinese population. *PLoS ONE* 10:e0133776. doi: 10.1371/journal.pone.0133776

Cheng, L., Wang, L., Li, N. N., Yu, W. J., Sun, X. Y., Li, J. Y., et al. (2016). SNCA rs356182 variant increases risk of sporadic Parkinson's disease in ethnic Chinese. *J. Neurol. Sci.* 368, 231–234. doi: 10.1016/j.jns.2016.07.032

Chiba-Falek, O., and Nussbaum, R. L. (2001). Effect of allelic variation at the NACP-Rep1 repeat upstream of the α-synuclein gene (SNCA) on transcription in a cell culture luciferase reporter system. *Hum. Mol. Genet.* 10, 3101–3109. doi: 10.1093/hmg/10.26.3101

Chiba-Falek, O., Touchman, J. W., and Nussbaum, R. L. (2003). Functional analysis of intra-allelic variation at NACP-Rep1 in the alpha-synuclein gene. *Hum. Genet.* 113, 426–431. doi: 10.1007/s00439-003-1002-9

Chun, H. S., Gibson, G. E., DeGiorgio, L. A., Zhang, H., Kidd, V. J., and Son, J. H. (2001). Dopaminergic cell death induced by MPP^+, oxidant and specific neurotoxicants shares the common molecular mechanism. *J. Neurochem.* 76, 1010–1021. doi: 10.1046/j.1471-4159.2001.00096.x

Costa, J., Lunet, N., Santos, C., Santos, J., and Vaz-Carneiro, A. (2010). Caffeine exposure and the risk of Parkinson's disease: a systematic review and meta-analysis of observational studies. *J. Alzheimers Dis.* 20(Suppl. 1), S221–S238. doi: 10.3233/JAD-2010-091525

Cronin, K. D., Ge, D., Manninger, P., Linnertz, C., Rossoshek, A., Orrison, B. M., et al. (2009). Expansion of the Parkinson disease-associated SNCA-Rep1 allele upregulates human α-synuclein in transgenic mouse brain. *Hum. Mol. Genet.* 18, 3274–3285. doi: 10.1093/hmg/ddp265

Dahodwala, N., Siderowf, A., Xie, M., Noll, E., Stern, M., and Mandell, D. (2009). Racial differences in the diagnosis of Parkinson's disease. *Mov. Disord.* 24, 1200–1205. doi: 10.1002/mds.22557

Dan, X., Wang, C., Zhang, J., Gu, Z., Zhou, Y., Ma, J., et al. (2016). Association between common genetic risk variants and depression in Parkinson's disease: A dPD study in Chinese. *Parkinsonism Relat. Disord.* 33, 122–126. doi: 10.1016/j.parkreldis.2016.09.029

Davila-Ortiz de Montellano, D. J., Rodriguez-Violante, M., Fresan, A., Monroy-Jaramillo, N., and Yescas-Gomez, P. (2016). Frequency of single nucleotide polymorphisms and alpha-synuclein haplotypes associated with sporadic Parkinson's disease in the Mexican population. *Rev. Neurol.* 63, 345–350.

Davis, A. A., Andruska, K. M., Benitez, B. A., Racette, B. A., Perlmutter, J. S., and Cruchaga, C. (2016). Variants in GBA, SNCA, and MAPT influence Parkinson disease risk, age at onset, and progression. *Neurobiol. Aging* 37, 209.e1–209.e7. doi: 10.1016/j.neurobiolaging.2015.09.014

De Palma, G., Mozzoni, P., Mutti, A., Calzetti, S., and Negrotti, A. (1998). Case-control study of interactions between genetic and environmental factors in Parkinson's disease. *Lancet* 352, 19–26. doi: 10.1016/S0140-6736(05)61332-3

Eisbach, S. E., and Outeiro, T. F. (2013). Alpha-Synuclein and intracellular trafficking: impact on the spreading of Parkinson's disease pathology. *J. Mol. Med.* 91, 693–703. doi: 10.1007/s00109-013-1038-9

Elbaz, A., Ross, O. A., Ioannidis, J. P. A., Soto-Ortolaza, A. I., Moisan, F., Aasly, J., et al. (2011). Independent and joint effects of the MAPT and SNCA genes in Parkinson disease. *Ann. Neurol.* 69, 778–792. doi: 10.1002/ana.22321

Emelyanov, A., Andoskin, P., Yakimovskii, A., Usenko, T., Nuzhnyi, E., Nikolaev, M., et al. (2013). SNCA, LRRK2, MAPT polymorphisms and Parkinson's disease in Russia. *Parkinsonism Relat. Disord.* 19, 1064–1065. doi: 10.1016/j.parkreldis.2013.06.003

Fahn, S. (2003). Description of Parkinson's disease as a clinical syndrome. *Ann. N. Y. Acad. Sci.* 991, 1–14. doi: 10.1111/j.1749-6632.2003.tb07458.x

Fahn, S., and Elton, R. (1987). "Members of the UPDRS development committee. the unified Parkinson's disease rating scale," in *Recent Developments in Parkinson's Disease*, eds S. Fahn, C. D. Marsden, D. B. Calne, and M. Goldstein (Florham Park, NJ: Mcmellam Health Care Information), 153–163.

Firestone, J. A., Smith-Weller, T., Franklin, G., Swanson, P., Longstrech, W. T., and Checkoway, H. (2005). Pesticides and risk of Parkinson disease. *Arch. Gerontol. Geriatr.* 62, 91–95. doi: 10.1001/archneur.62.1.91

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

Fuchs, J., Tichopad, A., Golub, Y., Munz, M., Schweitzer, K. J., Wolf, B., et al. (2008). Genetic variability in the SNCA gene influences alpha-synuclein levels in the blood and brain. *FASEB J.* 22, 1327–1334. doi: 10.1096/fj.07-9348com

Gao, J., Nalls, M. A., Shi, M., Joubert, B. R., Hernandez, D. G., Huang, X., et al. (2012). An exploratory analysis on gene-environment interactions for Parkinson disease. *Neurobiol. Aging* 33, 2528.e1–2528.e6. doi: 10.1016/j.neurobiolaging.2012.06.007

García, S., Chavira-Hernández, G., Gallegos-Arreola, M. P., Dávila-Maldonado, L., García Martínez, F., Montes Almanza, L. A., et al. (2016). The rs3857059 variant of the SNCA gene is associated with Parkinson's disease in Mexican Mestizos. *Arq. Neuropsiquiatr.* 74, 445–449. doi: 10.1590/0004-282X20160061

Giordano, S., Lee, J., Darley-Usmar, V. M., and Zhang, J. (2012). Distinct effects of rotenone, 1-methyl-4-phenylpyridinium and 6-hydroxydopamine on cellular bioenergetics and cell death. *PLoS ONE* 7:e44610. doi: 10.1371/journal.pone.0044610

Goris, A., Williams-Gray, C. H., Clark, G. R., Foltynie, T., Lewis, S. J. G., Brown, J., et al. (2007). Tau and α-synuclein in susceptibility to, and dementia in, Parkinson's disease. *Ann. Neurol.* 62, 145–153. doi: 10.1002/ana.21192

Guo, X. Y., Chen, Y. P., Song, W., Zhao, B., Cao, B., Wei, Q. Q., et al. (2014). SNCA variants rs2736990 and rs356220 as risk factors for Parkinson's disease but not for amyotrophic lateral sclerosis and multiple system atrophy in a Chinese population. *Neurobiol. Aging* 35, 2882.e1–2882.e6. doi: 10.1016/j.neurobiolaging.2014.07.014

Han, W., Liu, Y., Mi, Y., Zhao, J., Liu, D., et al. (2015). Alpha-synuclein (SNCA) polymorphisms and susceptibility to Parkinson's disease: a meta-analysis. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 168, 123–134. doi: 10.1002/ajmg.b.32288

Heckman, M. G., Soto-Ortolaza, A. I., Diehl, N. N., Carrasquillo, M. M., Uitti, R. J., Wszolek, Z. K., et al. (2012). Evaluation of the role of SNCA variants in survival without neurological disease. *PLoS ONE* 7:e42877. doi: 10.1371/journal.pone.0042877

Hindle, J. V., Hurt, C. S., Burn, D. J., Brown, R. G., Samuel, M., Wilson, K. C., et al. (2015). The effects of cognitive reserve and lifestyle on cognition and dementia in Parkinson's disease - a longitudinal cohort study. *Int. J. Geriatr. Psychiatry* 31, 13–23. doi: 10.1002/gps.4284

Hindle, J. V., Martyr, A., and Clare, L. (2014). Cognitive reserve in Parkinson's disease: a systematic review and meta-analysis. *Parkinsonism Relat. Disord.* 20, 1–7. doi: 10.1016/j.parkreldis.2013.08.010

Hoehn, M., and Yahr, M. (1967). Parkinsonism: onset, progression, and mortality. *Neurology* 17, 427–442. doi: 10.1212/WNL.17.5.427

Hong, D.-P., Fink, A. L., and Uversky, V. N. (2009). Smoking and Parkinson's disease: Does nicotine affect alpha-synuclein fibrillation? *Biochim. Biophys. Acta* 1794, 282–290. doi: 10.1016/j.bbapap.2008.09.026

Hu, F.-Y., Hu, W.-B., Liu, L., Yu, L.-H., Xi, J., He, X.-H., et al. (2010). Lack of replication of a previously reported association between polymorphism in the 3'UTR of the alpha-synuclein gene and Parkinson's disease in Chinese subjects. *Neurosci. Lett.* 479, 31–33. doi: 10.1016/j.neulet.2010.05.022

Huang, Y., Wang, G., Rowe, D., Wang, Y., Kwok, J. B. J., Xiao, Q., et al. (2015). SNCA gene, but not MAPT, influences onset age of Parkinson's disease in Chinese and Australians. *Biomed. Res. Int.* 2015:135674. doi: 10.1155/2015/135674

Hughes, R. N. (2004). The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neurosci. Biobehav. Rev.* 28, 497–505. doi: 10.1016/j.neubiorev.2004.06.006

Ibáñez, P., Bonnet, A., Débarges, B., Lohmann, E., Tison, F., Pollak, P., et al. (2004). Causal relation between α-synuclein gene duplication and familial Parkinson's disease. *Lancet* 364, 1169–1171. doi: 10.1016/S0140-6736(04)17104-3

Julian, L. J. (2011). Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). *Arthritis Care Res.* 63, S467–S472. doi: 10.1002/acr.20561

Kehagia, A., Barker, R. A., and Robbins, T. W. (2010). Neuropsychological and clinical heterogeneity of cognitive impairment and dementia in patients with Parkinson's disease. *Lancet Neurol.* 9, 1200–1213. doi: 10.1016/S1474-4422(10)70212-X

Lau, L. M. L., and Breteler, M. M. B. (2006). Epidemiology of Parkinson's disease. *Lancet Neurol.* 5, 525–535. doi: 10.1016/S1474-4422(06)70471-9

Lauterbach, E. C. (2004). The neuropsychiatry of Parkinson's disease and related disorders. *Psichatr. Clin. North Am.* 27, 801–825. doi: 10.1016/j.psc.2004.07.001

Lazzarini, A., Myers, R., Zimmerman, T. J., Mark, M., Golbe, L., Sage, J., et al. (1994). A clinical genetic study of Parkinson's disease: evidence for dominant transmission. *Neurology* 44, 499–506. doi: 10.1212/WNL.44.3_Part_1.499

Lee, H.-J., Khoshaghideh, F., Lee, S., and Lee, S.-J. (2006). Impairment of microtubule-dependent trafficking by overexpression of alpha-synuclein. *Eur. J. Neurosci.* 24, 3153–3162. doi: 10.1111/j.1460-9568.2006.05210.x

Li, X., Li, W., Liu, G., Shen, X., and Tang, Y. (2015). Association between cigarette smoking and Parkinson's disease: a meta-analysis. *Arch. Gerontol. Geriatr.* 61, 510–516. doi: 10.1016/j.archger.2015.08.004

Lima, M. M. S., Andersen, M. L., Reksidler, A. B., Ferraz, A. C., Vital, M. A. B. F., and Tufik, S. (2012). Paradoxical sleep deprivation modulates tyrosine hydroxylase expression in the nigrostriatal pathway and attenuates motor deficits induced by dopaminergic depletion. *CNS Neurol. Disord. Drug Targets* 11, 359–368. doi: 10.2174/187152712800792839

Linnertz, C., Saucier, L., Ge, D., Cronin, K. D., Burke, J. R., Jeffrey, N., et al. (2009). Genetic regulation of α-Synuclein mRNA expression in various human brain tissues. *PLoS ONE* 4:e7480. doi: 10.1371/journal.pone.0007480

Lotharius, J., and Brundin, P. (2002). Impaired dopamine storage resulting from alpha-synuclein mutations may contribute to the pathogenesis of Parkinson's disease. *Hum. Mol. Genet.* 11, 2395–2407. doi: 10.1093/hmg/11.20.2395

Markopoulou, K., Biernacka, J. M., Armasu, S. M., Anderson, K. J., Ahlskog, J. E., Chase, B. A., et al. (2014). Does α-synuclein have a dual and opposing effect in preclinical vs. clinical Parkinson's disease? *Parkinsonism Relat. Disord.* 20, 584–589. doi: 10.1016/j.parkreldis.2014.02.021

Mata, I., Shi, M., Agarwal, P., Chung, K., Edwards, K., Factor, S., et al. (2010). SNCA variant associated with Parkinson disease and plasma α-Synuclein level. *Arch. Neurol.* 67, 1350–1356. doi: 10.1001/archneurol.2010.279

Mata, I., Yearout, D., Alvarez, V., Coto, E., de Mena, L., Ribacoba, R., et al. (2011). Replication of MAPT and SNCA, but not PARK16-18, as susceptibility genes for Parkinson's disease. *Mov. Disord.* 26, 819–823. doi: 10.1002/mds.23642

Mccarthy, J. J., Linnertz, C., Saucier, L., Burke, J. R., Hulette, C. M., Welsh-Bohmer, K. A., et al. (2011). The effect of SNCA 3' region on the levels of SNCA-112 splicing variant. *Neurogenetics* 12, 59–64. doi: 10.1007/s10048-010-0263-4.The

Miyake, Y., Tanaka, K., Fukushima, W., Kiyohara, C., Sasaki, S., Tsuboi, Y., et al. (2012). SNCA polymorphisms, smoking, and sporadic Parkinson's disease in Japanese. *Parkinsonism Relat. Disord.* 18, 557–561. doi: 10.1016/j.parkreldis.2012.02.016

Mueller, J. C., Fuchs, J., Hofer, A., Zimprich, A., Lichtner, P., Illig, T., et al. (2005). Multiple regions of α-synuclein are associated with Parkinson's disease. *Ann. Neurol.* 57, 535–541. doi: 10.1002/ana.20438

Mullin, S., and Schapira, A. (2013). α-synuclein and mitochondrial dysfunction in Parkinson's disease. *Mol. Neurobiol.* 47, 587–597. doi: 10.1007/s12035-013-8394-x

Myhre, R., Toft, M., Kachergus, J., Hulihan, M. M., Aasly, J. O., Klungland, H., et al. (2008). Multiple alpha-synuclein gene polymorphisms are associated with Parkinson's disease in a Norwegian population. *Acta Neurol. Scand.* 118, 320–327. doi: 10.1111/j.1600-0404.2008.01019.x

Ozansoy, M., and Basak, A. N. (2012). The central theme of Parkinson's disease: α-synuclein. *Mol. Neurobiol.* 47, 460–465. doi: 10.1007/s12035-012-8369-3

Paillard, T., Rolland, Y., and de Souto Barreto, P. (2015). Protective effects of physical exercise in Alzheimer's disease and Parkinson's disease: a narrative review. *J. Clin. Neurol.* 11, 212–219. doi: 10.3988/jcn.2015.11.3.212

Pals, P., Lincoln, S., Manning, J., Heckman, M., Skipper, L., Hulihan, M., et al. (2004). Alpha-synuclein promoter confers susceptibility to Parkinson's disease. *Ann. Neurol.* 56, 591–595. doi: 10.1002/ana.20268

Pan, F., Ding, H., Dong, H., Ye, M., Liu, W., Cui, G., et al. (2013). Association of polymorphism in rs2736990 of the alpha-synuclein gene with Parkinson's disease in a Chinese population. *Neurol. India* 61, 360–364. doi: 10.4103/0028-3886.117595

Pan, F., Dong, H., Ding, H., Ye, M., Liu, W., Wu, Y., et al. (2012). SNP rs356219 of the α-synuclein (SNCA) gene is associated with Parkinson's disease in a Chinese Han population. *Parkinsonism Relat. Disord.* 18, 632–634. doi: 10.1016/j.parkreldis.2012.01.025

Perez, R. G., Waymire, J. C., Lin, E., Liu, J. J., Guo, F., and Zigmond, M. J. (2002). A role for alpha-synuclein in the regulation of dopamine biosynthesis. *J. Neurosci.* 22, 3090–3099.

Pihlstrøm, L., Axelsson, G., Anne, K., Dizdar, N., Fardell, C., Forsgren, L., et al. (2013). Supportive evidence for 11 loci from genome-wide association studies in Parkinson's disease. *Neurobiol. Aging* 34, 1708.e7–1708.e13. doi: 10.1016/j.neurobiolaging.2012.10.019

Rajput, A., Vllariño-Güell, C., Rajput, M. L., Ross, O. A., Soto-Ortolaza, A. I., Lincoln, S. J., et al. (2009). Alpha-synuclein polymorphisms are associated with Parkinson's disease in a Saskatchewan population. *Mov. Disord.* 24, 2411–2414. doi: 10.1002/mds.22768

Reijnders, J., Ehrt, U., Lousberg, R., Aarsland, D., and Leentjens, A. (2009). The association between motor subtypes and psychopathology in Parkinson's disease. *Parkinsonism Relat. Disord.* 15, 379–382. doi: 10.1016/j.parkreldis.2008.09.003

Ritz, B., Rhodes, S. L., Bordelon, Y., and Bronstein, J. (2012). α-Synuclein genetic variants predict faster motor symptom progression in idiopathic Parkinson disease. *PLoS ONE* 7:e36199. doi: 10.1371/journal.pone.0036199

Rodriguez-Oroz, M. C., Jahanshahi, M., Krack, P., Litvan, I., Macias, R., Bezard, E., et al. (2009). Initial clinical manifestations of Parkinson's disease: features and pathophysiological mechanisms. *Lancet Neurol.* 8, 1128–1139. doi: 10.1016/S1474-4422(09)70293-5

Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M., et al. (2009). Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat. Genet.* 41, 1303–1307. doi: 10.1038/ng.485

Schapira, A. H. V. (2007). Mitochondrial dysfunction in Parkinson's disease. *Cell Death Differ.* 14, 1261–1266. doi: 10.1038/sj.cdd.4402160

Semchuk, K. M., Love, E. J., and Lee, R. G. (1991). Parkinson's disease and exposure to rural environmental factors: a population based case-control study. *Can. J. Neurol. Sci.* 18, 279–286. doi: 10.1017/S0317167100031826

Sharma, M., Ioannidis, J. P. A., Aasly, J. O., Annesi, G., Brice, A., Van Broeckhoven, C., et al. (2012). Large-scale replication and heterogeneity in Parkinson disease genetic loci. *Neurology* 79, 659–667. doi: 10.1212/WNL.0b013e318264e353

Shih, I., Liew, Z., Krause, N., and Ritz, B. (2016). Lifetime occupational and leisure time physical activity and risk of Parkinson's disease. *Parkinsonism Relat. Disord.* 28, 112–117. doi: 10.1016/j.parkreldis.2016.05.007

Simón-Sánchez, J., Schulte, C., Bras, J. M., Sharma, M., Gibbs, J. R., Berg, D., et al. (2009). Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.* 41, 1308–1312. doi: 10.1038/ng.487

Sotiriou, S., Gibney, G., Baxevanis, A. D., and Nussbaum, R. L. (2009). A single nucleotide polymorphism in the 3′UTR of the SNCA gene encoding alpha-synuclein is a new potential susceptibility locus for Parkinson disease. *Neurosci. Lett.* 461, 196–201. doi: 10.1016/j.neulet.2009.06.034

Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M., and Goedert, M. (1998). α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proc. Natl. Acad. Sci. U.S.A.* 95, 6469–6473. doi: 10.1073/pnas.95.11.6469

Spillantini, M. G., Schmidt, M. L., Lee, V. M.-Y., Trojanowski, J. Q., and Goedert, M. R. J. (1997). α-synuclein in Lewy bodies. *Nature* 388, 839–840. doi: 10.1038/42166

Stern, Y. (2009). Cognitive reserve. *Neuropsychologia* 47, 2015–2028. doi: 10.1016/j.neuropsychologia.2009.03.004

Tabrizi, S. J., Orth, M., Wilkinson, J. M., Taanman, J. W., Warner, T. T., Cooper, J. M., et al. (2000). Expression of mutant alpha-synuclein causes increased susceptibility to dopamine toxicity. *Hum. Mol. Genet.* 9, 2683–2689. doi: 10.1093/hmg/9.18.2683

Trojanowski, J. Q., and Lee, V. M.-Y. (1998). Aggregation of neurofilament and α-synuclein proteins in Lewy bodies: implications for the pathogenesis of Parkinson disease and Lewy body dementia. *Arch. Neurol.* 55, 151–152. doi: 10.1001/archneur.55.2.151

Tröster, A. I., Stalp, L. D., Paolo, A. M., Fields, J. A., and Koller, W. C. (1995). Neuropsychological impairment in Parkinson's disease with and without depression. *Arch. Neurol.* 52, 1164–1169. doi: 10.1001/archneur.1995.00540360042014

Trotta, L., Guella, I., Soldà, G., Sironi, F., Tesei, S., Canesi, M., et al. (2012). SNCA and MAPT genes: independent and joint effects in Parkinson disease in the Italian population. *Parkinsonism Relat. Disord.* 135, 257–262. doi: 10.1016/j.parkreldis.2011.10.014

Uversky, V. N., Li, J., Bower, K., and Fink, A. L. (2002). Synergistic effects of pesticides and metals on the fibrillation of alpha-synuclein: implications for

Parkinson's disease. *Neurotoxicology* 23, 527–536. doi: 10.1016/S0161-813X(02)00067-0

Venda, L. L., Cragg, S. J., Buchman, V. L., and Wade-Martins, R. (2010). α-Synuclein and dopamine at the crossroads of Parkinson's disease. *Trends Neurosci.* 33, 559–568. doi: 10.1016/j.tins.2010.09.004

Verbaan, D., Boesveldt, S., Van Rooden, S. M., Visser, M., Marinus, J., MacEdo, M. G., et al. (2008). Is olfactory impairment in Parkinson disease related to phenotypic or genotypic characteristics? *Neurology* 71, 1877–1882. doi: 10.1212/01.wnl.0000336651.48596.c7

Wang, G., Huang, Y., Chen, W., Chen, S., Wang, Y., Qin, X., et al. (2016). Variants in the SNCA gene associate with motor progression while variants in the MAPT gene associate with the severity of Parkinson's disease. *Parkinsonism Relat. Disord.* 24, 89–94. doi: 10.1016/j.parkreldis.2015.12.018

Wang, G., Van Der Walt, J. M., Mayhew, G., Li, Y., Zu, S., Scott, W. K., et al. (2008). Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of alpha-synuclein. *Am. J. Hum. Gentetics* 82, 283–289. doi: 10.1016/j.ajhg.2007.09.021

Ward, A., Arrighi, H. M., Michels, S., and Cedarbaum, J. M. (2012). Mild cognitive impairment: disparity of incidence and prevalence estimates. *Alzheimers Dement.* 8, 14–21. doi: 10.1016/j.jalz.2011.01.002

Wider, C., Vilariño-Güell, C., Heckman, M. G., Jasinska-Myga, B., Ortolaza-Soto, A. I., Diehl, N. N., et al. (2011). SNCA, MAPT, and GSK3B in Parkinson disease: a gene-gene interaction study. *Eur. J. Neurol.* 18, 876–881. doi: 10.1111/j.1468-1331.2010.03297.x

Winkler, S., Hagenah, J., Lincoln, S., Heckman, M., Hugarvoll, K., Lohmann-Hedrich, K., et al. (2007). Alpha-Synuclein and Parkinson disease susceptibility. *Neurology* 69, 1745–1750. doi: 10.1212/01.wnl.0000275524.15125.f4

Wirdefeldt, K., Adami, H.-O., Cole, P., Trichopoulos, D., and Mandel, J. (2011). Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur. J. Epidemiol.* 26(Suppl. 1), S1–S58. doi: 10.1007/s10654-011-9581-6

Wu-Chou, Y.-H., Chen, Y.-T., Yeh, T.-H., Chang, H.-C., Weng, Y.-H., Lai, S.-C., et al. (2013). Genetic variants of SNCA and LRRK2 genes are associated with sporadic PD susceptibility: a replication study in a Taiwanese cohort. *Parkinsonism Relat. Disord.* 19, 251–255. doi: 10.1016/j.parkreldis.2012.10.019

Xu, W., Tan, L., and Yu, J. (2015). The link between the SNCA gene and parkinsonism. *Neurobiol. Aging* 36, 1505–1518. doi: 10.1016/j.neurobiolaging.2014.10.042

Yu, L., Xu, P., He, X., Hu, F., Lin, Z., Zhu, M., et al. (2010). SNP rs7684318 of the alpha-synuclein gene is associated with Parkinson's disease in the Han Chinese population. *Brain Res.* 1346, 262–265. doi: 10.1016/j.brainres.2010.05.069

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: 05 April 2017
doi: 10.3389/fnagi.2017.00093

Relationship between Cognitive and Sleep–wake Variables in Asymptomatic Offspring of Patients with Late-onset Alzheimer's Disease

Carolina Abulafia[1,2,3], Bárbara Duarte-Abritta[1], Mirta F. Villarreal[1,3], María S. Ladrón-de-Guevara[1,3], Celeste García[1], Geraldine Sequeyra[1], Gustavo Sevlever[1], Leticia Fiorentini[1], Karl-Jürgen Bär[4], Deborah R. Gustafson[5,6,7], Daniel E. Vigo[2,3] and Salvador M. Guinjoan[1,3,8,9]**

[1] FLENI Foundation Department of Psychiatry, Buenos Aires, Argentina, [2] Applied Neuroscience Laboratory, Institute for Biomedical Research, School of Medical Sciences, Universidad Católica Argentina, Buenos Aires, Argentina, [3] Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina, [4] Department of Psychiatry and Psychotherapy, Universitätsklinikum Jena, Friedrich-Schiller-Universität, Jena, Germany, [5] Department of Neurology, State University of New York - Downstate Medical Center, Brooklyn, NY, USA, [6] Neuropsychiatric Epidemiology Unit, University of Gothenburg, Gothenburg, Sweden, [7] Department of Health and Education, University of Skövde, Skövde, Sweden, [8] FLENI Teaching Unit, Department of Psychiatry and Mental Health, University of Buenos Aires School of Medicine, Buenos Aires, Argentina, [9] Department of Neurophysiology, University of Buenos Aires School of Psychology, Buenos Aires, Argentina

OPEN ACCESS

Edited by:
Agustin Ibanez, Institute of Cognitive and Translational Neuroscience, Argentina

Reviewed by:
Douglas Watt, Cambridge Health Alliance, USA
Trevor Ronald Norman, University of Melbourne, Australia

***Correspondence:**
Salvador M. Guinjoan
sguinjoan@fleni.org.ar
Daniel E. Vigo
dvigo@conicet.gov.ar

Received: *03 December 2016*
Accepted: *20 March 2017*
Published: *05 April 2017*

Citation:
Abulafia C, Duarte-Abritta B, Villarreal MF, Ladrón-de-Guevara MS, García C, Sequeyra G, Sevlever G, Fiorentini L, Bär K-J, Gustafson DR, Vigo DE and Guinjoan SM (2017) Relationship between Cognitive and Sleep–wake Variables in Asymptomatic Offspring of Patients with Late-onset Alzheimer's Disease. Front. Aging Neurosci. 9:93. doi: 10.3389/fnagi.2017.00093

Early neuropathological changes characteristic of late-onset Alzheimer's disease (LOAD) involve brain stem and limbic structures that regulate neurovegetative functions, including sleep–wake rhythm. Indeed, sleep pattern is an emerging biomarker and a potential pathophysiological mechanism in LOAD. We hypothesized that cognitively asymptomatic, middle-aged offspring of patients with LOAD (O-LOAD) would display a series of circadian rhythm abnormalities prior to the onset of objective cognitive alterations. We tested 31 children of patients with LOAD (O-LOAD) and 19 healthy individuals without family history of Alzheimer's disease (control subjects, CS) with basic tests of cognitive function, as well as actigraphy measures of sleep–wake rhythm, cardiac autonomic function, and bodily temperature. Unexpectedly, O-LOAD displayed subtle but significant deficits in verbal episodic memory (Rey Auditory Verbal Learning Test delayed recall 10.6 ± 0.4 vs. 8.6 ± 0.6, $t = 4.97$, $df = 49$, $p < 0.01$) and language (Weschler's vocabulary 51.4 ± 1.3 vs. 44.3 ± 1.5, $t = 2.49$, $df = 49$, $p < 0.001$) compared to CS, even though all participants had results within the clinically normal range. O-LOAD showed a phase-delayed rhythm of body temperature (2.56 ± 0.47 h vs. 3.8 ± 0.26 h, $t = 2.48$, $df = 40$, $p = 0.031$). Cognitive performance in O-LOAD was associated with a series of cardiac autonomic sleep–wake variables; specifically indicators of greater sympathetic activity at night were related to poorer cognition. The present results suggest sleep pattern deserves further study as a potential neurobiological signature in LOAD, even in middle-aged, at risk individuals.

Keywords: early diagnosis, late-onset Alzheimer's disease, circadian rhythms, cardiac autonomic control, actigraphy

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes up to 80% of all dementia cases worldwide (Alzheimer's Association, 2016). It is characterized by the coexistence of two neuropathological hallmarks, namely extracellular plaques of amyloid beta (Aβ) and intracellular neurofibrillary tangles made up of hyperphosphorylated Tau protein (Tau). Severe neurodegeneration and widespread neuroinflammation are ubiquitous albeit less specific neuropathological features of the disorder. Over 99% of AD cases are late-onset (i.e., the initial cognitive symptoms usually appear after 65 years of age); familial or early-onset AD cases result from fairly rare autosomal dominant mutations, and account for less than 1% of cases, including the patient of the original description of the disorder (Alzheimer, 1907). The relationship between Aβ- and Tau-related pathology in AD is complex and remains elusive in its details to this day. The amyloid cascade hypothesis (ACH) posits that AD begins with the deposition of Aβ, resulting from increased production, reduced clearance, or a combination or both. The deposition of Aβ would in turn trigger a cascade of neurodegenerative processes including Tau-related intracellular changes, loss of synapses, inflammation neurodegeneration, and eventually regional brain atrophy (Hardy and Higgins, 1992). The ACH got support from evidence that familial forms of AD were due to dominant mutations in enzymes participating in the metabolism of amyloid. Down syndrome [resulting in an extra copy of the gene coding for the amyloid precursor protein (APP) located in chromosome 21], which is associated with increased incidence and earlier onset of AD, lent further support to the ACH. However, the main conceptual problem of this hypothesis is a remarkable lack of correlation between Aβ deposits and cognitive changes, as well as the absence of a clear and orderly pattern of anatomical progression as the disease advances. Further, significant *in vivo* Aβ deposition is present in a substantial number of cognitively normal elderly individuals, and it is associated with gray matter (GM) changes not characteristic of the early phases of neurodegeneration of AD (Sepulcre et al., 2016). In spite of this evident lack of correlation between Aβ deposition and cognitive symptoms, a number of pharmacological agents targeting Aβ accumulation have failed or even resulted in worse outcomes than placebo. In fact, this group of experimental drugs (including anti-Aβ antibodies seeking to improve clearance of brain amyloid, and drugs interfering with the synthesis of new amyloid) have met with one of the highest failure rates in any therapeutic area in the history of medicine (Cummings et al., 2014).

On the other hand, Tau pathology in AD follows a highly predictable progression, predates Aβ accumulation in anatomical studies, and shows a parsimonious relationship with cognitive symptom development (Braak and Del Tredici, 2011; Walsh et al., 2017) being the basis for the most accepted staging system of AD pathology (Braak and Braak, 1991).

Early detection of cases of AD, before any cognitive symptoms emerge, is a challenge as important as discovering treatments aimed at the causes of AD. This should be accomplished before neuropathological changes are so advanced that they cannot be reversed. In fact neuropathological studies suggest changes characteristic of AD occur already in the third and fourth decades of life, involving phosphorylated Tau accumulation in selected groups of neurons, including those in the locus coeruleus, and diencephalic and allocortical limbic areas (Braak and Del Tredici, 2011; Buchhave et al., 2012). Such areas are involved in the regulation of vegetative functions, including autonomic nervous system regulation and diurnal variation (Braak et al., 2011). Sleep–wake cycle regulation in particular, depends on intrinsic circadian rhythm generation by diencephalic structures, in addition to a series of environmental cues of sleep–wake cycle (Buijs et al., 2016). Thus, alterations in sleep could represent, in theory, early manifestations of late-onset AD (LOAD)-related neuropathological changes in the brain.

Sleep abnormalities have emerged as potential early biomarkers of LOAD status (see Mander et al., 2016 for a review on this topic). AD-related changes in sleep have also been proposed as possible neuropathogenic mediators that perpetuate the cycle of amyloid deposition, neurofibrillar changes, and neurodegeneration ultimately resulting in cognitive and functional deterioration (Lim et al., 2013; Mander et al., 2015). In addition, previous evidence suggests that in cognitively asymptomatic, middle-aged individuals, amyloid deposition is associated with poor sleep (Sprecher et al., 2015).

We hypothesized that circadian alterations could be an early functional manifestation of AD neuropathology in at-risk individuals. To this end, we studied a group of cognitively normal, middle-aged individuals with at least one parent diagnosed with LOAD, which accounts for over 99% of all cases of AD (Dubois et al., 2016). We specifically predicted that cognitively asymptomatic offspring of LOAD patients (O-LOAD) would display abnormalities in sleep, circadian autonomic activity and diurnal variation, and circadian rhythm of body temperature as compared with healthy individuals without a family history of AD. Moreover, we expected to find correlations between such circadian alterations (in the form of decreased amplitude of diurnal variation, phase alterations, sleep efficiency, and greater sympathetic or lesser parasympathetic activity), and cognitive performance in verbal memory in O-LOAD but not healthy individuals.

MATERIALS AND METHODS

Design and Sample

This was a cross-sectional study, where cognitive and chronobiological measures were compared between a sample of AD offspring (O-LOAD) and control subjects (CS). The study protocol was performed in accordance with the Declaration of Helsinki, and approved by the Bioethics Committee of FLENI Foundation, Argentina. All participants provided their written informed consent for the study.

A total of 31 O-LOAD participated in the study along with 19 healthy subjects with no family history of AD (CS), consecutively selected and comparable in gender, age, and education level. The inclusion criteria for O-LOAD were as follows: (1) To have at least one parent diagnosed with probable LOAD according

to the DSM-5, (2) to be 40–65 years old at the time of recruitment, and (3) seven or more years of formal education. The CS group had the same inclusion criteria except for item 1). The exclusion criteria for O-LOAD and CS were as follows: (1) Mini Mental State Examination (MMSE) score <25, (2) compromised intellectual level based education and employment history, (3) evidence of current progressive neurologic disease or likely to impair cognitive performance, (4) history of substance abuse (alcohol, marijuana, stimulants, benzodiazepines, or other drugs), and (5) Hachinski score >7 (to filter data suggestive of vascular-derived cognitive impairment).

Cognitive Assessment

The neuropsychological tests selected for this study have been widely validated and are frequently used in clinical practice and thus require no detailed explanation. They comprise a concise battery aimed at assessing the most salient cognitive domains impaired in AD: episodic memory and language. Neuropsychological evaluation was performed in a single session of approximately 90 min by an experienced neuropsychologist (CA). All evaluations were performed between 12.00 and 17.00 h. The MMSE (Folstein et al., 1975) and the Clock Drawing Test subtest from the 7 Minute Screen test (Solomon et al., 1998) were included as screening measurements; whereas MMSE is insensitive to even prodromal AD, we incorporated it as a widely used screening instrument (Ravaglia et al., 2005), to be complemented with more sensitive tests. Thus, verbal episodic memory was assessed by the Rey Auditory Verbal Learning Test (RAVLT; Rey, 1964; Schmidt, 1996). Semantic memory, or "the knowledge of words" was measured by the semantic fluency task ("animals" category) (Spreen and Benton, 1969) and the vocabulary subtest of the intelligence battery WAIS-III (Wechsler, 1997). Additionally, the Cognitive Reserve Questionnaire (CRQ) was administered to all participants. This technique proves to be quick and useful screening to assess the most relevant elements associated to the cognitive reserve (education level, parent's education level, additional academic courses completed, professional activity, musical education, fluent languages, reading activity, and ingenuity games) (Rami et al., 2011). Finally, the presence and severity of depressive symptoms was measured by the Beck Depression Inventory (self-report) and by the Hamilton Depression Rating Scale (HDRS) completed by a clinician.

All participants were cognitively asymptomatic and their neuropsychological testing yielded normal values, so none of the individuals met criteria for mild cognitive impairment or dementia.

Assessment of Neurovegetative Function

Two questionnaires were used to measure quality of sleep: Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS). The PSQI is a self-report questionnaire that assesses sleep problems in the last month. A PSQI score greater than 4 points is defined as "poor sleep quality." The ESS measures daytime sleepiness. An ESS score greater than 10 represents "excessive daytime sleepiness" (Diez et al., 2011).

Objective evaluation of the sleep–wake cycle was performed by actigraphy. Subjects wore the actigraph (MicroMini-Motionlogger, Ambulatory Monitoring Inc, NY, USA) on their wrist for 7 days. During this time, they were also requested to complete a Daily Sleep Diary where they reported sleep habits including sleep onset and offset. Time spent in bed, time awake, and sleep efficiency were calculated using Action W 2.5 (Ambulatory Monitoring Inc, NY, USA) (Bellone et al., 2016).

Distal skin temperature was measured as a proxy for the circadian rhythm of core temperature. There is an approximate 12-h phase difference between distal skin temperature and core body temperature and both measures behave in the same manner, reaching its maximum levels during the sleeping period and decreasing during waking hours (Sarabia et al., 2008). For this study, 16 mm × 6 mm Thermochron iButton® DS1291H (Dallas Maxim) sensors were placed next to the actigraph. Temperature samples were obtained every 10 min for 7 days (van Marken Lichtenbelt et al., 2006). The Chronos-Fit software was used to fit a cosine curve with a 24-h period to the data as an estimate of the circadian pattern, using the partial Fourier series method. From that model, the following measures were derived: % rhythm: represents the percentage of variation in the data explained by the fitted model; MESOR (midline estimating statistic of rhythm) is a rhythm adjusted mean; amplitude is the difference between the maximum and the MESOR; acrophase is the time at which the maximum of the rhythm occurs (van Marken Lichtenbelt et al., 2006; Refinetti et al., 2007).

Autonomic nervous system circadian rhythm was measured by heart rate variability (HRV) analysis described in detail elsewhere (Vigo et al., 2005, 2010). Fluctuations in heartbeats are cyclical and have different frequencies. High frequency (HF) modifications respond to respiratory mechanisms and are a marker of parasympathetic activity. Low frequency (LF) fluctuations respond to the baroreflex and present sympathetic and parasympathetic influences. There are also very low frequency (VLF) oscillations, which possibly respond to hormonal or thermal oscillations.

Participants underwent a 24-h digital electrocardiogram (ECG) study (Holtech, Servicios Computados SA, Buenos Aires). The data obtained were processed to calculate the time elapsed between R waves (RR intervals). HRV evaluation consisted of time domain and frequency domain analyses. For the time domain analysis, the following measures were calculated: RRM (mean duration of RR intervals in milliseconds) quantifies the mean heart rate, SDNN (standard deviation of RR intervals in milliseconds) represents a coarse quantification of overall variability, and RMSSD (square root of the mean squared differences of successive normal RR) measures short-term heart rate variations. For the frequency domain analysis, the discrete wavelet transform (DWT) was chosen over the traditional fast Fourier transform because it is not affected by discontinuities or non-stationarities. Before applying the DWT, the linear trend and the mean value were subtracted from the signal. Moreover, it was evenly sampled with a frequency of 2.4 Hz through a spline interpolation algorithm and zero padded to the next higher power of two. The signal was analyzed by a six-level wavelet decomposition with a Daubechies four-wavelet function. After

undergoing such a decomposition process, wavelet levels A6 and D1–D6 make up the total power (TP, 0–0.5 Hz), wavelet levels A6 and D6 closely represent the VLF band (0–0.0375 Hz), wavelet levels D4–D5 reflect the LF band (0.0375–0.15 Hz), and wavelet levels D2–D3 approximate to the HF band (0.15–0.6 Hz). In DWT, the square of the standard deviation of wavelet coefficients at each level matches the spectral power of that level. The obtained values are formulated as the natural logarithm of TP, HF, LF, and VLF; normalized units of LF [LF/(TP − VLF)/100] and HF [HF/(TP − VLF)/100]; and the ratio between LF and HF (Vigo et al., 2012).

For the circadian analysis, the ECG recording was segmented in 30-min fragments, which were then averaged according to the sleep or wake periods as defined by actigraphy. Furthermore, sleep–wake autonomic differences were calculated. Daytime sleep periods were classified as naps and excluded from the daytime average.

Structural MRI

MRI images were acquired on a 3 T GE Signa HDxt MRI machine with an eight-channel head coil. A high resolution T1 3D fast SPGR-IR (TR = 6.604 ms, TE = 2.796 ms, TI = 450) image was acquired. Image correction (ASSET) with acceleration factor = 2; acquisition matrix = 256 × 256; FOV = 24 cm; slice thickness = 1.2 mm; 120 axial contiguous slices.

The acquired images were converted from DICOM format to NIFTI using the SPM12 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (MathWorks Inc., Sherborn, MA, USA). The CAT12 tool[1] for the SPM12 was used to process the images (Preprocessing → Segment Data Batch with default parameter values). A quality control of the images derived from the segmentation was carried out and the relative volumes of GM and white matter (WM) were obtained for each subject.

Statistical Analysis

Continuous variables were summarized by means and standard errors. Categorical variables were summarized as frequencies and percentages. Differences between groups were calculated by using *t*-test for independent samples, and sex differences in groups by means of the chi-square test. Correlations of chronobiological measures with cognitive and clinical data were evaluated using Pearson correlation coefficients. We report two-tailed signification at $p < 0.05$ and also Bonferroni correction for multiple comparisons. All statistical analysis was performed using the SPSS version 22.0 software (SPSS Inc.).

RESULTS

Both groups were comparable in age (CS = 54.2 ± 2.2 years vs. O-LOAD = 53.8 ± 1.6 years) and sex (CS 73.7% women vs. O-LOAD 71%). **Table 1** shows the demographic and clinical characteristics of O-LOAD and CS. Groups had similar years of education, Hachinski score, and depressive symptoms (**Table 1**).

[1]http://dbm.neuro.uni-jena.de/cat/

Whereas all participants had normal cognitive performance, as a group, O-LOAD showed lower episodic memory as evidenced by performance on the RAVLT (**Table 2**). The WAIS vocabulary item was also lower among the O-LOAD (**Table 2**).

O-LOAD showed a delayed phase in the circadian rhythm of body temperature compared to CS (**Table 1**). Measures of sleep–wake cycle in the actigraphy, cardiac autonomic variables, and relative GM and WM brain volumes were similar in both groups as well (**Table 1**).

In CS, relative GM volume showed a direct correlation with learning in RAVLT ($r = 0.769$, $p = 0.001$). We did not observe a relationship between GM or WM volume and cognitive performance variables in O-LOAD.

Table 2 shows significant correlations between HRV and its diurnal variations and cognitive values in O-LOAD. Learning and recall values on the RAVLT displayed robust correlations with a series of cardiac autonomic activity measures and their diurnal variations. In general, greater HRV and a greater night–day difference in HRV resulted in better verbal memory results. More specifically, an increased sympathovagal balance at night was associated with poorer cognitive results on verbal memory, clock drawing and MMSE score (**Table 2**). All associations between frequency-domain HRV and RAVLT variables survived Bonferroni corrections for multiple tests (**Table 2**, bold *r*, *p*). These associations were not present in CS (not shown). Sleep efficiency measured with actigraphy in O-LOAD was associated with greater cognitive reserve ($r = 0.495$, $p = 0.007$). Such correlations were again not present in CS (not shown).

DISCUSSION

The main findings of the present study include (1) lower performance in O-LOAD as compared to CS in measures of verbal memory and language, even though performance in both groups falls within the normal range, (2) a delayed phase of the circadian rhythm of body temperature in O-LOAD, (3) the presence of a relationship between sleep efficiency and verbal memory in O-LOAD, and (4) overall HRV, decreased night–day differences in HRV, and increased sympathovagal balance at night are all related to decreased verbal memory in O-LOAD.

The present observations of a relationship between indicators of healthy circadian and neurovegetative function and cognitive abilities in O-LOAD lend support to the hypothesis that sleep characteristics are associated with changes in cognition typical of AD, and that such a relationship is present over a decade prior to the expected onset of cognitive symptoms. This is compatible with the view that ongoing sleep changes, if persistent, could contribute to the appearance of cognitive symptoms characteristic of AD. Further, we observed a phase delay in the diurnal rhythm of body temperature in O-LOAD, pointing to a basic alteration in the circadian system in this group. Moreover, our findings extend this observation to sleep–wake differences in sympathovagal balance, another limbic phenotype with a potential relationship with early AD pathological changes. HRV is a non-invasive measure of cardiac autonomic output, ultimately reflecting complex regulation at the central autonomic network

TABLE 1 | Demographic and clinical data.

	CS		O-LOAD			
	Mean or frequency	**SE or %**	**Mean or frequency**	**SE or %**	***t***	***p***
Demographic and clinical data						
n	19		31			
Education	17.1	1.2	16.3	0.4	10.732	0.564
CRQ	17.2	0.7	15.3	0.7	1.227	0.071
Hachinski score	1.1	0.3	1.2	0.2	1.172	0.667
BDI-II	9.1	1.9	8.8	1.2	0.068	0.903
HDRS	7.9	1.3	8.8	1.3	0.927	0.635
PSQI	5.7	0.9	6.3	0.7	0.431	0.548
ESS	8.9	1.0	9.3	0.8	0.044	0.808
Neuropsychological tests						
n	19		31			
MMSE	29.3	0.2	28.9	0.2	1.152	0.103
Clock	5.9	0.3	6.0	0.2	0.280	0.806
Vocabulary	51.4	1.3	44.3	1.5	2.486	0.001
Semantic fluency	21.4	1.3	20.7	0.7	3.474	0.644
RAVLT	45.6	2.2	42.9	1.3	2.271	0.293
RAVLT D	10.6	0.4	8.6	0.6	4.974	0.009
Brain volume						
n	15		27			
RGM (%)	42.83	0.52	42.74	0.31	1.158	0.880
RWM (%)	34.19	0.37	34.46	0.30	0.342	0.582
Actigraphy						
n	17		28			
Start time (hh:mm)	00:43	00:14	00:00	00:14	0.669	0.097
End time (hh:mm)	07:55	00:14	07:55	00:14	1.096	0.922
Duration (min)	443.91	12.47	473.70	11.79	0.033	0.090
Efficiency (%)	95.85	0.92	95.64	0.46	0.618	0.839
Distal temperature						
n	15		26			
% rhythm	26.69	3.95	26.40	2.60	0.157	0.952
MESOR	33.03	0.14	32.16	1.15	1.584	0.456
Amplitude (°C)	0.99	0.11	0.96	0.09	0.052	0.841
Acrophase (time)	2.56	0.47	3.80	0.26	2.478	0.031
HRV						
n	16		23			
Wake						
RRM (ms)	778.49	20.66	794.71	19.38	0.096	0.571
SDNN (ms)	59.99	4.66	63.69	4.38	0.107	0.567
RMSSD (ms)	30.15	2.74	34.28	3.57	0.313	0.365
ln VLF (WPC)	14.90	0.07	15.04	0.06	0.016	0.128
ln LF (WPC)	10.30	0.13	10.54	0.13	0.251	0.194
ln HF (WPC)	7.71	0.14	7.87	0.12	0.030	0.407
L/H	15.10	1.14	16.59	1.23	0.212	0.380
LF (nu)	0.16	0.06	0.10	0.03	2.951	0.414
HF (nu)	0.84	0.06	0.90	0.03	2.951	0.414
Sleep						
RRM (ms)	902.90	31.37	926.33	25.43	0.100	0.566
SDNN (ms)	57.00	5.74	62.20	4.68	0.463	0.488
RMSSD (ms)	31.42	3.19	34.17	2.58	0.886	0.508
ln VLF (WPC)	15.14	0.07	15.31	0.10	0.762	0.167

(Continued)

TABLE 1 | Continued

	CS		O-LOAD		*t*	*p*
	Mean or frequency	**SE or %**	**Mean or frequency**	**SE or %**		
ln LF (WPC)	10.30	0.14	10.48	0.09	2.169	0.263
ln HF (WPC)	7.88	0.16	7.97	0.10	2.048	0.606
L/H	12.32	0.85	14.12	1.30	2.340	0.253
LF (nu)	0.22	0.06	0.22	0.06	0.403	0.946
HF (nu)	0.78	0.06	0.78	0.06	0.403	0.946
Sleep–wake difference						
RRM (ms)	130.67	26.37	127.99	20.88	0.067	0.937
SDNN (ms)	−1.28	3.56	−2.07	4.14	0.961	0.886
RMSSD (ms)	1.87	2.41	−0.32	3.71	0.723	0.623
ln VLF (WPC)	0.26	0.06	0.26	0.12	2.445	0.997
ln LF (WPC)	0.03	0.08	−0.07	0.14	3.134	0.522
ln HF (WPC)	0.20	0.10	0.11	0.11	1.268	0.806
L/H	−2.98	0.99	−2.68	1.22	0.576	0.850
LF (nu)	0.07	0.07	0.12	0.05	0.109	0.558
HF (nu)	−0.07	0.07	−0.12	0.05	0.109	0.558

CRQ, Cognitive Reserve Questionnaire; BDI-II, Beck Depression Inventory, second edition; HDRS, Hamilton Depression Rating Scale; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index; MMSE, Mini Mental State Examination; RAVLT, Rey Auditory Verbal Learning Test learning curve; RAVLT D, Rey Auditory Verbal Learning Test delayed recall; RGM, relative gray matter; RWM, relative white matter; HRV, heart rate variability; RRM, mean duration of RR intervals; SDNN, standard deviation of RR intervals; RMSSD, square root of the mean squared differences of successive normal RR; WPC, power wavelet coefficients corresponding to: VLF, very low frequency; LF, low frequency; HF, high frequency; L/H, relationship between LF and HF.

TABLE 2 | Correlation coefficients between HRV and neuropsychological measurements.

	SDNN	RMSSD	LF	HF	L/H
Wake					
CRQ	ns	ns	−0.459, 0.028	−0.437, 0.037	ns
RAVLT	**0.557, 0.006**	**0.659, 0.001**	**0.523, 0.010**	**0.562, 0.005**	ns
RAVLT D	0.504, 0.014	**0.579, 0.004**	0.489, 0.018	**0.541, 0.008**	ns
Sleep					
Clock	ns	ns	ns	ns	−0.452, 0.035
MMSE	ns	ns	ns	ns	−0.524, 0.012
RAVLT	ns	ns	ns	ns	**−0.559, 0.007**
RAVLT D	ns	ns	ns	ns	−0.508, 0.041
Sleep–wake					
MMSE	ns	ns	ns	ns	−0.522, 0.013
RAVLT	−0.484, 0.023	−0.445, 0.038	**−0.613, 0.002**	ns	**−0.744, <0.001**
RAVLT D	ns	ns	**−0.575, 0.005**	ns	**−0.713, <0.001**

Shown for each variable are: r, p. CRQ, Cognitive Reserve Questionnaire; MMSE, Mini Mental State Examination; RAVLT, Rey Auditory Verbal Learning Test learning curve; RAVLT D, Rey Auditory Verbal Learning Test delayed recall. In bold are marked r, p surviving Bonferroni correction for multiple measurements.

(see Kemp and Quintana, 2013 for a comprehensive discussion on the origins and interpretation of HRV).

In the present study, we observed a relationship between overall HRV and amplitude of the sleep–wake differences of HRV and better cognition in O-LOAD. More specifically, greater sympathetic prevalence [as denoted by increased low frequency/high frequency (L/H)] at night was associated with more widespread poor results in cognition, including not only verbal memory but overall cognition (as reflected in the MMSE score) and visuospatial/executive function (clock drawing score).A potential link between abnormal sleep–wake variation and poor cognition in the present sample of O-LOAD might be the reported association between reduced parasympathetic activity and decreased sleep quality (Burton et al., 2010), which in turn predicts cognitive decline (Mander et al., 2016). Another potential link may be the increased susceptibility to stress (e.g., Stenfors et al., 2016). However, normal anxiety and depression symptomatology in O-LOAD argue against this possibility. Alternatively, recent data suggest that vascular and endocrine changes might mediate the relationship between cardiac autonomic activity and cognition (Kemp et al., 2016). Results in the study by Kemp et al. (2016) might not be easily comparable to the present study, as they studied HRV in 10-min samples and the cognitive test was TMT-B.

Moreover, the normal Hachinski score observed in our O-LOAD sample makes a similar pathophysiologic link less likely in our sample. However, other published results emphasize the need to further investigate the mechanisms linking HRV and cognition. In the case of O-LOAD, the hypothesis that limbic changes underlie both cognitive changes and top-down abnormalities in the control of peripheral autonomic function needs to be tested with the help of structural and functional regional brain measurements focusing on structures known to be affected early in AD.

We could not confirm our prediction of unaffected cognition in this middle-aged, asymptomatic sample of O-LOAD patients. Whereas performance in all participants fell in the range considered normal, delayed recall, learning in the 5th trial, and recuperation on the RAVLT were lower in O-LOAD than in CS. This finding was unexpected given that average age was around 53 years, i.e., approximately a decade prior to the age of expected onset of symptoms. This lends support to the hypothesis that significant AD pathology may be present in at-risk individuals several years prior to cognitive symptom onset (Braak et al., 2011). Such structural changes could affect limbic functions as suggested by the associations between cognition and sleep–wake cycle abnormalities described herein. However, brain anatomical and functional correlates of these findings will have to be confirmed in future studies addressing brain volume and indicators of amyloid deposition in regions known to bear the early impact of AD pathology.

The present conclusions are limited by a series of factors. The relatively small number of participants would require that the present findings be confirmed in larger samples; in particular, we might have overlooked differences between groups in variables of limbic functioning due to small sample size. The only available structural measure was total GM and WM in the encephalon, adjusted for intracranial volume. There were no significant differences in these general measures of neurodegeneration, but regional volumes and brain function measurements were not ascertained so, as stated, the meaning of the present clinical and physiological findings in terms of AD pathology remains to be determined. Albeit significant, correlation values suggest limbic phenotypes explain only up to 25% of the variance in cognitive values, leaving a substantial variability explained by other factors. Last, we observed a trend toward a better cognitive reserve in controls compared to O-LOAD (**Table 1**). Albeit not statistically significant, a better cognitive reserve could explain at least in part the better performance in memory and vocabulary in this group, unrelated to the fact of not having a family history of LOAD.

In sum, the present preliminary results extend previous observations of a relationship between sleep structure and cognition in patients with minor and major neurocognitive disorder due to AD, to cognitively asymptomatic, middle-aged offspring of individuals with AD. Further, it extends the observation to a peripheral indicator of the activity of the central autonomic network, and specifically links autonomic activity during sleep with cognitive performance. The relationship of these observations with specific structural alterations in limbic areas and pattern of amyloid deposit in this group remains unsettled and opened to further investigation. Further, the potential utility of variables studied herein as early biomarkers of AD status would necessitate long-term follow-up in order to detect those individuals who progress to the development of clinical characteristics of AD.

AUTHOR CONTRIBUTIONS

SMG, DEV, DRG, KJB, and MFV designed the initial project of this manuscript. CA, BDA, MFV, DEV, SMG, GuS, and LF made the final research plan. CA, BDA, MSLDG, CG, and GeS carried out all the experiments. CA, BDA, DEV, MFV, and SMG ran the statistical analyses and made a definitive interpretation of the findings. CA, DEV, and SMG wrote the first version of the manuscript. All authors edited and approved the final version of the manuscript.

ACKNOWLEDGMENTS

CA is a doctoral research fellow from CONICET. SDG. BD-A is a doctoral research fellow from Agency of Scientific Promotion. Funding was provided by Agency of Scientific Promotion (grants PICT-2012-0984 and PICT-2014-0633). The funding agency did not have a role in the development of the study, analysis of data, and writing of the manuscript.

REFERENCES

Alzheimer, A. (1907). Über eine eigenartige Erkrankung der Hirnrinde. *Allgem. Z. Psychiatr. Gerichtl. Med.* 64, 146–148.

Alzheimer's Association (2016). Alzheimer's disease facts and figures. *Alzheimers Dement.* 12, 459–509. doi: 10.1016/j.jalz.2016.03.001

Bellone, G. J., Plano, S. A., Cardinali, D. P., Pérez Chada, D., Vigo, D. E., and Golombek, D. A. (2016). Comparative analysis of actigraphy performance in healthy young subjects. *Sleep Sci.* 9, 272–279. doi: 10.1016/j.slsci.2016.05.004

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* 82, 239–259. doi: 10.1007/BF00308809

Braak, H., and Del Tredici, K. (2011). The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathol.* 121, 171–181. doi: 10.1007/s00401-010-0789-4

Braak, H., Thal, D. R., Ghebremedhin, E., and Del Tredici, K. (2011). Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J. Neuropathol. Exp. Neurol.* 70, 960–969. doi: 10.1097/NEN.0b013e318232a379

Buchhave, P., Minthon, L., Zetterber, H., Wallin, A. K., Blennow, K., and Hansson, O. (2012). Cerebrospinal fluid levels of beta-amyloid 1-42, but not of tau, are fully changed 5 to 10 years before the onset of Alzheimer dementia. *Arch. Gen. Psychiatry* 69, 98–106. doi: 10.1001/archgenpsychiatry.2011.155

Buijs, F. N., León-Mercado, L., Guzmán-Ruiz, M., Guerrero-Vargas, N. N., Romo-Nava, F., and Buijs, R. M. (2016). The circadian system: a regulatory feedback network of periphery and brain. *Physiology* 31, 170–181. doi: 10.1152/physiol.00037.2015

Burton, A., Rahman, K., Kadota, Y., Lloyd, A., and Vollmer-Conna, U. (2010). Reduced heart rate variability predicts poor sleep quality in a case-control study of chronic fatigue syndrome. *Exp. Brain Res.* 204, 71–78. doi: 10.1007/s00221-010-2296-1

Cummings, J. L., Morstorf, T., and Zhong, K. (2014). Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimers Res. Ther.* 6, 37. doi: 10.1186/alzrt269

Diez, J. J., Vigo, D. E., Pérez Lloret, S., Rigters, S., Role, N., Cardinali, D. P., et al. (2011). Sleep habits, alertness, cortisol levels, and cardiac autonomic activity in short-distance bus drivers: differences between morning and afternoon shifts. *J. Occup. Environ. Med.* 53, 806–811. doi: 10.1097/JOM.0b013e318221c6de

Dubois, B., Hampel, H., Feldman, H. H., Scheltens, P., Aisen, P., Andrieu, S., et al. (2016). Preclinical Alzheimer's disease: definition, natural history, and diagnostic criteria. *Alzheimers Dement.* 12, 292–323. doi: 10.1016/j.jalz.2016.02.002

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

Hardy, J. A., and Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184–185. doi: 10.1126/science.1566067

Kemp, A. H., and Quintana, D. S. (2013). The relationship between mental and physical health: insights from the study of heart rate variability. *Int. J. Psychophysiol.* 89, 288–296. doi: 10.1016/j.ijpsycho.2013.06.018

Kemp, A. H., Rodríguez-López, S., Passos, V. M. A., Bittencourt, M. S., Dantas, E. M., Mill, J. G., et al. (2016). Insulin resistance and carotid intima-media thickness mediate the association between resting-state heart rate variability and executive function: a path modelling study. *Biol. Psychol.* 117, 216–224. doi: 10.1016/j.biopsycho.2016.04.006

Lim, A. S., Kowgier, M., Yu, L., Buchman, A. S., and Bennett, D. A. (2013). Sleep fragmentation and the risk of incident Alzheimer's disease and cognitive decline in older persons. *Sleep* 36, 1027–1032. doi: 10.5665/sleep.2802

Mander, B. A., Marks, S. M., Vogel, J. W., Rao, V., Lu, B., Saletin, J. M., et al. (2015). Beta-amyloid disrupts human NREM slow waves and related hippocampus-dependent memory consolidation. *Nat. Neurosci.* 18, 1051–1057. doi: 10.1038/nn.4035

Mander, B. A., Winer, J. R., Jagust, W. J., and Walker, M. P. (2016). Sleep: a novel mechanistic pathway, biomarker and treatment target in the pathology of Alzheimer's disease? *Trends Neurosci.* 39, 552–566. doi: 10.1016/j.tins.2016.05.002

Rami, L., Valls-Pedret, C., Bartrés-Faz, D., Caprile, C., Solé-Padullés, C., Castellvi, M., et al. (2011). Cognitive reserve questionnaire. Scores obtained in a healthy elderly population and in one with Alzheimer's disease. *Rev. Neurol.* 52, 195–201.

Ravaglia, G., Maioli, F., Martelli, M., Brunetti, N., Bastagli, L., and Mariani, E. (2005). Screening for mild cognitive impairment in elderly ambulatory patients with cognitive complaints. *Aging Clin. Exp. Res.* 17, 374–379. doi: 10.1007/BF03324625

Refinetti, R., Lissen, G. C., and Halberg, F. (2007). Procedures for numerical analysis of circadian rhythms. *Biol. Rhythm Res.* 38, 275–325. doi: 10.1080/09291010600903692

Rey, A. (1964). *L'Examen Clinique en Psychologie*. Paris: Presses Universitaires de France.

Sarabia, J. A., Rol, M. A., Mendiola, P., and Madrid, J. A. (2008). Circadian rhythm of wrist temperature in normal-living subjects a candidate of new index of the circadian system. *Physiol. Behav.* 95, 570–580. doi: 10.1016/j.physbeh.2008.08.005

Schmidt, M. (1996). *Rey Auditory and Verbal Learning Test: A Handbook*. Los Angeles, CA: Western Psychological Services.

Sepulcre, J., Schultz, A. P., Sabuncu, M., Gomez-Isla, T., Chhatwal, J., Becker, A., et al. (2016). In vivo tau, amyloid, and gray matter profiles in the aging brain. *J. Neurosci.* 36, 7364–7374. doi: 10.1523/JNEUROSCI.0639-16.2016

Solomon, P. R., Hirschoff, A., Kelly, B., Relin, M., Brush, M., and Deveaux, R. D. (1998). A 7 minute neuron cognitive screening battery highly sensitive to Alzheimer's disease. *Arch. Neurol.* 55, 349–355. doi: 10.1001/archneur.55.3.349

Sprecher, K. E., Bendlin, B. B., Racine, A. M., Okonkwo, O. C., Christian, B. T., Koscik, R. L., et al. (2015). Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults. *Neurobiol. Aging* 36, 2568–2576. doi: 10.1016/j.neurobiolaging.2015.05.004

Spreen, O., and Benton, A. L. (1969). *Neurosensory Centre Comprehensive Examination for Aphasia: Manual of Directions*. Victoria, BC: University of Victoria, Neuropsychology Laboratory.

Stenfors, C. U. D., Hanson, L. M., Theorell, T., and Osika, W. S. (2016). Executive cognitive functionig and cardiac autonomic regulation in a population-based sample of working adults. *Front. Psychol.* 7:1536. doi: 10.3389/fpsyg.2016.01536

van Marken Lichtenbelt, W. D., Daanen, H. A., Wouters, L., Fronczek, R., Raymann, R. J., Severens, N. M., et al. (2006). Evaluation of wireless determination of skin temperature using iButtons. *Physiol. Behav.* 88, 489–497. doi: 10.1016/j.physbeh.2006.04.026

Vigo, D. E., Dominguez, J., Guinjoan, S. M., Scaramal, M., Ruffa, E., Solerno, J., et al. (2010). Nonlinear analysis of heart rate variability within independent frequency components during the sleep-wake cycle. *Auton. Neurosci.* 154, 84–88. doi: 10.1016/j.autneu.2009.10.007

Vigo, D. E., Guinjoan, S. M., Scaramal, M., Siri, L. N., and Cardinali, D. P. (2005). Wavelet transform shows age-related changes of heart rate variability within independent frequency components. *Auton. Neurosci.* 2005, 94–100. doi: 10.1016/j.autneu.2005.10.004

Vigo, D. E., Ogrinz, B., Wan, L., Bersenev, E., Tuerlinckx, F., Van den Bergh, O., et al. (2012). Sleep-wake differences in heart rate variability during a 105-day simulated mission to Mars. *Aviat. Space Environ. Med.* 83, 125–130. doi: 10.3357/ASEM.3120.2012

Walsh, C., Drinkenburg, W. H. I. M., and Ahnaou, A. (2017). Neurophysiological assessment of neural network plasticity and connectivity: progress towards early functional biomarkers for disease interception therapies in Alzheimer's disease. *Neurosci. Biobehav. Rev.* 73, 340–358. doi: 10.1016/j.neubiorev.2016.12.020

Wechsler, D. A. (1997). *Wechsler Adult Intelligence Scale—III*. New York, NY: Psychological Corporation.

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: 07 March 2017
doi: 10.3389/fnagi.2017.00054

Differences on Brain Connectivity in Adulthood Are Present in Subjects with Iron Deficiency Anemia in Infancy

Cecilia Algarin[1], Keerthana Deepti Karunakaran[2], Sussanne Reyes[1], Cristian Morales[1], Betsy Lozoff[3], Patricio Peirano[1] and Bharat Biswal[2]*

[1]*Sleep and Neurofunctional Laboratory, Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile,* [2]*Department of Biomedical Engineering, New Jersey Institute of Technology, University Heights, Newark, NJ, USA,* [3]*Center for Human Growth and Development, University of Michigan, Ann Arbor, MI, USA*

Iron deficiency continues to be the most prevalent micronutrient deficit worldwide. Since iron is involved in several processes including myelination, dopamine neurotransmission and neuronal metabolism, the presence of iron deficiency anemia (IDA) in infancy relates to long-lasting neurofunctional effects. There is scarce data regarding whether these effects would extend to former iron deficient anemic human adults. Resting state functional magnetic resonance imaging (fMRI) is a novel technique to explore patterns of functional connectivity. Default Mode Network (DMN), one of the resting state networks, is deeply involved in memory, social cognition and self-referential processes. The four core regions consistently identified in the DMN are the medial prefrontal cortex, posterior cingulate/retrosplenial cortex and left and right inferior parietal cortex. Therefore to investigate the DMN in former iron deficient anemic adults is a particularly useful approach to elucidate de long term effects on functional brain. We conducted this research to explore the connection between IDA in infancy and altered patterns of resting state brain functional networks in young adults. Resting-state fMRI studies were performed to 31 participants that belong to a follow-up study since infancy. Of them, 14 participants were former iron deficient anemic in infancy and 17 were controls, with mean age of 21.5 years (±1.5) and 54.8% were males. Resting-state fMRI protocol was used and the data was analyzed using the seed based connectivity statistical analysis to assess the DMN. We found that compared to controls, former iron deficient anemic subjects showed posterior DMN decreased connectivity to the left posterior cingulate cortex (PCC), whereas they exhibited increased anterior DMN connectivity to the right PCC. Differences between groups were also apparent in the left medial frontal gyrus, with former iron deficient anemic participants having increased connectivity with areas included in DMN and dorsal attention networks. These preliminary results suggest different patterns of functional connectivity between former iron deficient anemic and control young adults. Indeed, IDA in infancy, a common nutritional problem among human infants, may turn out to be important for understanding the mechanisms of cognitive alterations, common in adulthood.

Keywords: iron deficiency anemia, infancy, long-lasting effects, brain connectivity, resting state networks, default mode network

OPEN ACCESS

Edited by:
Agustin Ibanez, Institute of Cognitive and Translational Neuroscience, Argentina

Reviewed by:
Cathy W. Levenson, Florida State University, USA
James Connor, Penn State Milton S. Hershey Medical Center, USA

****Correspondence:***
Cecilia Algarin
calgarin@inta.uchile.cl

Received: *17 November 2016*
Accepted: *23 February 2017*
Published: *07 March 2017*

Citation:
Algarin C, Karunakaran KD, Reyes S, Morales C, Lozoff B, Peirano P and Biswal B (2017) Differences on Brain Connectivity in Adulthood Are Present in Subjects with Iron Deficiency Anemia in Infancy. Front. Aging Neurosci. 9:54. doi: 10.3389/fnagi.2017.00054

INTRODUCTION

Iron deficiency anemia (IDA) continues to be the world most prevalent micronutrient deficit. Its frequency is greater in children and pregnant women and can affect up to 70% of population in many countries, particularly economically developing countries (Swaminathan et al., 2013). Even in developed countries, the prevalence of IDA in children can be as high as 4% (Jáuregui-Lobera, 2014). In Chile, until as late as 1999, about 30% of the children under 2 years of age presented with IDA, often as a result of poor maternal health condition or poor dietary intake. Public health policies of "*fortification*" such as availability of formula milk at government health centers, caused prevalence of IDA in infants under 18 months of age to drop to 10% in a period of 10 years (Brito et al., 2013). Although the decreasing incidence is promising, it should be emphasized that iron is an important nutrient for brain development, and the impact of IDA in infancy development, even after iron therapy is of clinical relevance. Therefore, understanding the neural mechanisms through which the developing brain is coping with a nutrient deficiency may help improve therapeutic strategies aimed at optimizing brain functional integrity.

Studies using rodent models indicate that early IDA modifies myelin protein profile, specifically the proteolipid protein and MBP 21 (myelin basic protein), both of them required for myelin compaction (Ortiz et al., 2004). Further, alteration in genes regulating dendritic morphology appears to alter experience-depending brain synaptic plasticity, derailing thus memory, learning and other developmental milestones (Georgieff, 2008).

In line with these results, there is mounting evidence that IDA in human infants is associated with long-lasting negative outcomes on several neurofunctional and cognitive domains, extending into adolescence (Algarín et al., 2013). We have shown that children who were properly treated for IDA during infancy, had delayed latencies in auditory and visual evoked potentials at preschool age. This slower neuronal transmission in both sensory systems is consistent with an impaired myelination process (Algarín et al., 2003).

Animal models have shown that early iron deficiency modifies the dopaminergic neurotransmission system, altering the functioning of the hippocampus and frontal cortex, among other brain regions (Beard and Connor, 2003). Moreover in rats, dopamine synaptic connections develop quickly during the first 15 days of post-natal life; iron deficiency throughout this period would likely influence these synaptic connections (Ward et al., 2007). Dopaminergic neurotransmission system is critically involved in executive functions which encompass a heterogeneous repertoire of abilities related to monitoring and controlling thought and action, including self-regulation, inhibitory motor, cognitive control, planning future actions, attentional flexibility and error detection and correction capacity, among others (Prencipe et al., 2011; Lantrip et al., 2016). These higher cognitive functions have been well studied in humans using both event-related potentials (ERPs) and functional magnetic resonance imaging (fMRI).

Using ERPs, measure brain electrical activity when a subject performs a task, we found that at 10 years of age, former IDA (FIDA) children exhibited longer reaction time and lower P300 amplitude in the Go/No-Go task, which evaluates inhibitory motor control (Algarín et al., 2013). These results are consistent with subsequent research using a recognition memory paradigm (also at 10 years of age) that found slower reaction time and smaller ERP response to new/old tasks with an almost equal level of performance accuracy, suggesting that the effects of IDA in infancy persisted into late childhood despite apparently normal behavior (Congdon et al., 2012).

Neurophysiological and imaging assessments are complementary methods for the study of brain functions. There has been much progress regarding typical and atypical human brain activities at the group level using fMRI and resting state fMRI (rs-fMRI; Dosenbach et al., 2010). Resting state networks (RSN) are defined by a set of functionally interacting spatially distant regions at rest (Biswal, 2012). The networks are known to be organized in different functional systems that correlate to behavioral performance, allowing for further understanding of the significance of functional connectivity. Several studies on the developing brain show that the extent of brain regions exhibiting resting state functional connectivity increases from 2 weeks of age up to 2 years old (Lin et al., 2008). Fair et al. (2008) showed that functional connectivity between ventro-medial prefrontal cortex and posterior cingulate cortex (PCC) differs among children, adolescents and young adults, whereas, regions involved with motor and conflict monitoring differs little throughout these periods. These results indicate that the motor system and goal directed behavior develop earlier than other cognitive systems. A recent report from Marek et al. (2015) showed that network connectivity decreases in early adolescence (13–15 years) and then increases from late adolescence to adulthood, with the exception of the link between two of the most recognized networks, the Default Mode Network (DMN) and fronto-parietal network.

An important characteristic regarding the RSN, is the strength of the correlation between functional and structural connectivity. From rodent model, the results of the study conducted by Hübner et al. (2017) inducing brain demyelination, showed that functional connectivity was also altered, and the areas most affected were the posterior centers of DMN. In humans, myelination and functional connectivity develop parallel from birth until late adolescence (Stevens et al., 2009). Greicius et al. (2009) showed that there was not functional connectivity between hemispheres in absence of corpus callosum, reinforcing the concept that structural neural connections are required for functional connectivity. This hypothesis is further supported by studies showing that the strength of functional and structural connectivity is correlated in both healthy and patients with cognitive dysfunction (Hagmann et al., 2010). As an example, clinical evidence of the interaction between myelin disorder and functional connectivity alteration has been reported in patients with Multiple Sclerosis; subjects with an active lesion have increased functional connectivity that might be secondary to a compensatory mechanism for limiting and repairing the injury (Droby et al., 2016).

Although there is some data regarding long-lasting cognitive effects in FIDA human adults (Lukowski et al., 2010), to our knowledge, there are no studies using rs-fMRI studies. Given that the DMN is a core brain network, the assessment of DMN in FIDA adults is particularly imperative in order to understand the long-lasting effects of IDA in infancy on neural functioning in adulthood. To achieve this, we used resting state fMRI to compare baseline whole-brain connectivity in both groups. We hypothesized that FIDA participants will present altered brain networks connectivity relative to control participants.

MATERIALS AND METHODS

Participants were part of a longitudinal cohort study, which began recruitment in 1990 in order to conduct research on the behavioral, neuro-functional development and sleep-wake patterns effects of IDA in infancy. Six cognitive follow-ups were conducted at 12 and 18 months, 3, 5, 10 and 16 years old. The follow-ups included, among other measurements, cognitive assessment, academic achievements, socioeconomical and nutritional status. In brief, inclusion criteria were healthy full-term infants with birth weights ≥3.0 kg, no perinatal complications, and no acute or chronic illnesses. Infants were assessed for IDA at 6, 12 and 18 months. Anemia was defined as venous hemoglobin ≤100 g/L at 6 months and <110 g/L at 12 and 18 months. Iron deficiency was defined as at least two out of three iron measures in the iron-deficient range (mean cell volume <70 fl, erythrocyte protoporphyrin >100 μg/dL red blood cells [1.77 μmol/L], serum ferritin <12 μg/L). For each IDA infant, a non-anemic (venous hemoglobin ≥115 g/L) infant of the same age was randomly selected, constituting the "control group". After initial testing, all infants were given supplemental iron for 6–12 months (Lozoff et al., 2003). No participant presented IDA thereafter.

In year 2014, all the cohort members who participated in the previous follow-ups and were approximately 22 years old were invited to perform a brain magnetic resonance. The present study involved 31 healthy young adult participants (17 controls and 14 FIDA) with a mean age of 21.5 years (±1.5 years), and 55% were male.

For current and background characteristics of the subjects we compared the Graffar scale (socio-ecomomical status; Richaud et al., 2013), performed the Spanish version of Manual for the State-Trait Anxiety Inventory (STAI "Self-Evaluation Questionnaire"; Guillén-Riquelme and Buela-Casal, 2011), and established current occupation (employee, studying or unemployed).

The research protocol was approved by the Institutional Review Boards of the University of Michigan Medical Center, Ann Arbor, Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, and the Office of Protection from Research Risks, NIH. Participants signed an informed consent.

Imaging Parameters

fMRI was performed using Siemens Magnetom Skyra 3T scanner with parameters at TR = 2000 ms, TE = 35 ms with 16, 7.040-mm slices, 1-mm slice gap, and an in-plane resolution of 2.553 × 2.553 mm^2. Total of 200 time points were collected for every subject. For the anatomical T1 scan, imaging was performed on the same scanner with TR = 20 ms, TE = 4.92 ms with 303 slices and field of view (FOV) of 192 × 499 mm^2.

Preprocessing

For all participants functional and anatomical image preprocessing was performed using statistical parametric mapping software (SPM 8 implemented using Matlab R2012b). After manually performing anterior commissure alignment for each participant, a realignment step was accomplished to correct for head motion. During this step, all scans of the participant were realigned to their first scan of the first session. The details of the transformation in six directions (*x*, *y*, *z*, roll, pitch and yaw) were stored as translation and rotation parameters. All participants were within the acceptable head motion range (less than 2 mm). After realignment, functional images were co-registered to the anatomical image (T1-weighted image) of that participant. Following successful co-registration of anatomical and functional images, segmentation was performed with SPM8 toolbox, and a probability map of gray matter, white matter (WM) and cerebrospinal fluid (CSF) was obtained. Simultaneously, a field vector storing information on the transformation from native subject space to standard template (Montreal Neurologic Institute, MNI template) was calculated. For each participant, the functional image was transformed to MNI space based on the deformation field and re-sampled to 3 mm isotropic voxel size. A binary mask of WM and CSF was created when a threshold for the WM/CSF maps obtained from segmentation step at $p > 0.98$ was applied (Biswal et al., 2010). In order to remove the confounding effects of WM, CSF and head motion, they were included as nuisance variables in linear regression model. The nuisance variable for linear regression included five principal components of WM and CSF obtained from WM and CSF masks. The regression input also included six time series describing head motion in six directions, six time series describing head motion in previous time points and 12 time series describing quadratics

TABLE 1 | Characteristics of Control and FIDA groups.

	Control (*n* = 17)	FIDA (*n* = 14)	*p*-value
Age (year)	21.3 (20.9–21.5)	21.2 (21.0–21.6)	0.150
Males (%)[a]	8 (47)	9 (64)	0.337
Occupation[b]			
Employed (%)	13 (42)	6 (19)	0.145
Student (%)	4 (13)	5 (16)	
Unemployed (%)	0 (0)	3 (10)	
STAI scale			
Negative emotion	29.1 (26.5–31.7)	26.0 (23.1–28.8)	0.925
Positive emotion	8.8 (7.3–10.2)	10.5 (8.8–12.1)	0.173
Anxiety	6.2 (5.0–7.4)	6.7 (5.4–8.0)	0.601
Socioeconomic status			
Graffar score (infancy)	28.6 (24.3–32.9)	28.9 (25.0–32.8)	0.916
Graffar score (5 years)	26.3 (22.9 29.7)	24.5 (21.4–27.6)	0.426
Graffar score (15 years)	34.0 (30.8–37.1)	33.6 (30.8–36.4)	0.865

Abbreviations: FIDA, former iron-deficiency anemia; STAI, state-trait anxiety inventory. Values are expressed as mean (confidence intervals). T-test, [a]chi square test, [b]and estimating missing cell frequencies (Graf et al., 1997).

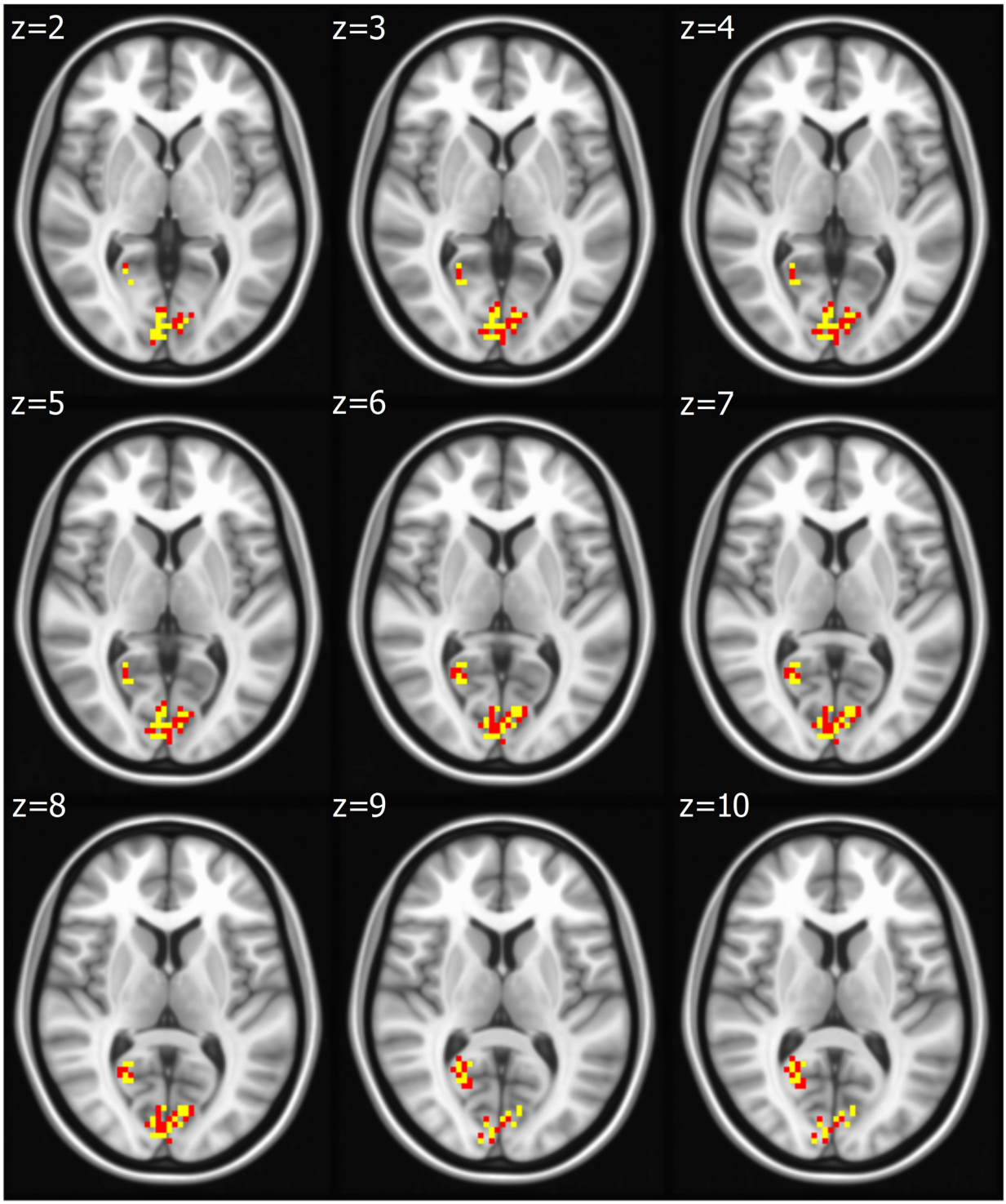


FIGURE 1 | Right posterior cingulate cortex (PCC) connectivity in Controls. Right PCC connectivity greater in Control group. Red indicates regions with significance $p < 0.02$ and yellow indicates regions with significance $p < 0.005$.

of motion. The residual of linear regression was temporally bandpass filtered at 0.01–0.1 Hz and spatially smoothened using a Gaussian kernel of 6 mm full width at half maximum. This filtered and smoothened data was used for further analysis. Acquisition, processing and analysis were performed to whole brain.

Seed Based Connectivity

To analyze the intrinsic functional connectivity of PCC with the whole brain, two spheres of 5 mm radius was generated and used as a seed for left and right PCC seeds using MNI coordinates. RSN were obtained from coherent fluctuations of 0.01–0.1 Hz frequency occurring at spatially distinct regions through underlying monosynaptic or polysynaptic anatomical connections. Therefore, such RSN can be derived using any seed within a network. Seeds of 5 mm radius were also generated for left and right medial frontal gyrus (MFG), another major component of DMN. For all four seeds, the average blood oxygen level-dependent (BOLD) time series was calculated for each seed region and a whole brain voxel wise Pearson's r correlation was performed for each subject. Subject level correlation maps were later converted to z-maps using r-z Fisher transformation.

Regions of Interest (ROI)-Based Connectivity

In addition to seed based correlation of the PCC and MFG, a total of 160 regions of interests (ROIs) were created based on the list defined by Dosenbach et al. (2010). All ROIs were sorted based on RSN's: 1. Cerebellar, 2. Cingulo-Opercular, 3. Default mode, 4. Fronto-Parietal, 5. Occipital, and 6. Sensoriomotor networks. Using the predefined MNI coordinates, a 5 mm radius sphere was created surrounding the peak voxel sampled at the same voxel dimensions of participants BOLD fMRI image. Nine ROIs were discarded as they were not within the brain mask of at least one subject. Using the remaining 151 ROI as mask, an average BOLD signal for each ROI was calculated for all participants. Pearson's r correlations were calculated between the BOLD signals of each ROI with remaining ROI. Hence a 151*151 correlation matrix was generated for each participant. Each 151*151 correlation matrix was transformed into Z-matrices using Fisher's r-z transformation.

Statistical Analysis

Seed Based analysis

The z-maps were concatenated to create two 4D functional z-maps, one for each group. An unpaired two-sample two tailed *t*-test was performed on the 4D inputs with gender as covariate using Functional MRI of the Brain Software Library randomized implementation. All statistical comparisons were performed only for voxels that were present across all subjects. Further, Monte-Carlo simulation was implemented to perform cluster based thresholding on the uncorrected voxel-wise p-maps to correct for multiple comparison problem.

ROI Based Connectivity

An independent sample *t*-test was performed on the ROI pairs to compare the connectivity between FIDA and control groups at $p < 0.001$. Due to the small sample size, the statistical threshold was set to lenient with no additional correction for multiple comparison problems.

RESULTS

Control and FIDA groups were similar in current characteristics, including socioeconomic status, emotion scales and gender (**Table 1**).

Seed Based Connectivity Analysis

Posterior Cingulate Cortex

Seed based analysis of left and right PCC resulted in a connectivity map involving core anatomical regions of DMN, including bilateral ventral medial prefrontal cortex, posterior cingulate/retrosplenial cortex, lateral temporal cortex, dorsal medial prefrontal cortex and hippocampal formation. Randomization method performed on both left and right PCC z-maps resulted in voxel-based p-maps for contrast 1) Control > FIDA and 2) FIDA > Control. P-maps of right PCC thresholded at $p < 0.02$ with Monte Carlo cluster size = 38.9 voxels resulted in two clusters greater in Control (**Figure 1** and **Table 2**) and one cluster greater in FIDA (**Figure 2** and **Table 2**) participants. Similarly, randomization results of left PCC threshold at $p < 0.02$ with Monte Carlo cluster size = 40.3 voxels resulted in five clusters greater in Controls and no clusters greater in FIDA (**Figure 3** and **Table 3**). Regions showing decreased connectivity to left and right PCC in FIDA group included regions of posterior DMN such as cuneus, PCC, parahippocampal gyrus, medial temporal gyrus and lingual gyrus. In contrast, regions presenting increased connectivity to right PCC in FIDA group included regions of anterior DMN and anterior cingulate cortex.

Medial Frontal Gyrus (MFG)

Seed based analysis of left and right MFG resulted in a connectivity map involving core anatomical regions of DMN. Randomization method performed on both left and right MFG z-maps resulted in voxel-based p-maps for contrasts 1)

TABLE 2 | Connectivity to right posterior cingulate cortex (PCC) in Control and FIDA groups.

Control group > FIDA group		
Cluster size (voxels)	**Region**	***X, Y, Z* coordinates in TLRC space**
140 (36)	Right lingual gyrus (R.cuneus)	+3.0, −85.0, +1.0 (+6, −88, +11)
48	Right posterior cingulate cortex	+18.0, −58.0, +16.0
FIDA group > Control group		
Cluster size (voxels)	**Region**	***X, Y, Z* coordinates in TLRC space**
89 (19)	Left anterior cingulate cortex (L.ACC)	−3.0, +29.0, +7.0 (−9, +38, +16)

List of clusters with significantly greater connectivity to right PCC (−10, +54, +14) in 1) Control group and 2) FIDA group at voxel wise threshold $p < 0.02$ with multiple comparison correction using cluster threshold = 38.9 voxels. Values within parenthesis indicate regions with significance ($p < 0.005$ at cluster size = 16.7).

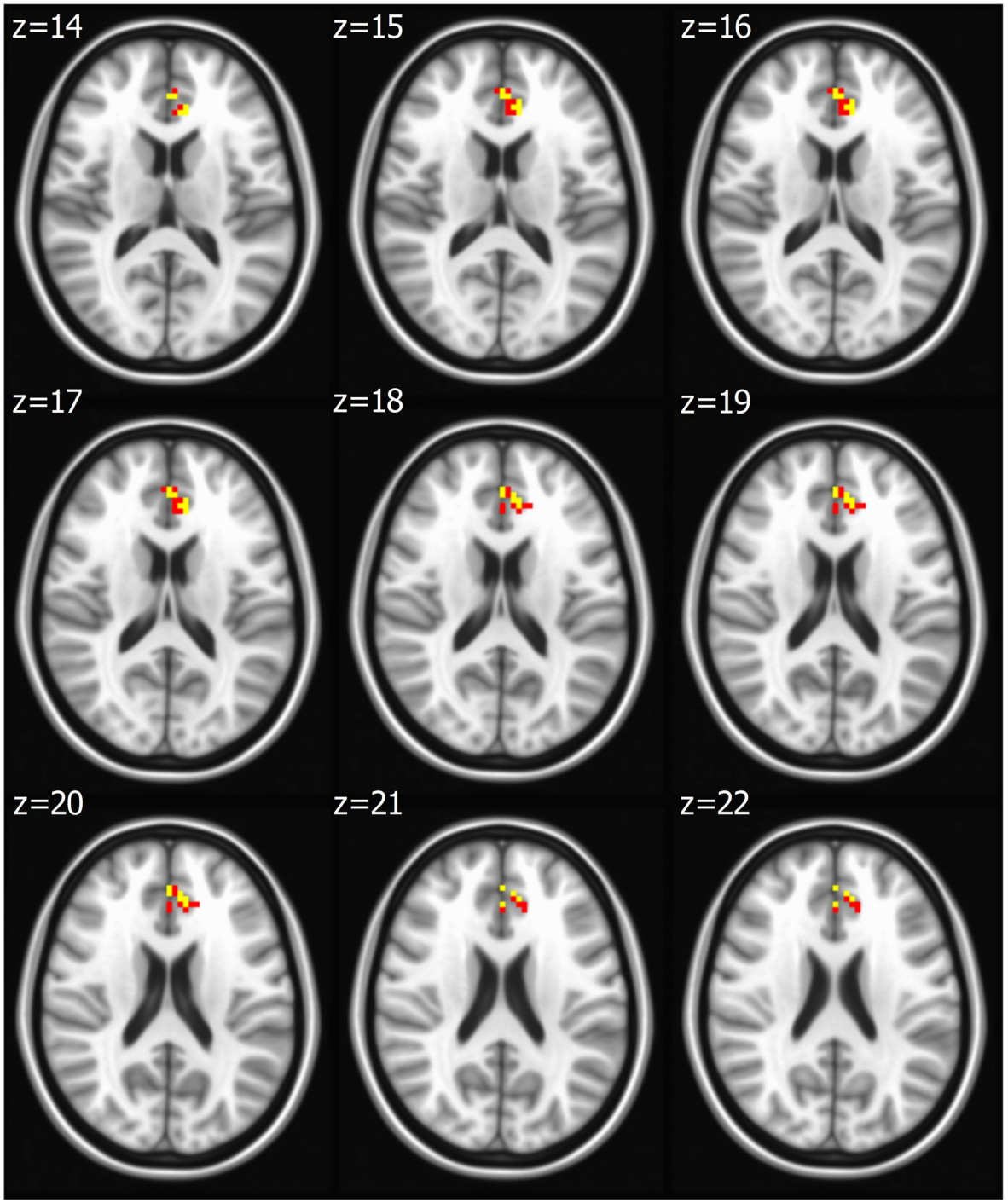


FIGURE 2 | Right PCC connectivity in former iron-deficiency anemia (FIDA). Right PCC connectivity greater in FIDA group. Red indicates regions with significance $p < 0.02$ and yellow indicates regions with significance $p < 0.005$.

Control > FIDA and 2) FIDA> Control. P-maps of both left and right MFG in the contrast Control > FIDA were not statistically significant after Monte Carlo cluster thresholding at $p < 0.02$. However, the FIDA > Control contrast condition of left MFG threshold at $p < 0.02$ with Monte Carlo cluster size = 38.3 voxels resulted in nine clusters greater in FIDA. The regions exhibiting increased connectivity to left MFG in FIDA group included DMN and dorsal attention networks. The list of regions and their coordinates mainly includes the intraparietal lobule and precuneus (**Table 4** and **Figure 4**).

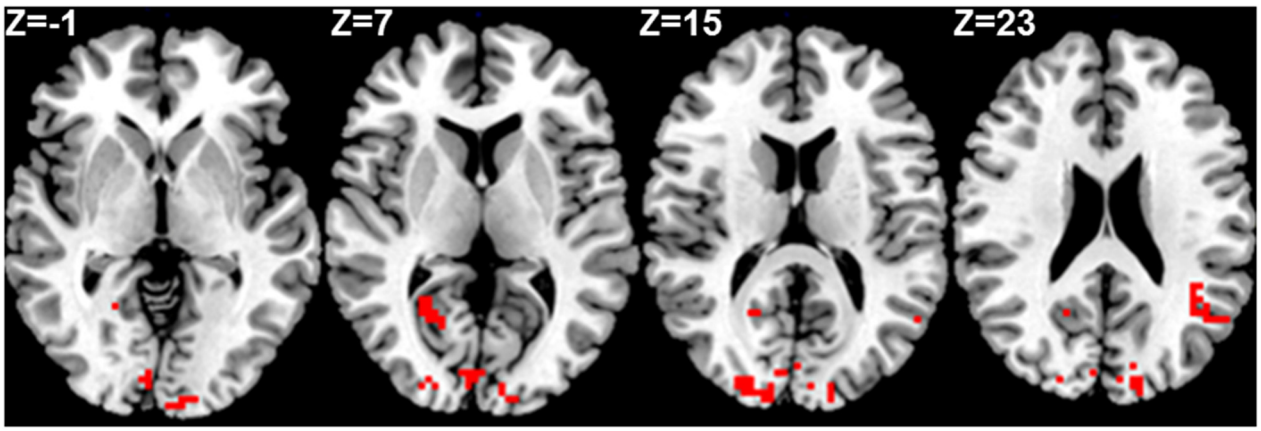


FIGURE 3 | Left PCC connectivity in Control group. Red indicates regions with significance $p < 0.005$.

ROI Based Connectivity Analysis

Control and FIDA groups showed greater connectivity within Cerebellum, DMN and Occipital networks as compared to between network connectivity. Based on the connectivity matrix, FIDA participants presented a scattered decrease in negative connectivity between Cingulo-opercular, Fronto-parietal and Sensory motor networks with DMN, and decrease in positive connectivity within DMN network. Otherwise, they exhibited a sparse increase in positive connectivity within Cingulo-opercular and Fronto-parietal networks (**Figure 5**).

DISCUSSION

These preliminary findings based on specific rs-fMRI assessments suggest different patterns of functional connectivity between FIDA and control young adults.

Seed based connectivity results show that FIDA participants have decreased connectivity of the left PCC with posterior DMN regions, relative to control participants. The PCC is a highly connected and metabolically active brain region and a component of several RSN. The link between PCC and DMN is of particular importance due to the possibility of the PCC's role as a "hub" interacting with multiple brain networks to allocate cognitive resources to different tasks (Leech and Sharp, 2014).

Our findings are reminiscent of DMN patterns observed in patients with neurodegenerative diseases, as Alzheimer disease, frontotemporal dementia, multiple sclerosis, autism and Parkinson disease (Sandrone and Catani, 2013). In fact, DMN regions showing decreased connectivity to left and right PCC in FIDA group included regions of posterior DMN such as cuneus, PCC, and parahippocampal gyrus, is also found in a majority of patients with Alzheimer disease and other types of dementia (Chhatwal et al., 2013).

Furthermore, several studies of subjects at high risk for these disorders, including healthy relatives of patients with late onset of Alzheimer disease, have demonstrated that DMN patterns, particularly PCC and medial temporal cortex changes, are present before the disorder's onset and could even early marker of the disease (Wang et al., 2012). For instance, a study in autosomal dominant Alzheimer disease showed alterations in DMN connectivity in both symptomatic and asymptomatic carriers of pathogenic mutations, with a higher connectivity

TABLE 3 | Connectivity to left PCC in Control group.

Control group > FIDA group		
Cluster size (voxels)	**Region**	***X, Y, Z* coordinates in TLRC space**
61	Left cuneus	−15.0, −94.0, +6.0
46	Left cuneus	−0.0, −79.0, +19.0
46	Left middle temporal gyrus	−50.0, −58.0, +23.0
45	Right parahippocampal gyrus	+24.0, −52.0, +7.0
42	Right cuneus	+21.0, −91.0, +13.0

List of clusters with significantly greater connectivity to left PCC (+10, +54, +14) in control group at voxel wise threshold $p < 0.02$ with multiple comparison correction using cluster threshold = 40.3 voxels.

TABLE 4 | Connectivity to left medial frontal gyrus (MFG) in FIDA group.

Cluster size (voxels)	Region	*X, Y, Z* coordinates in TLRC space
93	Left inferior parietal lobule	−50.0, −35.0, +28.0
77	Right cuneus	+27.0, −79.0, +21.0
73	Left middle frontal gyrus	−33.0, +23.0, +22.0
73	Left cuneus	−21.0, −91.0, +22.0
52 (20)	Right posterior cingulate cortex (R.PCC)	+12.0, −55.0, +16.0 (+12, −55, +16)
51	Left precuneus	−30.0, −61.0, +34.0
50	Left cuneus	−3.0, +82.0, +34.0
45	Left paracentral lobule	−3.0, −34.0, +49.0
41	Right superior parietal lobule	+33.0, −52.0, +58.0

List of clusters with significantly greater connectivity to left medial frontal gyrus (+6, −47, −5) in FIDA group at voxel wise threshold $p < 0.02$ with multiple comparisons correction using cluster threshold = 39.4 voxels. Values within parenthesis indicate regions significant at $p < 0.005$ at cluster size = 16.7.

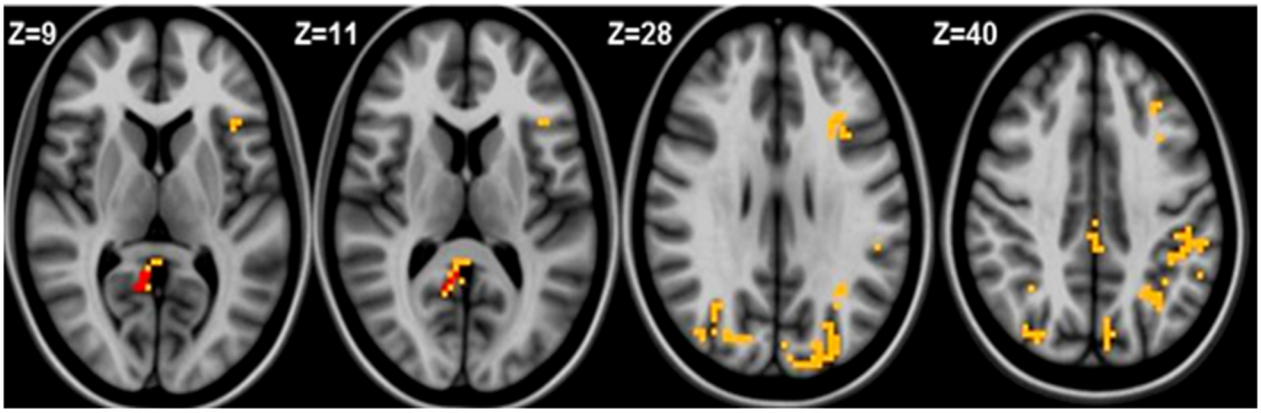


FIGURE 4 | Left medial frontal gyrus (MFG) connectivity in FIDA group. Red indicates regions with significance $p < 0.005$.

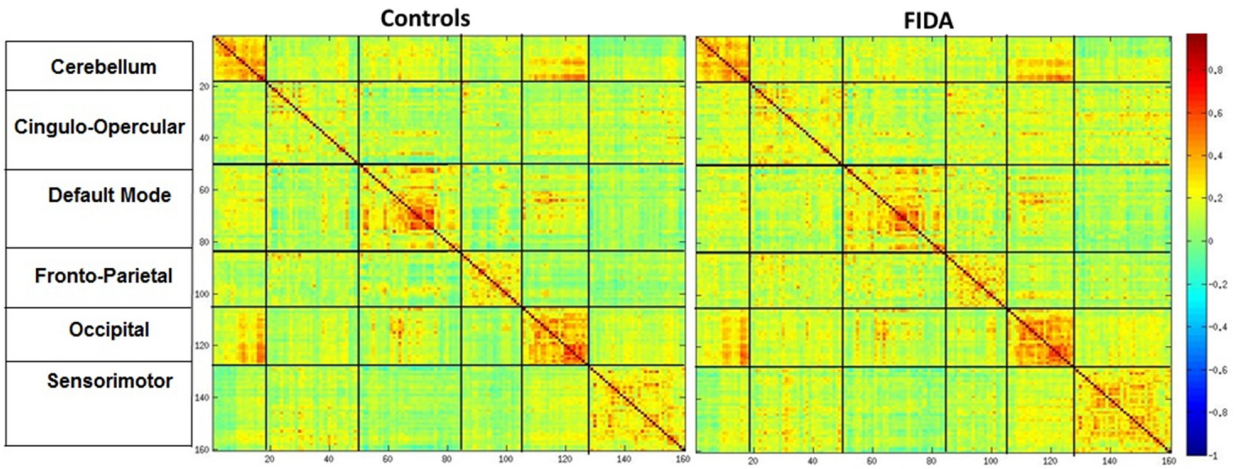


FIGURE 5 | Mean connectivity matrix. Mean Connectivity matrix in Controls and FIDA groups. Color bar indicates Pearsons r correlation ranging from 1 to −1, hot colors indicating positive correlation between two regions. Vertical and horizontal partitions represent functional connectivity networks.

decrease in symptomatic carriers. Of note, subtle decreases in DMN connectivity were observable before symptom onset (Chhatwal et al., 2013).

Otherwise from the field of cognitive development, individuals with attention-deficit and hyperactivity disorder—a condition related to impairment of dopamine neurotransmission system—are characterized by a decreased connectivity between precuneus and other DMN components (Somandepalli et al., 2015), and a negative relationship between the activity in dorsal anterior cingulate cortex and DMN (Castellanos et al., 2008). Correspondingly, studies in patients with schizophrenia, another illness associated with an alteration in the dopamine system, show decreased strength in DMN nodes connectivity, suggesting that these patterns could underlie symptoms such as impaired self-awareness (Dodell-Feder et al., 2014). A recent study in healthy subjects compared the neurological soft signs (NSS) score and the grade of connectivity among cortical and subcortical areas related to motor function. The results showed a negative correlation between NSS score (lower score means better performance) and resting state-activity of right posterior cingulate. NSS have been found in psychiatric disorders of neurodevelopmental origin such as schizophrenia, autism and borderline personality, among others conditions that involve dopamine and myelin alterations (Thomann et al., 2015).

Throughout brain development network properties increase integration and decrease segregation, thus WM maturation might play an important role in circuit formation or changes in their strengths (Hagmann et al., 2010). There are several studies showing a close relationship and a highly complex interaction between structural connectivity (myelin tracts) and functional connectivity (Greicius et al., 2009). Based on these results we speculate that a derailment in myelination process may reshape

the connectivity map and explain in part, our finding of a connectivity absence in the posterior region of DMN in FIDA participants.

The effects of IDA in infancy on dopamine neurotransmission system could be explore assessing the executive functions, i.e., inhibitory control, set-shifting and planning, high cognitive abilities that relies on the integrity of frontostriatal circuits, in which the dopaminergic system plays a key role. At 10 years old, FIDA participants from our longitudinal study, showed a mild impairment performing an inhibitory motor task (Algarín et al., 2013). A follow-up study performed in Costa Rica indicated that FIDA at 19 years presented greater difficulty performing executive functions test (Lukowski et al., 2010). In the present study at 22 years old, a decreased DMN connectivity characterized FIDA subjects. Therefore, it is reasonable to argue that different DMN patterns in FIDA young adults might also refer to a dopaminergic dysregulation.

The MFG—another key node of DMN (Passow et al., 2015)—is involved in decision making and reasoning processes (Talati and Hirsch, 2005). In the present study the FIDA group demonstrated increased connectivity to the left MFG including DMN and dorsal attention networks, primarily included intraparietal lobule and precuneus. Sang et al. (2015) reported greater nodal centrality in MFG and parietal lobule regions in patients with Parkinson disease and proposed that these areas are highly connected to compensate for deficits caused by the disease.

Given that we did not perform a task based fMRI, it is not possible to determine whether the increased connectivity between brain circuits at rest relates to a decreased activation or suppression of DMN during tasks requiring higher cognitive resources. However, we could suggest that differences in intrinsic functional organization in the resting state, may contribute to change the interactions among specific brain regions while performing a task.

Finally, a recent study in preschool age children showed that early life stressful events are associated with greater activity of rs-fMRI in left middle frontal gyrus, independent of other variables as poverty, violence exposure and exposure to a traumatic event (Demir-Lira et al., 2016). These results provide further support for the hypothesis that environment factors like micronutrient deficits (IDA), could generate persistent effects on neurofunctional domains, including resting state connectivity, more than 20 years after the nutritional insult (Lozoff et al., 2003).

An important limitation for the interpretation of the results is the lower spatial resolution of axial slicing of rs-fMRI and the small sample size. Notwithstanding, differences in brain connectivity between groups in specific regions were significant. The correlation analysis among connectivity results and behavioral tasks are not presented in this manuscript. Given the small sample size, one of our goals is to increase the number of participants to correlate rs-fMRI findings with behavioral results in future studies.

In conclusion, the results of the rs-fMRI study show altered brain connectivity patterns in otherwise healthy FIDA young adults. Based on the hypothesis of long-lasting effects of IDA in infancy on myelination and dopaminergic system functioning, it should be possible to design studies with specific fMRI imaging measures known to depend on these systems and processes. Whether altered brain connectivity in FIDA participants influences the progression of specific cognitive functions and neurodegenerative diseases with advancing age is part of our ongoing research challenges.

AUTHOR CONTRIBUTIONS

CA conceptualized and designed the study, drafted the initial manuscript, and participated in critical revision of the manuscript. KDK carried out the initial analyses, and participated in critical revision of the manuscript. SR and CM led the participant recruitment, coordinated and supervised data collection and participated in critical revision of the manuscript. BL and PP conceptualized the study and critically reviewed the manuscript. BB designed the data collection tools, conceptualized the study and critically reviewed the manuscript. All authors approved the final manuscript as submitted.

FUNDING

This study received a grant from the National Institutes of Health, Bethesda, MD, USA (R01 HD33487). The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Child Health and Human Development or the National Institutes of Health.

ACKNOWLEDGMENTS

We would like to express our gratitude to the young adults whose ongoing participation made this follow-up possible. We also thank the technicians from Neuroradiology Unit, Clínica Alemana, Santiago, for providing facilities to perform fMRI studies during the course of this research. Drivers are also acknowledged for providing careful transportation to participants.

REFERENCES

Algarín, C., Nelson, C. A., Peirano, P., Westerlund, A., Reyes, S., and Lozoff, B. (2013). Iron-deficiency anemia in infancy and poorer cognitive inhibitory control at age 10 years. *Dev. Med. Child Neurol.* 55, 453–458. doi: 10.1111/dmcn.12118

Algarín, C., Peirano, P., Garrido, M., Pizarro, F., and Lozoff, B. (2003). Iron deficiency anemia in infancy: long-lasting effects on auditory and visual system functioning. *Pediatr. Res.* 53, 217–223. doi: 10.1203/00006450-200302000-00004

Beard, J. L., and Connor, J. R. (2003). Iron status and neural functioning. *Annu. Rev. Nutr.* 23, 41–58. doi: 10.1146/annurev.nutr.23.020102.075739

Biswal, B. B. (2012). Resting state fMRI: a personal history. *Neuroimage* 62, 938–944. doi: 10.1016/j.neuroimage.2012.01.090

Biswal, B. B., Mennes, M., Zuo, X. N., Gohel, S., Kelly, C., Smith, S. M., et al. (2010). Toward discovery science of human brain function.

Proc. Natl. Acad. Sci. U S A 107, 4734–4739. doi: 10.1073/pnas.0911855107

Brito, A., Olivares, M., Pizarro, T., Rodríguez, L., and Hertrampf, E. (2013). Chilean complementary feeding program reduces anemia and improves iron status in children aged 11 to 18 months. *Food Nutr. Bull.* 34, 378–385. doi: 10.1177/156482651303400402

Castellanos, F. X., Margulies, D. S., Kelly, C., Uddin, L. Q., Ghaffari, M., Kirsch, A., et al. (2008). Cingulate-precuneus interactions: a new locus of dysfunction in adult attention-deficit/hyperactivity disorder. *Biol. Psychiatry* 63, 332–337. doi: 10.1016/j.biopsych.2007.06.025

Chhatwal, J. P., Schultz, A. P., Johnson, K., Benzinger, T. L., Jack, C. J. Jr., Ances, B. M., et al. (2013). Impaired default network functional connectivity in autosomal dominant Alzheimer disease. *Neurology* 81, 736–744. doi: 10.1212/WNL.0b013e3182a1aafe

Congdon, E. L., Westerlund, A., Algarin, C. R., Peirano, P. D., Gregas, M., Lozoff, B., et al. (2012). Iron deficiency in infancy is associated with altered neural correlates of recognition memory at 10 years. *J. Pediatr.* 160, 1027–1033. doi: 10.1016/j.jpeds.2011.12.011

Demir-Lira, Ö. E., Voss, J. L., O'Neil, J. T., Briggs-Gowan, M. J., Wakschlag, L. S., and Booth, J. R. (2016). Early-life stress exposure associated with altered prefrontal resting-state fMRI connectivity in young children. *Dev. Cogn. Neurosci.* 19, 107–114. doi: 10.1016/j.dcn.2016.02.003

Dodell-Feder, D., DeLisi, L. E., and Hooker, C. I. (2014). The relationship between default mode network connectivity and social functioning in individuals at familial high-risk for schizophrenia. *Schizophr. Res.* 156, 87–95. doi: 10.1016/j.schres.2014.03.031

Dosenbach, N. U. F., Nardos, B., Cohen, A. L., Fair, D. A., Power, J. D., Church, J. A., et al. (2010). Prediction of individual brain maturity using fMRI. *Science* 329, 1358–1361. doi: 10.1126/science.1194144

Droby, A., Yuen, K. S. L., Muthuraman, M., Reitz, S.-C., Fleischer, V., Klein, J., et al. (2016). Changes in brain functional connectivity patterns are driven by an individual lesion in MS: a resting-state fMRI study. *Brain Imaging Behav.* 10, 1117–1126. doi: 10.1007/s11682-015-9476-3

Fair, D. A., Cohen, A. L., Dosenbach, N. U. F., Church, J. A., Miezin, F. M., Barch, D. M., et al. (2008). The maturing architecture of the brain's default network. *Proc. Natl. Acad. Sci. U S A* 105, 4028–4032. doi: 10.1073/pnas.0800376105

Georgieff, M. K. (2008). The role of iron in neurodevelopment: fetal iron deficiency and the developing hippocampus. *Biochem. Soc. Trans.* 36, 1267–1271. doi: 10.1042/BST0361267

Graf, R. G., Alf, E. F., Williams, S., and Okolo, I. (1997). A computer program for estimating missing cell frequencies in chi square tests for association. *InterStat.* [Accessed July 14, 2016].

Greicius, M. D., Supekar, K., Menon, V., and Dougherty, R. F. (2009). Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cereb. Cortex* 19, 72–78. doi: 10.1093/cercor/bhn059

Guillén-Riquelme, A., and Buela-Casal, G. (2011). Actualización psicométrica y funcionamiento diferencial de los items en el state trait anxiety inventory (STAI). *Psicothema* 23, 510–515.

Hagmann, P., Sporns, O., Madan, N., Cammoun, L., Pienaar, R., Wedeen, V. J., et al. (2010). White matter maturation reshapes structural connectivity in the late developing human brain. *Proc. Natl. Acad. Sci. U S A* 107, 19067–19072. doi: 10.1073/pnas.1009073107

Hübner, N. S., Mechling, A. E., Lee, H.-L., Reisert, M., Bienert, T., Hennig, J., et al. (2017). The connectomics of brain demyelination: functional and structural patterns in the cuprizone mouse model. *Neuroimage* 146, 1–18. doi: 10.1016/j.neuroimage.2016.11.008

Jáuregui-Lobera, I. (2014). Iron deficiency and cognitive functions. *Neuropsychiatr. Dis. Treat.* 10, 2087–2095. doi: 10.2147/NDT.s72491

Lantrip, C., Isquith, P. K., Koven, N. S., Welsh, K., and Roth, R. M. (2016). Executive function and emotion regulation strategy use in adolescents. *Appl. Neuropsychol. Child* 5, 50–55. doi: 10.1080/21622965.2014.960567

Leech, R., and Sharp, D. J. (2014). The role of the posterior cingulate cortex in cognition and disease. *Brain* 137, 12–32. doi: 10.1093/brain/awt162

Lin, W., Zhu, Q., Gao, W., Chen, Y., Toh, C., Styner, M., et al. (2008). Functional connectivity MR imaging reveals cortical functional connectivity in the developing brain. *Am. J. Neuroradiol.* 29, 1883–1889. doi: 10.3174/ajnr.a1256

Lozoff, B., De Andraca, I., Castillo, M., Smith, J. B., Walter, T., and Pino, P. (2003). Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. *Pediatrics* 112, 846–854.

Lukowski, A. F., Koss, M., Burden, M. J., Jonides, J., Nelson, C. A., Kaciroti, N., et al. (2010). Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long-term deficits in executive function and recognition memory. *Nutr. Neurosci.* 13, 54–70. doi: 10.1179/147683010X12611460763689

Marek, S., Hwang, K., Foran, W., Hallquist, M. N., and Luna, B. (2015). The contribution of network organization and integration to the development of cognitive control. *PLoS Biol.* 13:e1002328. doi: 10.1371/journal.pbio.1002328

Ortiz, E., Pasquini, J., Thompson, K., Felt, B., Butkus, G., Beard, J., et al. (2004). Effect of manipulation of iron storage, transport, or availability on myelin composition and brain iron content in three different animal models. *J. Neurosci. Res.* 77, 681–689. doi: 10.1002/jnr.20207

Passow, S., Specht, K., Adamsen, T. C., Biermann, M., Brekke, N., Craven, A. R., et al. (2015). Default-mode network functional connectivity is closely related to metabolic activity. *Hum. Brain Mapp.* 36, 2027–2038. doi: 10.1002/hbm.22753

Prencipe, A., Kesek, A., Cohen, J., Lamm, C., Lewis, M. D., and Zelazo, P. D. (2011). Development of hot and cool executive function during the transition to adolescence. *J. Exp. Child. Psychol.* 108, 621–637. doi: 10.1016/j.jecp.2010.09.008

Richaud, M., Mestre, M., Lemos, V., Tur, A., Ghiglione, M., and Samper, P. (2013). La influencia de la cultura en los estilos parentales en contextos de vulnerabilidad social. *Av. Psicol. Latinoam.* 31, 419–431.

Sandrone, S., and Catani, M. (2013). Journal Club. Default-mode network connectivity in cognitively unimpaired patients with Parkinson disease. *Neurology* 81, e172–e175. doi: 10.1212/01.wnl.0000436943.62904.09

Sang, L., Zhang, J., Wang, L., Zhang, J., Zhang, Y., Li, P., et al. (2015). Alteration of brain functional networks in early-stage parkinson's disease: a resting-state fMRI study. *PLoS One* 10:e0141815. doi: 10.1371/journal.pone.0141815

Somandepalli, K., Kelly, C., Reiss, P. T., Zuo, X.-N., Craddock, R., Yan, C.-G., et al. (2015). Short-term test-retest reliability of resting state fMRI metrics in children with and without attention-deficit/hyperactivity disorder. *Dev. Cogn. Neurosci.* 15, 83–93. doi: 10.1016/j.dcn.2015.08.003

Stevens, M. C., Skudlarski, P., Pearlson, G. D., and Calhoun, V. D. (2009). Age-related cognitive gains are mediated by the effects of white matter development on brain network integration. *Neuroimage* 48, 738–746. doi: 10.1016/j.neuroimage.2009.06.065

Swaminathan, S., Edward, B. S., and Kurpad, A. V. (2013). Micronutrient deficiency and cognitive and physical performance in Indian children. *Eur. J. Clin. Nutr.* 67, 467–474. doi: 10.1038/ejcn.2013.14

Talati, A., and Hirsch, J. (2005). Functional specialization within the medial frontal gyrus for perceptual go/no-go decisions based on "what," "when," and "where" related information: an fMRI study. *J. Cogn. Neurosci.* 17, 981–993. doi: 10.1162/0898929054475226

Thomann, P. A., Hirjak, D., Kubera, K. M., Stieltjes, B., and Wolf, R. C. (2015). Neural network activity and neurological soft signs in healthy adults. *Behav. Brain Res.* 278, 514–519. doi: 10.1016/j.bbr.2014.10.044

Wang, L., Roe, C. M., Snyder, A. Z., Brier, M. R., Thomas, J. B., Xiong, C., et al. (2012). Alzheimer disease family history impacts resting state functional connectivity. *Ann. Neurol.* 72, 571–577. doi: 10.1002/ana.23643

Ward, K. L., Tkac, I., Jing, Y., Felt, B., Beard, J., Connor, J., et al. (2007). Gestational and lactational iron deficiency alters the developing striatal metabolome and associated behaviors in young rats. *J. Nutr.* 137, 1043–1049.

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 December 2016
doi: 10.3389/fnagi.2016.00299

Mitochondrial Functional Changes Characterization in Young and Senescent Human Adipose Derived MSCs

*Bernd R. Stab II[1], Laura Martinez[1], Adriana Grismaldo[1], Alejandra Lerma[1], María L. Gutiérrez[1,2], Luis A. Barrera[2], Jhon J. Sutachan[1] and Sonia L. Albarracín[1]**

[1] Department of Nutrition and Biochemistry, School of Sciences, Pontificia Universidad Javeriana, Bogotá, Colombia, [2] Institute for the Study of Inborn Errors of Metabolism, Faculty of Sciences, Pontificia Universidad Javeriana, Bogotá, Colombia

Mitochondria are highly dynamic organelles that in response to the cell's bio-energetic state continuously undergo structural remodeling fission and fusion processes. This mitochondrial dynamic activity has been implicated in cell cycle, autophagy, and age-related diseases. Adult tissue-derived mesenchymal stromal/stem cells present a therapeutic potential. However, to obtain an adequate mesenchymal stromal/stem cell number for clinical use, extensive *in vitro* expansion is required. Unfortunately, these cells undergo replicative senescence rapidly by mechanisms that are not well understood. Senescence has been associated with metabolic changes in the oxidative state of the cell, a process that has been also linked to mitochondrial fission and fusion events, suggesting an association between mitochondrial dynamics and senescence. In the present work, we studied the mitochondrial structural remodeling process of mesenchymal stromal/stem cells isolated from adipose tissue *in vitro* to determine if mitochondrial phenotypic changes were associated with mesenchymal stromal/stem cell senescence. For this purpose, mitochondrial dynamics and oxidative state of stromal/stem cell were compared between young and old cells. With increased cell passage, we observed a significant change in cell morphology that was associated with an increase in β-galactosidase activity. In addition, old cells (population doubling seven) also showed increased mitochondrial mass, augmented superoxide production, and decreased mitochondrial membrane potential. These changes in morphology were related to slightly levels increases in mitochondrial fusion proteins, Mitofusion 1 (MFN1), and Dynamin-related GTPase (OPA1). Collectively, our results showed that adipose tissue-derived MSCs at population doubling seven developed a senescent phenotype that was characterized by metabolic cell changes that can lead to mitochondrial fusion.

Keywords: adipose derived mesenchymal stromal cells, mitochondria, reactive oxygen species, senescence, fission and fusion

OPEN ACCESS

Edited by:
Lucas Sedeño, Institute of Cognitive Neurology (INECO), Argentina

Reviewed by:
Christian Gonzalez-Billault, University of Chile, Chile
Jingwen Niu, Temple University, USA

****Correspondence:***
Sonia L. Albarracín
albarra@javeriana.edu.co

Received: *04 August 2016*
Accepted: *22 November 2016*
Published: *15 December 2016*

Citation:
Stab BR II, Martinez L, Grismaldo A, Lerma A, Gutiérrez ML, Barrera LA, Sutachan JJ and Albarracín SL (2016) Mitochondrial Functional Changes Characterization in Young and Senescent Human Adipose Derived MSCs. Front. Aging Neurosci. 8:299. doi: 10.3389/fnagi.2016.00299

INTRODUCTION

Mesenchymal stromal/stem cells (MSC) were described by Friedenstein almost 50 years ago as plastic adherent fibroblast-like cells (Friedenstein et al., 1968, 1970, 1976). Caplan in 1991 referred to MSC as cells with regenerative potential in tissues of mesenchymal origin such as bone, cartilage, muscle, ligament, tendon, adipose, and stroma, thus he coined the term "mesenchymal stem

cell" (Caplan, 1991). Multilineage differentiation potential was demonstrated by Pittenger et al. identifying this population as adult stem cells with stable phenotype (Pittenger et al., 1999). MSCs are highly metabolically, with a vast array of molecules secreted into the extracellular matrix (ECM), in addition to cytokines (Keating, 2012). Therefore, for the past 15 years MSCs have become very popular because of their therapeutic potential in tissue regeneration and cancer treatment (Schipani and Kronenberg, 2009; Dong and Caplan, 2012; Droujinine et al., 2013). The number of clinical trials on MSCs has been rising since 2004, including phase I–IV clinical studies for myocardial infarction, graft versus host disease, diabetes, spinal cord injury, and others. However, numerous scientific issues remain to be resolved before the establishment of clinical standards and government regulations (Wei et al., 2013).

Most therapeutic protocols require *ex vivo* cell expansion, guaranteeing reproducible, cost-effective, and good manufacturing practices. Although state of the art protocols describing systems such as microcarrier-based stirred cultured system have been reported (Carmelo et al., 2014), less is known about MSC expansion and senescence. To describe the phenotype acquired by MSC population after sequential cell passaging, characterized by low proliferation, and loss of clonogenic and differentiation potential, some researchers have used proteomic analysis to understand molecular mechanisms underlying replicative senescence (Madeira et al., 2012). Others have described epigenetic modifications during *in vitro* culture, where cells acquire DNA methylation changes at specific genomic sites (Schellenberg et al., 2014). Furthermore, telomere length and telomerase activity has been a hallmark during MSC expansion protocols (Parsch et al., 2004). Indeed it can be used as a method to track cellular changes upon long term culture (Wagner et al., 2010).

In addition to telomere shortening and telomerase activity inducing senescence, free radical, and mitochondrial theory are notable theories on aging (Andreyev et al., 2005; Romano et al., 2010). Decreased mitochondrial function is critical in the aging process and has been associated with age-related disorders (Seo et al., 2010). Mitochondria have been described as the major producers of free radical and concurrently the principal target of free radical action (Harman, 1972). The mitochondrial free radical theory of aging proposes reactive oxygen species (ROS), produced as by-products during normal metabolism results in oxidative damage (Sanz and Stefanatos, 2008). In response to a cell's bio-energetic state mitochondria are constantly remodeled. This process is known as mitochondrial dynamics, and is an integral part of many cellular responses. These dynamics are characterized by fission and fusion events that allow mitochondrial changes in orientation, number, and/or size within the cell. These tightly regulated processes allow constant remodeling of mitochondria (Hoppins et al., 2007). Conditions such as hypoxia, stress, and aging have been reported to impact mitochondrial dynamics leading to cellular dysfunction. Recently, mitochondrial fusion has been shown to induce senescent-like phenotypes in human cell cultures (Lee et al., 2007).

Mitochondrial fusion has been proposed to occur through two independent mechanisms in mammalian cells by which the inner and outer membrane fuse separately (Griffin et al., 2006). Fusion is believed to impart functional protection for the mitochondria by allowing them to exchange contents that might alleviate damaged constituents and promote repair (Seo et al., 2010). There are currently three established fusion proteins, Mitofusion proteins 1/2 (Mfn1/2) and optic atrophy protein 1 (Opa1). Over expression of Mfn 1/2 has been shown to inhibit apoptosis and promote cell survival (Sugioka et al., 2004). Furthermore, Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis (Sugioka et al., 2004). Mitochondrial fission participates in mitosis. In addition, it is thought to contribute to and even regulate apoptosis, as well as participate in the removal of dysfunctional mitochondria via mitophagy (Seo et al., 2010). In humans there are currently two established fission proteins, dynamin-related protein 1 (Drp1) and fission 1 protein (Fis1).

Reports in the literature suggest mitochondrial dynamics might play a role in senescence and aging, and could induce or alleviate these phenotypes (Ziegler et al., 2015). We proposed to study the mitochondrial structural remodeling process during *in vitro* culture. We set out to characterize possible fission and fusion events to establish the relationship between mitochondrial dynamics and replicative senescence.

MATERIALS AND METHODS

MSC Isolation and Population Doubling Determination

Mesenchymal stromal/stem cells were isolated from processed lipoaspirate. Adipose tissue was collected after informed signed consent from females ($n = 3$) undergoing cosmetic surgery with the approval of the Bioethics Committee at the Pontificia Universidad Javeriana. Processed lipoaspirate MSCs were isolated and characterized as previously described (Gutiérrez et al., 2013). Cells were passaged after reaching 80% confluency. Cell Media was changed twice a week, and MSCs were harvested and passaged according to the standardized protocol and re-seeded at a density of 10^4 cells/cm^2. Population doubling (PD) was established as follows:

$$PD = \log(N/Ni) \times 3.32 \quad (1)$$

N = number of viable cells, Ni = initial seeded number of cells.

MSCs Characterization

Cells at passage one were trypsinized and 96×10^3 cells were seeded in six well plates and incubated with complete media for 24 h. Adipogenesis and osteogenesis was evaluated with media supplemented with inducers according to Gutiérrez et al. (2013).

Senescent Phenotype

Mesenchymal stromal/stem cells were serially passaged until they reached a state of senescence defined as the inability to achieve 80% confluency after feedings over a 4 week period, verified by the senescence-associated β-galactosidase assay.

Senescence-Associated β-Galactosidase Assay

After reaching 80% confluency cells were detached and re-seeded at a 3×10^4/6 well plate and incubated at 37°C, 5% CO_2 for 24 h. Histochemical detection of β-galactosidase expression at pH 6.0 was only present in senescent cells and not pre-senescent cells, evidenced by x-gal conversion into a blue stain. Beta-galactosidase activity was evaluated following manufacturer's instructions (Senescence, β-galactosidase kit, Millipore, Massachusetts, USA). Last, SA β-gal cell stain count was performed under light microscopy and percentage of positive cells was calculated.

Mitochondrial Dynamics Characterization

Mitochondrial Mass

MSCs were stained with MitoTracker Green FM (Invitrogen, Carlsbad CA, USA) to quantify functional mitochondrial mass. Briefly, 48 h prior to staining 1×10^4 cells were seeded using 35 mm glass bottom culture dishes, containing 14 mm microwell (MatTek, MA, USA). Cells were incubated for 30 min with DMEM supplemented with 80 nM MitoTracker Green FM, followed by 5 μM CellTracker Red CMTPX solutions to stain the cytoplasm (Invitrogen), and Hoechst (Invitrogen) to define cell nucleus. Cells were observed *in vivo,* and 2D and 3D images were collected by confocal microscopy using an Olympus FV1000 with an excitation/emission range of 400/545 for MitoTracker Green FM and 577/602 nm for CellTracker Red CMTPX. Z-stack parameters were as follows: ∼15 z-axis slices at ∼0.50 μm intervals with a final Z-stack thickness of ∼7.5 μm. Mitochondrial mass was determined by data obtained from confocal microscopy in voxel units.

Mitochondrial Membrane Potential

Briefly, cells were collected as described for β-galactosidase assay and cultured for 48 h. We performed the established protocol for Mitoprobe™ JC-1 Assay Kit for Flow Cytometry (Molecular Probes, Invitrogen USA). We added 200 μM JC-1 and incubated for 30 min at 37°C. To determine mitochondrial membrane potential flow cytometry analysis was performed in a Guava easyCyte Flow cytometer (EMD Millipore, Billerica MA USA) at 488 nm excitation and 530 and 580 nm emission range with bandpass emission filters. Non-cellular debris and dead cells were gated out based on the light-scattering properties in the Side- and Forward- scatter parameters, and approximately 10×10^3 events from live cells were collected for each analysis. Exposure to 50 μM carbonyl-cyanide 3-chlorophenylhydrazone (CCCP) (Sigma Aldrich, St Louis MO USA) for 10 min was used as a control to set the threshold of fluorescence intensity for cells with intact mitochondrial membrane potentials. Results are presented as the percent of all cells with Mitoprobe fluorescence greater than the threshold set by CCCP.

Mitochondrial Anion Superoxide Production

After reaching 80% confluency cells were detached and re-seeded at a 3×10^4/6 well plate and incubated at 37°C, 5% CO_2 for 48 h. MitoSOX Red mitochondrial superoxide indicator for live-cell imaging (Molecular Probes, Invitrogen) assay was performed as an indicator of mitochondrial superoxide production. As a positive control 50 μM rotenone (Sigma) was used for 1 h. As a negative control we used 1X PBS without marker. Analysis was performed in Guava cytometer at 510/580 excitation/emission ranges. Results are presented as arbitrary units of fluorescence.

Fusion and Fission Protein Identification

Cells were harvested as described above in the cell culture method and lysed with lysis buffer (50 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 1% Triton X-100, and protease inhibitor cocktail). Sample protein concentration was determined by Bradford assay (Pierce). Equivalent amounts of proteins (25 μg) were separated by SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted. The following antibodies: MFN1 (Abcam, MA USA), OPA1 (Abcam, MA USA), DRP (Abcam, MA USA), and FIS1 (Abcam, MA USA), were used to determine if fusion or fission processes at the selected time-points were taking place. Immunoblots were visualized by the enhanced chemiluminescence system (JPI Healthcare Co, Plainview NY USA), β-actin (Abcam, MA USA) was used as the control and ProSieve QuadColor Protein Marker, 4.6–300 kDa as a reference for protein identification (Lonza, Allendale NJ USA). Protein quantification was performed in ImageJ (NIH) by normalizing mitochondrial dynamic protein to β-actin. Difference is reported as arbitrary units.

RESULTS

MSC Characterization and Senescent Phenotype

In order to characterize the effects of consecutive cell passaging on human MSCs proliferative capacity and mitochondrial metabolism, samples from three independently isolated healthy donors were cultured for seven consecutive passages (P1–P7) using DMEM supplemented with 10% FBS. Initial characterization at two population doublings (PD2) of these adipose derived-mesenchymal stromal/stem cells showed that these cells had high capacity of adherence to plastic, fibroblast-like morphology, and specific cell surface antigen expression of CD73, CD90, and CD105, and lack of CD34 expression (**Figures 1A,B**). Furthermore, adipose derived-mesenchymal stromal/stem were able to differentiate into adipogenic and osteogenic linages (**Figures 1C,D**) (Dominici et al., 2006; Gutiérrez et al., 2013).

MSCs isolated from adipose tissue reached population doublings seven (PD7) after 130 days in culture and their growth was characterized by having a logarithmic proliferation rate during the first 40 days followed by a plateau phase in which MSCs were unable to achieve a 80% confluency over a 4 week period (**Figure 2A**). Upon light microscopy examination, significant changes in morphology were observed during consecutive passaging expansion. Comparison of MSCs morphology between PD 2 and 7 showed an increase in cell size, as well as a visible augmentation in cell granularity for PD7 cells. Namely, over 60% of MSCs at PD7 acquired a flattened and widened phenotype that was accompanied with a severe decrease in cell proliferation, suggesting that cells at PD7

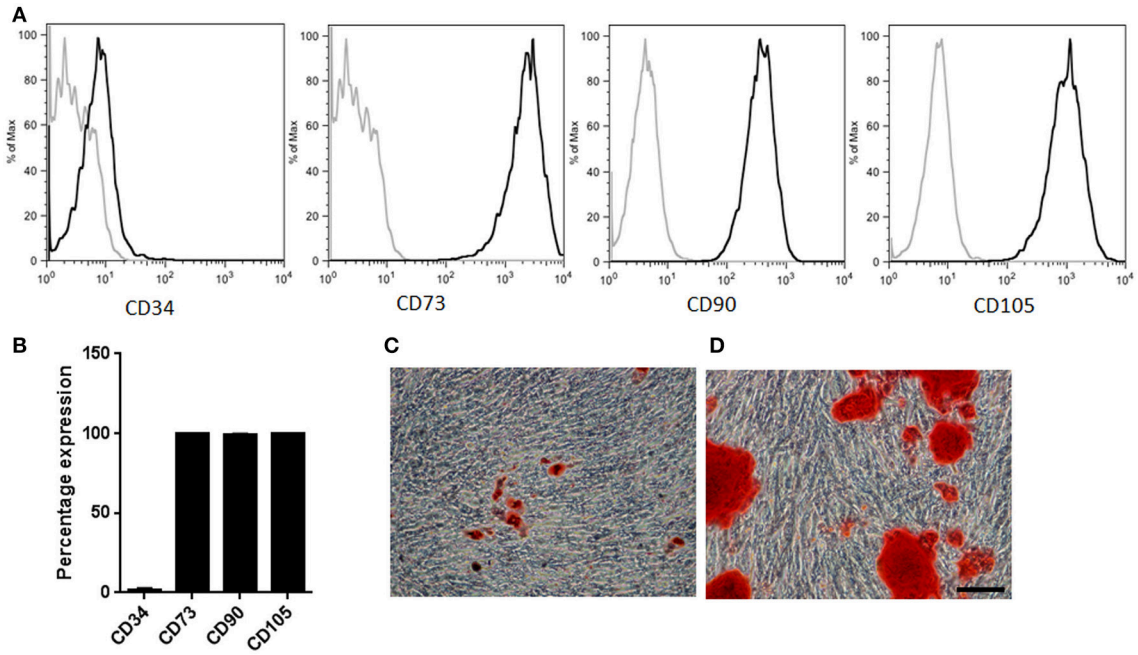


FIGURE 1 | MSCs characterization. (A) Representative sample illustrating immunophenotypic profile. Antibodies against CD34, CD73, CD90, and CD105 (thick gray lines) with isotype control (thin gray lines). **(B)** Percentage expression corresponding to the average profile of all cell types ($n = 3$). **(C)** Representative sample of MSCs induced into adipogenic lineage differentiation. Neutral fat lipid droplets were detected by Oil red O stain **(D)** Representative sample of MSCs induced into osteogenic differentiation. Extracellular calcium deposits were determined by Alizarin Red stain. Scale bar 100 μm.

were in a senescent state (**Figure 2B**). To further corroborate a senescent phenotype at PD7, we used the senescence-associated β-galactosidase stained assay (SA β-gal) with optimum activity at pH 6.0. In MSCs cultured up to PD7 we observed a marked increase in SA β-gal activity compared with PD2 (**Figures 2C,D**). Hence, we evidenced that MSCs cultured up to PD7 after 130 days in culture exhibited a senescent phenotype.

Mitochondrial Dynamics Characterization

It is well established that MSCs can reach a senescent phenotype during *in vitro* proliferation and lose their differentiation potential, with alteration of their metabolic profile (Wagner et al., 2010; Geissler et al., 2015). Senescence has been associated with metabolic changes in the oxidative state of the cell, a process that has been linked to mitochondrial fusion and fission events (Mitra, 2013; Geissler et al., 2015). During the process of fusion and fission mitochondria can adjust their size, shape, and organization inside the cell (Westermann, 2010; Mitra, 2013). Significance of mitochondrial configuration changes has only recently begun to be understood, and might play a role in the regulation of senescence processes in MSCs. Therefore, we characterized mitochondrial changes for MSCs cultured at lower passage and higher passage number (PD2 vs. PD7) in adipose derived MSCs.

Morphological differences between young and senescent cells, as was described for bright-field microscopy, were also observed by using the CellTracker fluorescent dye (**Figure 3**). Digital rendering of Z-stack depicting the cell cytoplasm through CellTracker red evidenced young cell fibroblast morphology (**Figure 3A**) in contrast to senescent cells (**Figure 3D**), where an increased in cytoplasmic cell surface was observed. Likewise, morphological analysis of mitochondria at PD2 cells -using the mitochondrial marker MitoTracker green showed small tubular mitochondria forming a slightly interconnected network (**Figures 3B,C**). In contrast, PD7 cells had large tubular mitochondria forming an intricate network that was uniformly distributed in the cytoplasm (**Figures 3E,F**).

To further corroborate if mitochondrial morphological changes were associated with mitochondrial volume, we quantified mitochondrial mass. Our results showed that PD7 MSCs had a larger mass (1286 ± 160) compared with PD2 (663.5 ± 104). These results suggest that increased adipose derived MSCs cell expansion lead to a senescent state characterized by an augmented mitochondrial network and mass. Mitochondrial dynamics fusion and fission are critical for organelle inheritance and maintenance of mitochondrial functions (Westermann, 2010). It has been described that increased mitochondrial mass could be associated with fusion processes. Therefore, we evaluated if changes in mitochondrial mass observed were due to changes in the levels of proteins involved in fusion and fission processes. Results showed a slight tendency for an increased level in fusion proteins GTPase, Mitofusion 1 (MFN1) and Dynamin-related GTPase OPA1 (**Figure 4A**), and in fission protein Fission 1 (FIS1) (**Figure 4B**). In contrast, fission protein DRP1 did not have important protein level difference between young and old populations. Collectively, our results suggest a tendency

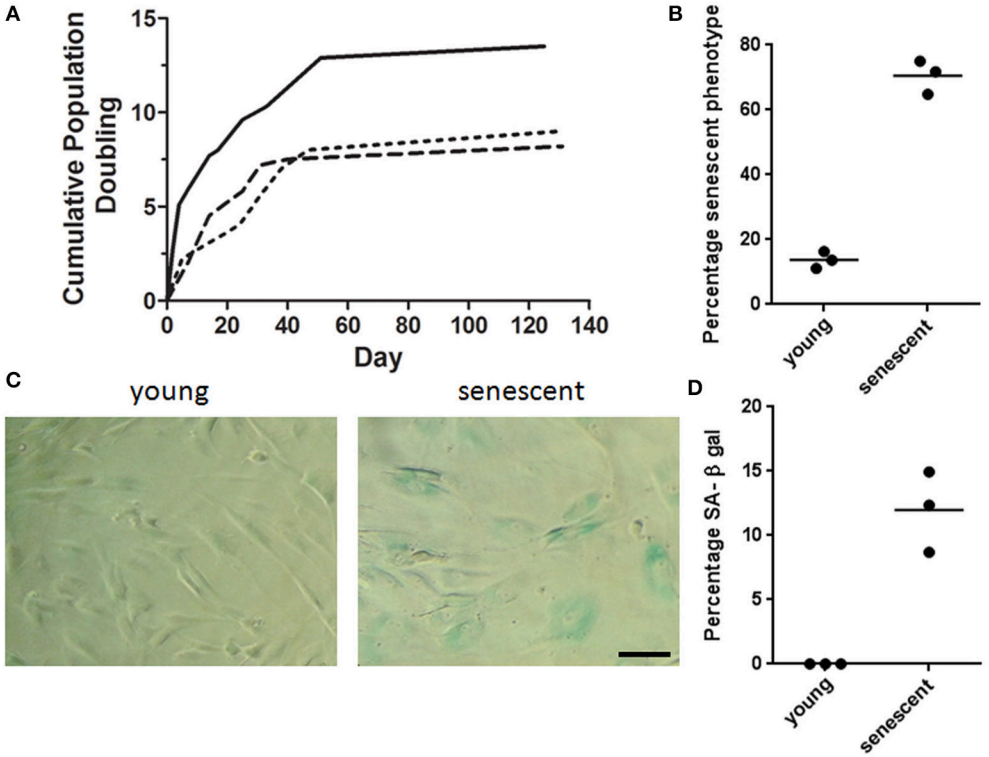


FIGURE 2 | MSCs senescent associated phenotype. (A) Cumulative Population doubling for adipose derived MSCs. Adipose tissue derived MSCs were cultured for a total of 131 days. Cumulative PD were calculated at the end of every passage in relation to cell number of the first passage ($n = 3$). **(B)** Percentage senescent cells determined by cell count/field. Over 60% of MSCs at PD7 acquired a flattened and widened phenotype. **(C)** Representative brightfield images of young and senescent MSCs. Young cells are spindle shaped and slender in contrast to senescent cells with a widened and flat morphology. Cells presenting blue staining indicate the presence of β-galactosidase activity in senescent cells. Scale bar 100 μm. **(D)** Quantification of β-galactosidase positive cells for young (PD2) and senescent cells (PD7).

for fusion events in senescent cells, favoring the formation of mitochondrial networks. In response to mitochondrial stress fusion possibly protects the cell by maintaining a functional population of mitochondrial within the cell.

Mitochondrial Metabolic Characterization

It has been shown that mitochondrial fusion is a mechanism used by the cell to dilute out mitochondrial dysfunction, respond to high energy demands, and maintain proper cell function. Since we observed mitochondrial mass changes in senescent MSCs associated with increased fusion, we then proceeded to evaluate mitochondria functionality by assaying mitochondrial membrane potential ($\Delta\psi m$) and ROS production (Zorov et al., 2014).

Mitochondrial functionality by membrane potential was quantified by using the FACS JC-1 fluorescence dye. This cationic carbocyanine dye accumulates in the mitochondria in a low monomeric concentration yielding a green fluorescence when the membrane potential is low. On the other hand, it aggregates at high concentrations with a red fluorescence emission at high membrane potentials. Our results showed a 47% reduction in $\Delta\psi m$ for cells at PD7 in comparison with cells at PD2 (**Figure 5A**). These results suggest that senescent MSCs have a diminished mitochondrial energetic capacity, which may lead to a lower activity in mitochondrial respiration and less ATP synthesis.

Subsequently, we characterized ROS production in young and senescent MSCs to evaluate the activity of mitochondrial respiration in these cells. Reactive oxygen species (ROS) are electron transporter chain (ETC) by-products of the cell's oxidative metabolism. A normal balance in ROS production and inactivation is mediated by different enzymatic systems and exogenous molecules capable of ROS detoxification to prevent oxidative stress damage (Sanz and Stefanatos, 2008). If mitochondria homeostasis is not sustained, higher ROS levels are produced. This in turn results in longer mitochondrial permeability transition pore (mPTP) openings releasing a ROS burst leading to mitochondria destruction. If these events are propagated from mitochondrion to mitochondrion, they might result in cell apoptosis (Zorov et al., 2014). Furthermore, reports have shown prolonged elongated mitochondria result in higher ROS production and lower mitochondrial respiration activity, resulting in cellular senescence (Yoon et al., 2007; Wagner et al., 2008).

Mitochondria superoxide production was evaluated by FACS analysis, where the reagent permeated live cells, selectively

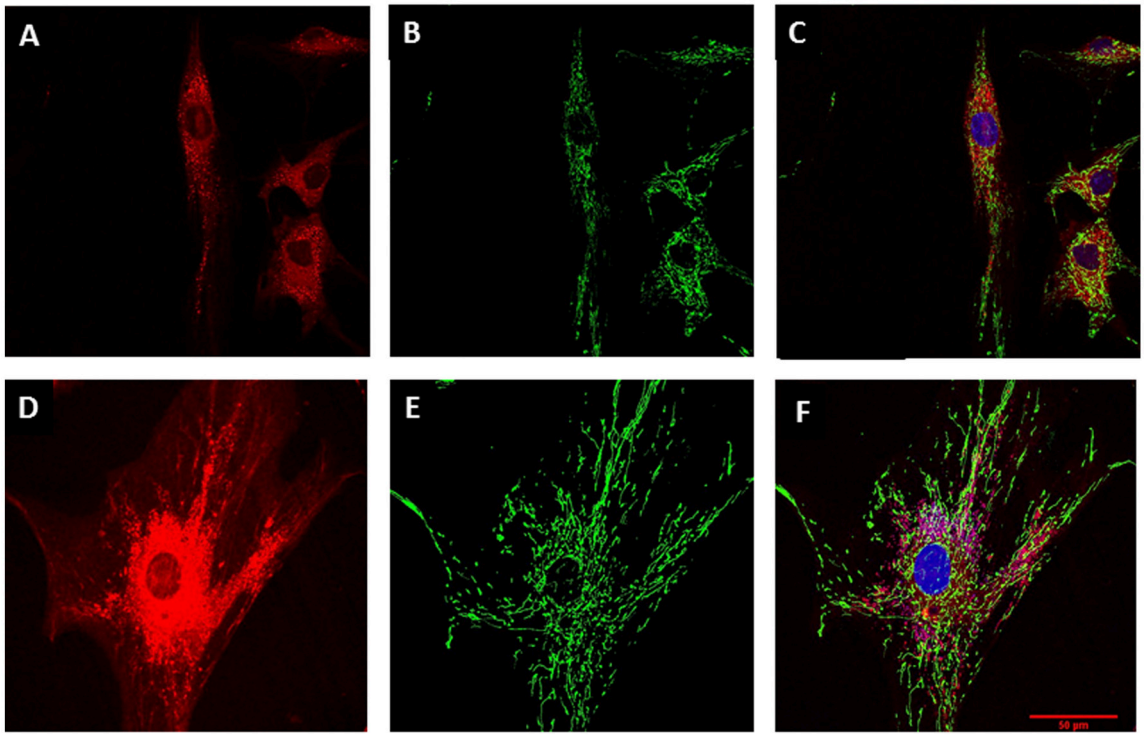


FIGURE 3 | Mitochondrial characterization. MSC cell morphology and mitochondrial mass evaluation by confocal microscopy. **(A)** MSC at PD2 cytoplasm revealed by CellTracker red. **(B)** Mitochondrial stain with Mito Tracker green for MSCs at PD2. **(C)** Merge for **(A)** and **(C)** with nucleus stained with Hoechst (blue). **(D)** Larger cytoplasm in MSC at PD7 revealed by CellTracker red. **(E)** Mitochondrial stain with Mito Tracker green for MSCs at PD7. **(F)** Merge for **(A)** and **(C)** with nucleus stained with Hoechst (blue). Scale bar 50 µm for all images.

targeting mitochondria, and rapidly oxidizing to produce a highly fluorescent product. Our data determined anion superoxide production was 2.37 fold higher for senescent MSCs cultured up to PD7 compared with young MSCs (**Figure 5B**). Collectively, these results show that old MSCs have a decreased mitochondrial membrane potential, and increased ROS production, while young MSC have a conserved membrane mitochondrial potential and decreased ROS production. These results suggest that senescent MSC could have deficiencies in ATP production and an increase in oxidative stress. Old MSCs can compensate these mitochondrial changes with an augmentation in mass and interconnected mitochondrial network favored by the fusion process. Together, our results suggest possible mitochondrial functional changes that could be associated with senescence.

DISCUSSION

Mesenchymal stromal/stem cells have a great potential for therapeutic use; and human adipose tissue is an ideal autologous source of MSCs for diverse regenerative medicine and tissue engineering strategies (Caplan and Correa, 2011; Jin et al., 2013; Choudhery et al., 2014). Mesenchymal stem/stromal cells have been widely described in the literature for their therapeutic potential as anti-inflammatory, immuno-modulatory, and trophic support (Caplan and Correa, 2011; Choudhery et al., 2014). Most therapeutic protocols require *ex vivo* cell expansion to attain desired numbers, generally described as 1×10^6/kg weight (Wagner et al., 2010). This requirement induces deficiency in cell proliferation capacity, loss of clonogenic potential, and impairment in differentiation potential (Madeira et al., 2012; Jin et al., 2013). Moreover, cells acquire DNA methylation changes at specific genomic sites brought about by epigenetic modifications during *in vitro* culture (Bentivegna et al., 2016). Therefore, MSCs proliferation and senescence are important issues to be considered for clinical safety and efficacy.

Our results demonstrate that for adipose tissue-derived MSCs a senescent phenotype was observed for cells cultured up to seven population doublings. This was evidenced by changes in cell morphology, decreased cell proliferation, and positive SA β-gal stain as previously described (Wagner et al., 2008). In addition to this characterization, we defined mitochondrial functional changes possibly related to replicative senescence to shed light on potential mitochondrial associated aging process. Senescent MSCs had an increased mitochondrial mass and strongly interconnected network that distributed uniformly in the cytoplasm, suggesting potentiation of fusion processes. Therefore, characteristic mitochondrial tubular shape was observed. In addition to slightly increased fusion associated proteins, MFN-1 and OPA1.

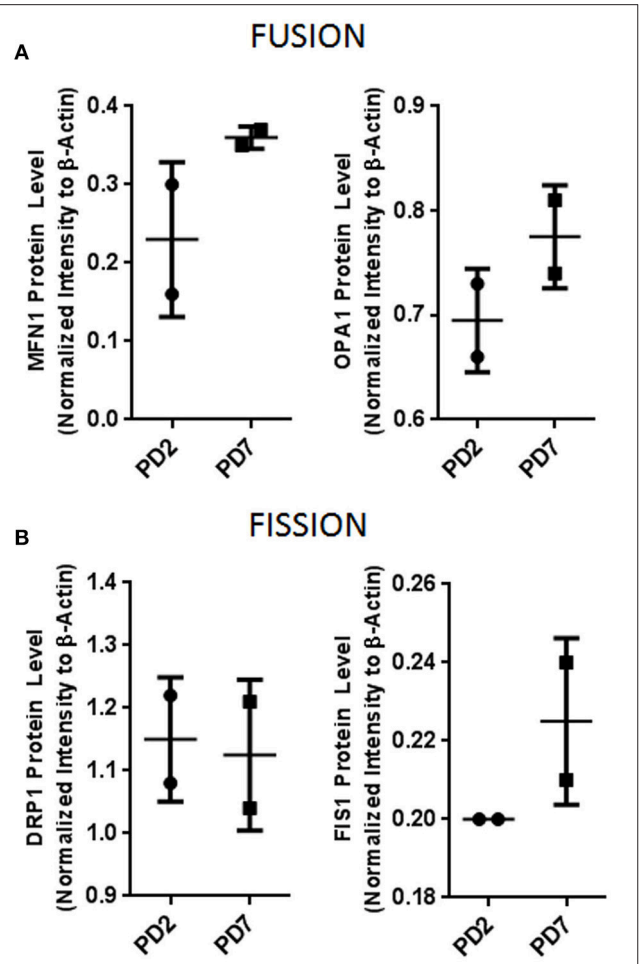


FIGURE 4 | Mitochondrial dynamics protein evaluation. (A) Mitochondrial fusion proteins MFN1 and OPA1. **(B)** Mitochondrial fission proteins DRP1 and FIS1. Bar graphs depict protein expression as an arbitrary unit when protein of interest was normalized to β-actin. MFN1, Mitofusion1; OPA1, Optic atrophy 1; DRP1, Dynamin-1-like protein; FIS1, Mitochondrial fission 1.

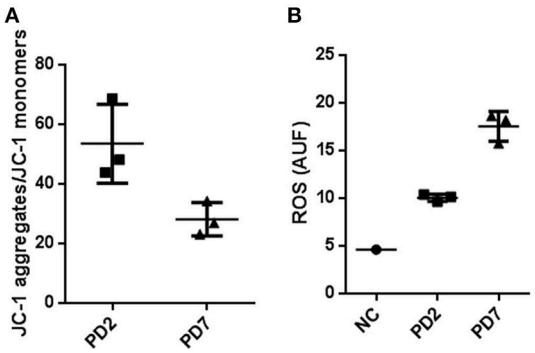


FIGURE 5 | Mitochondrial membrane potential and Reactive oxygen species production in young and senescent MSCs. (A) FACS analysis to determine membrane potential changes by means of JC-1, a lipophilic cationic dye selectively entering into mitochondria. JC-1 accumulation in mitochondria, due to concentration-dependent formation of red fluorescent JC-1 aggregates was higher for MSCs cultured up to PD2 compared with PD7 MSCs. Results are presented as JC-1 aggregates/JC-1 monomer **(B)**. Reactive oxygen species production in young and senescent MSCs was compared by Mitosox Red fluorescent intensity production quantified by flow cytometry and expressed as arbitrary units fluorescence ROS (AUF). As a positive control rotenone at 50 μM was used.

Mitochondrial dynamics are defined by the capacity of this organelle to continuously fuse or divide (Westermann, 2010). The balance between these two processes is responsible for mitochondria shape, distribution, inheritance, and functionality. If this delicate balance is lost, mitochondrial and cellular functions are affected as well (Detmer and Chan, 2007; Terman et al., 2010). Mitochondria can continually exchange contents through membrane fusion. This process controls organelle shape and is critically important for maintaining mitochondrial network function (Suen et al., 2008; Correia-Melo et al., 2016). We observed changes suggesting fusion events in senescent MSCs, based on a slightly higher MFN1 and OPA1protein levels. The most relevant proteins involved in mitochondrial fusion process are three GTPase dynamin-like proteins: Mitofusion 1 (MFN1) and 2 (MFN2), located in the outer mitochondrial membrane, and optic atrophy protein 1 (OPA1), in the inner membrane (Westermann, 2010). It is conceivable that under normal conditions outer membrane fusion is coordinated with inner membrane fusion. Even though we did not evaluate MFN2, our data evidenced slightly increased outer and inner mitochondrial membrane protein expression in aged MSCs. Under these conditions senescent MSCs would possibly use fusion processes to connect neighboring depolarized mitochondria and unite their contents to maintain membrane potential (Ikeda et al., 2015). Mitochondrial fusion has been reported to be beneficial for cardiomyocytes under stress conditions, since it consolidates the mitochondria's ability to supply energy (Ikeda et al., 2015). It is plausible this increased mitochondrial networking could be serving as a protective mechanism. It has been suggested mitochondrial fusion may protect neurons form excessive mitochondrial stress by maintaining a functional population (Ikeda et al., 2015).

In contrast, mitochondrial fission can segregate damaged mitochondria from intact ones, and the injured part of the mitochondria is phagocytized by mitophagy (Ikeda et al., 2015). Dynamin-related protein 1 (DRP1) is a master regulator of mitochondrial division (Westermann, 2010). Recruitment of DRP1 from the cytosol and assembly in the mitochondria depends partly on mitochondrial fission protein (FIS1). Both proteins are located on the external mitochondrial membrane. Our findings demonstrated younger cells expressed insignificant increased levels of DRP1, while senescent MSCs had a negligible increment in FIS1 expression.

Studies from in mouse embryonic fibroblasts (MEF) evidenced maintenance of mitochondrial morphological dynamics during cell stress can occur either by blocking fission (via dominant negative mutant-Drp1) or enhancing fusion (via Mfn1, Mfn2), suppressing mitochondrial injury and subsequent apoptosis. Furthermore, it has been reported Mfn1 and Mfn2 can maintain mitochondrial morphology during cell stress, and prevent mitochondrial outer membrane permeabilization and

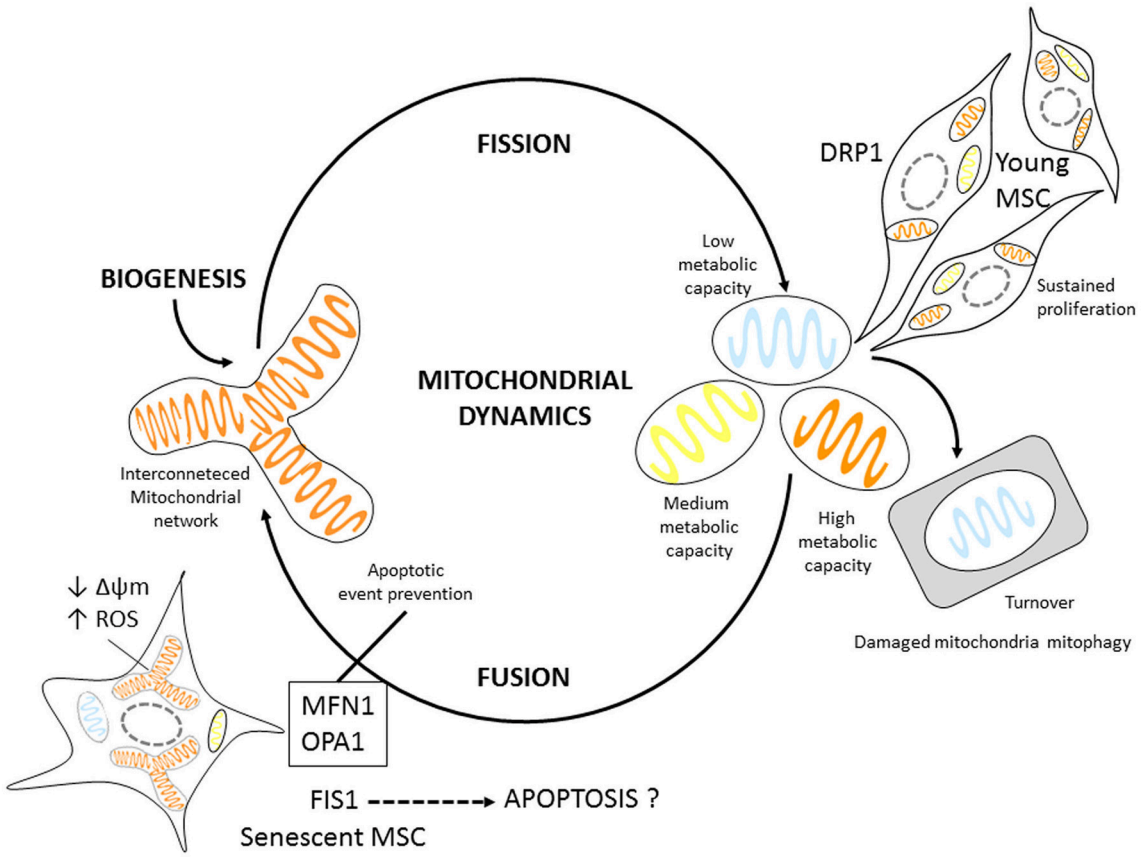


FIGURE 6 | Mitochondrial dynamic Model proposal for MSCs. Young MSCs can readily undergo proliferation. Mitotic cells have slightly higher DRP1 expression, possibly regulating mitochondrial fission. Senescent MSCs display mitochondrial fusion. In addition they have larger mitochondrial volume; express proteins associated with fusion events most likely as an adaptation mechanism to energetic changes, such as increased ROS production, and decreased $\Delta\psi m$ possibly to prevent apoptosis.

apoptosis (Brooks et al., 2011). In senescent MSCs, a hypothesis proposing fusion process enhancement mediated by Mfn1, could suppress apoptosis. Future studies associating intrinsic apoptosis signaling pathways would elucidate these mechanisms in senescent MSCs.

Among the principal pathways toward senescence reported in the literature associated with senescence are telomere shortening and ROS species production by mitochondria. To date to the best of our knowledge no study has addressed mitochondrial functional changes associated changes with replicative senescence in adipose derived MSCs. Mitochondria are involved in multiple anabolic and catabolic reactions, such as citric acid cycle and β-oxidation, in addition to their prominent role in metabolic energy production. Furthermore, they participate in developmental processes of aging (Westermann, 2010).

Our data show that old MSCs have a decreased mitochondrial membrane potential, and increased ROS production, while young MSC have a conserved membrane mitochondrial potential and decreased ROS production. These results suggest that senescent MSCs could have deficiencies in ATP production and an increase in oxidative stress.

The mitochondrial crisis can be described as a decrease in electron chain transport, diminished membrane potential $\Delta\psi m$, decreased oxidation, increased ROS production, and diminish mitochondrial function. Our data suggest a mitochondrial impairment could have occurred in senescent MSCs. Other authors have described for human fibroblast under oxidative stress during replicative senescence, ROS associated with increased mitochondrial mass (Lee et al., 2002). For the fibroblast study increase in mitochondrial mass was attributed to sublethal increased levels of oxidative stress. It is likely increased ROS production resulted in augmented mitochondrial mass, contributing to MSCs senescent phenotype. Furthermore, since Harman in 1956 proposed the free-radical theory of aging, where cumulative damage to molecules by ROS leads to irreversible cell damage and functional decline (Harman, 1956), this theory has also been extended including mutations and deletions in mitochondrial DNA. These accumulated age related mutations and deletions lead to impaired function of the respiratory chain

and increase ROS production; generating a vicious cycle (Seo et al., 2010).

In conclusion, our study describes mitochondrial functional changes associated with adipose derived MSCs senescent phenotype after PD7. Increased MFN1 expression in PD7 MSCs, favoring fusion, could be suggestive of a protective mechanism against cells undergoing apoptotic events. Furthermore, metabolically MFN1 could potentiate membrane potential allowing the "senescent phenotype" MSC to adapt to stress. Our data suggest that the aging process could be associated with impaired mitochondrial function and energy metabolism in MSCs. However, further studies are required to define metabolic changes that accompany the mitochondrial crisis here described. Results derived from this study should be taken into account when considering MSC expansion for therapeutic use; as our data revealed mitochondrial functional changes associated with replicative senescence in cells with higher PDs.

Our findings propose fusion and fission processes could be regulated under stress conditions for PD7 MSCs (**Figure 6**). Mitochondrial dynamics might be aiding in regulating mitochondrial ATP cellular levels and minimizing damaged mitochondrial DNA (mtDNA) accumulation during aging. We show fusion is important in senescent MSCs, perhaps to protect cells against oxidative stress and energy failure. During aging mitochondrial capability to generate ATP declines and mutations in mitochondrial and nuclear DNA accumulate (Stewart and Chinnery, 2015). However, to date the relationship between mitochondrial dynamics, and energy metabolism in young and senescent MSCs is still poorly understood and should be taken into account when considering MSCs for therapeutic applications.

ETHICS STATEMENT

Approved by the Bioethics Committee at the Pontificia Universidad Javeriana. Each donor carefully read the informed consent to donate a lipoaspirate sample during voluntary cosmetic surgery procedure. Any questions were answered by the researcher. The consent was signed by the donor with a witness.

AUTHOR CONTRIBUTION

BS designed the work, acquired, and interpreted the data. LM, AG, and AL acquired and analyzed the data. MG, LB, JS, and SA interpreted the data, drafted and revised the work.

FUNDING

This work was funded by Pontificia Universidad Javeriana-Vicerrectoría de Investigación, Projects 00004468 and 00007027, Mitochondrial fission and fusion: a new pathway toward senescence in Stem Cells.

ACKNOWLEDGMENTS

The authors thank Pontificia Universidad Javeriana for financial support and the subjects for sample donation.

REFERENCES

Andreyev, A. Y., Kushnareva, Y. E., and Starkov, A. A. (2005). Mitochondrial metabolism of reactive oxygen species. *Biochemistry* 70, 200–214.

Bentivegna, A., Roversi, G., Riva, G., Paoletta, L., Redaelli, S., Miloso, M., et al. (2016). The effect of culture on human bone marrow mesenchymal stem cells: focus on DNA methylation profiles. *Stem Cells Int.* 2016:5656701. doi: 10.1155/2016/5656701

Brooks, C., Cho, S. G., Wang, C. Y., Yang, T., and Dong, Z. (2011). Fragmented mitochondria are sensitized to Bax insertion and activation during apoptosis. *Am. J. Physiol. Cell Physiol.* 300, C447–C455. doi: 10.1152/ajpcell.00402.2010

Caplan, A. I. (1991). Mesenchymal stem cells. *J. Orthop. Res.* 9, 641–650. doi: 10.1002/jor.1100090504

Caplan, A. I., and Correa, D. (2011). The MSC: an injury drugstore. *Cell Stem Cell* 9, 11–15. doi: 10.1016/j.stem.2011.06.008

Carmelo, J. G., Fernandes-Platzgummer, A., Cabral, J. M. S., and da Silva, C. L. (2014). Scalable *ex vivo* expansion of human mesenchymal stem/stromal cells in microcarrier-based stirred culture systems. *Methods Mol. Biol.* 1283, 147–159. doi: 10.1007/7651_2014_100

Choudhery, M. S., Badowski, M., Muise, A., Pierce, J., and Harris, D. T. (2014). Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. *J. Transl. Med.* 12:8. doi: 10.1186/1479-5876-12-8

Correia-Melo, C., Marques, F. D., Anderson, R., Hewitt, G., Hewitt, R., Cole, J., et al. (2016). Mitochondria are required for pro-ageing features of the senescent phenotype. *EMBO J.* 35, 724–742. doi: 10.15252/embj.201592862

Detmer, S. A., and Chan, D. C. (2007). Functions and dysfunctions of mitochondrial dynamics. *Nat. Rev. Mol. Cell Biol.* 8, 870–879. doi: 10.1038/nrm2275

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., et al. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8, 315–317. doi: 10.1080/14653240600855905

Dong, F., and Caplan, A. I. (2012). Cell transplantation as an initiator of endogenous stem cell-based tissue repair. *Curr. Opin. Organ Transplant.* 17, 670–674. doi: 10.1097/MOT.0b013e328359a617

Droujinine, I. A., Eckert, M. A., and Zhao, W. (2013). To grab the stroma by the horns: from biology to cancer therapy with mesenchymal stem cells. *Oncotarget* 4, 651–664. doi: 10.18632/oncotarget.1040

Friedenstein, A. J., Chailakhjan, R. K., and Lalykina, K. S. (1970). The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet.* 3, 393–403. doi: 10.1111/j.1365-2184.1970.tb00347.x

Friedenstein, A. J., Gorskaja, J. F., and Kulagina, N. N. (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Exp. Hematol.* 4, 267–274.

Friedenstein, A. J., Petrakova, K. V., Kurolesova, A. I., and Frolova, G. P. (1968). Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation* 6, 230–247. doi: 10.1097/00007890-196803000-00009

Geissler, S., Textor, M., Kuhnisch, J., Konnig, D., Klein, O., Ode, A., et al. (2015). Functional comparison of chronological and *in vitro* aging: differential role of the cytoskeleton and mitochondria in mesenchymal stromal cells. *PLoS ONE* 7:e52700. doi: 10.1371/journal.pone.0052700

Griffin, E. E., Detmer, S. A., and Chan, D. C. (2006). Molecular mechanism of mitochondrial membrane fusion. *Biochim. Biophys. Acta* 1763, 482–489. doi: 10.1016/j.bbamcr.2006.02.003

Gutiérrez, M. L., Guevara, J. M., Echeverri, O. Y., Garzón-Alvarado, D. A., and Barrera, L. A. (2013). Aggrecan catabolism during mesenchymal stromal cell *in vitro* chondrogenesis. *Anim. Cells Syst.* 17, 243–249. doi: 10.1080/19768354.2013.812537

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

Hoppins, S., Lackner, L., and Nunnari, J. (2007). The machines that divide and fuse mitochondria. *Annu. Rev. Biochem.* 76, 751–780. doi: 10.1146/annurev.biochem.76.071905.090048

Ikeda, Y., Shirakabe, A., Brady, C., Zablocki, D., Ohishi, M., and Sadoshima, J. (2015). Molecular mechanisms mediating mitochondrial dynamics and mitophagy and their functional roles in the cardiovascular system. *J. Mol. Cell. Cardiol.* 78, 116–122. doi: 10.1016/j.yjmcc.2014.09.019

Jin, H. J., Bae, Y. K., Kim, M., Kwon, S. J., Jeon, H. B., Choi, S. J., et al. (2013). Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. *Int. J. Mol. Sci.* 14, 17986–18001. doi: 10.3390/ijms140917986

Keating, A. (2012). Mesenchymal stromal cells: new directions. *Cell Stem Cell* 10, 709–716. doi: 10.1016/j.stem.2012.05.015

Lee, H. C., Yin, P. H., Chi, C. W., and Wei, Y. H. (2002). Increase in mitochondrial mass in human fibroblasts under oxidative stress and during replicative cell senescence. *J. Biomed. Sci.* 9, 517–526. doi: 10.1007/BF02254978

Lee, S., Jeong, S. Y., Lim, W. C., Kim, S., Park, Y. Y., Sun, X., et al. (2007). Mitochondrial fission and fusion mediators, hFis1 and OPA1, modulate cellular senescence. *J. Biol. Chem.* 282, 22977–22983. doi: 10.1074/jbc.M700679200

Madeira, A., da Silva, C. L., dos Santos, F., Camafeita, E., Cabral, J. M., and Sa-Correia, I. (2012). Human mesenchymal stem cell expression program upon extended ex-vivo cultivation, as revealed by 2-DE-based quantitative proteomics. *PLoS ONE* 7:e43523. doi: 10.1371/journal.pone.0043523

Mitra, K. (2013). Mitochondrial fission-fusion as an emerging key regulator of cell proliferation and differentiation. *Bioessays* 35, 955–964. doi: 10.1002/bies.201300011

Parsch, D., Fellenberg, J., Brummendorf, T. H., Eschlbeck, A. M., and Richter, W. (2004). Telomere length and telomerase activity during expansion and differentiation of human mesenchymal stem cells and chondrocytes. *J. Mol. Med.* 82, 49–55. doi: 10.1007/s00109-003-0506-z

Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., et al. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* 284, 143–147. doi: 10.1126/science.284.5411.143

Romano, A. D., Serviddio, G., de Matthaeis, A., Bellanti, F., and Vendemiale, G. (2010). Oxidative stress and aging. *J. Nephrol.* 23(Suppl. 15), S29–S36.

Sanz, A., and Stefanatos, R. K. (2008). The mitochondrial free radical theory of aging: a critical view. *Curr. Aging Sci.* 1, 10–21. doi: 10.2174/1874609810801010010

Schellenberg, A., Mauen, S., Koch, C. M., Jans, R., de Waele, P., and Wagner, W. (2014). Proof of principle: quality control of therapeutic cell preparations using senescence-associated DNA-methylation changes. *BMC Res. Notes* 7:254. doi: 10.1186/1756-0500-7-254

Schipani, E., and Kronenberg, H. M. (2009). "Adult mesenchymal stem cells," in *StemBook [Internet]* (Cambridge, MA: Harvard Stem Cell Institute), 1–31. doi: 10.3824/stembook.1.38.1

Seo, A. Y., Joseph, A. M., Dutta, D., Hwang, J. C., Aris, J. P., and Leeuwenburgh, C. (2010). New insights into the role of mitochondria in aging: mitochondrial dynamics and more. *J. Cell Sci.* 123, 2533–2542. doi: 10.1242/jcs.070490

Stewart, J. B., and Chinnery, P. F. (2015). The dynamics of mitochondrial DNA heteroplasmy: implications for human health and disease. *Nat. Rev. Genet.* 16, 530–542. doi: 10.1038/nrg3966

Suen, D. F., Norris, K. L., and Youle, R. J. (2008). Mitochondrial dynamics and apoptosis. *Genes Dev.* 22, 1577–1590. doi: 10.1101/gad.1658508

Sugioka, R., Shimizu, S., and Tsujimoto, Y. (2004). Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis. *J. Biol. Chem.* 279, 52726–52734. doi: 10.1074/jbc.M408910200

Terman, A., Kurz, T., Navratil, M., Arriaga, E. A., and Brunk, U. T. (2010). Mitochondrial turnover and aging of long-lived postmitotic cells: the mitochondrial-lysosomal axis theory of aging. *Antioxid. Redox Signal.* 12, 503–535. doi: 10.1089/ars.2009.2598

Wagner, W., Bork, S., Lepperdinger, G., Joussen, S., Ma, N., Strunk, D., et al. (2010). How to track cellular aging of mesenchymal stromal cells? *Aging* 2, 224–230. doi: 10.18632/aging.100136

Wagner, W., Horn, P., Castoldi, M., Diehlmann, A., Bork, S., Saffrich, R., et al. (2008). Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PLoS ONE* 3:e2213. doi: 10.1371/journal.pone.0002213

Wei, X., Yang, X., Han, Z. P., Qu, F. F., Shao, L., and Shi, Y. F. (2013). Mesenchymal stem cells: a new trend for cell therapy. *Acta Pharmacol. Sin.* 34, 747–754. doi: 10.1038/aps.2013.50

Westermann, B. (2010). Mitochondrial fusion and fission in cell life and death. *Nat. Rev. Mol. Cell Biol.* 11, 872–884. doi: 10.1038/nrm3013

Yoon, Y. G., Haug, C. L., and Koob, M. D. (2007). Interspecies mitochondrial fusion between mouse and human mitochondria is rapid and efficient. *Mitochondrion* 7, 223–229. doi: 10.1016/j.mito.2006.11.022

Ziegler, D. V., Wiley, C. D., and Velarde, M. C. (2015). Mitochondrial effectors of cellular senescence: beyond the free radical theory of aging. *Aging Cell* 14, 1–7. doi: 10.1111/acel.12287

Zorov, D. B., Juhaszova, M., and Sollott, S. J. (2014). Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* 94, 909–950. doi: 10.1152/physrev.00026.2013

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: 13 February 2017
doi: 10.3389/fnagi.2017.00016

Non-Invasive Brain Stimulation: A New Strategy in Mild Cognitive Impairment?

*Agustina Birba[1,2], Agustín Ibáñez[1,2,3,4,5], Lucas Sedeño[1,2], Jesica Ferrari[1], Adolfo M. García[1,2,6] and Máximo Zimerman[1]**

[1]*Laboratory of Experimental Psychology and Neuroscience (LPEN), Institute of Cognitive and Translational Neuroscience (INCyT), INECO Foundation, Favaloro University, Buenos Aires, Argentina,* [2]*National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina,* [3]*Universidad Autónoma del Caribe, Barranquilla, Colombia,* [4]*Center for Social and Cognitive Neuroscience (CSCN), School of Psychology, Universidad Adolfo Ibañez, Santiago de Chile, Chile,* [5]*Centre of Excellence in Cognition and its Disorders, Australian Research Council (ARC), Sydney, NSW, Australia,* [6]*Faculty of Education, National University of Cuyo (UNCuyo), Mendoza, Argentina*

Non-invasive brain stimulation (NIBS) techniques can significantly modulate cognitive functions in healthy subjects and patients with neuropsychiatric disorders. Recently, they have been applied in patients with mild cognitive impairment (MCI) and subjective cognitive impairment (SCI) to prevent or delay the development of Alzheimer's disease (AD). Here we review this emerging empirical corpus and discuss therapeutic effects of NIBS on several target functions (e.g., memory for face-name associations and non-verbal recognition, attention, psychomotor speed, everyday memory). Available studies have yielded mixed results, possibly due to differences among their tasks, designs, and samples, let alone the latter's small sizes. Thus, the impact of NIBS on cognitive performance in MCI and SCI remains to be determined. To foster progress in this direction, we outline methodological approaches that could improve the efficacy and specificity of NIBS in both conditions. Furthermore, we discuss the need for multicenter studies, accurate diagnosis, and longitudinal approaches combining NIBS with specific training regimes. These tenets could cement biomedical developments supporting new treatments for MCI and preventive therapies for AD.

Keywords: mild cognitive impairment, non-invasive brain stimulation, neuroenhancement, transcranial magnetic stimulation, transcranial direct current stimulation

OPEN ACCESS

Edited by:
Catarina Oliveira, University of Coimbra, Portugal

Reviewed by:
Yury (Juri) Kropotov, Institute of the Human Brain of Russian Academy of Sciences, Russia
Panteleimon Giannakopoulos, University of Geneva, Switzerland

****Correspondence:***
Máximo Zimerman
mzimerman@ineco.org.ar

Received: *27 October 2016*
Accepted: *20 January 2017*
Published: *13 February 2017*

Citation:
Birba A, Ibáñez A, Sedeño L, Ferrari J, García AM and Zimerman M (2017) Non-Invasive Brain Stimulation: A New Strategy in Mild Cognitive Impairment? Front. Aging Neurosci. 9:16. doi: 10.3389/fnagi.2017.00016

INTRODUCTION

In the face of an aging society, an urgent need has emerged to prevent and treat dementia, in general, and Alzheimer's disease (AD), in particular (Ferri et al., 2006). Despite wide-ranging research efforts to elucidate the putative pathological mechanisms of dementia, available treatments remain limited (Alzheimer's Association, 2012). Promisingly, however, various factors have been shown to boost the dynamics of relevant neural networks, delaying and even reducing clinical symptoms (Fregni and Pascual-Leone, 2007). Therefore, interventions that enhance neuroplastic responses in early disease stages might be particularly useful to attenuate the rate of cognitive decline in AD patients.

A key model to assess this possibility is afforded by patients with mild cognitive impairment (MCI). This construct has evolved over the past two decades to denote a cognitive state intermediate between normal aging and very early dementia (Petersen and Negash, 2008). Patients with

this condition feature objective memory impairment and other cognitive deficits, but their dysfunction is not so severe as to compromise daily activities (Petersen and Negash, 2008). MCI comprises four clinical subtypes: (i) single-domain amnestic MCI (aMCI); (ii) multiple-domain aMCI; (iii) single-domain non-amnestic MCI; and (iv) multiple-domain non-amnestic MCI (Winblad et al., 2004; Anstey et al., 2008; Albert et al., 2011). These subtypes differ in etiology and outcome: whereas both forms of aMCI imply a high chance of progression to AD, non-amnestic varieties involve considerable risk of conversion to non-AD dementia (Winblad et al., 2004; Anstey et al., 2008; Albert et al., 2011).

A second relevant model can be found in individuals featuring subjective cognitive impairment (SCI). This notion encompasses people who report everyday concerns about their cognitive functioning, even in the absence of objectively established impairments. Given its association with subsequent cognitive decline, SCI represents a potential prodromal state of AD occurring even before MCI (Reisberg and Gauthier, 2008).

In sum, aMCI and SCI are likely to anticipate incipient dementia. Despite the high between-patient variability and the uncertainty about their causative physiopathological mechanisms, both conditions offer highly relevant models to implement interventions aimed to boost cognitive performance through modulations of neuronal activity and connectivity. In this sense, non-invasive brain stimulation (NIBS) techniques have emerged as a potentially useful alternative. These tools can selectively enhance adaptive activity patterns, suppress maladaptive patterns, and influence learning and skill acquisition processes by inducing synaptic plasticity and network reorganization (Hummel and Cohen, 2006; Nitsche et al., 2008). In particular, NIBS can have a direct impact on memory mechanisms (including working, episodic, and associative memory) in young adults, elderly adults and patients with neurological dysfunctions (Manenti et al., 2012; Elder and Taylor, 2014).

Building on these antecedentes, a number of studies have assessed the impact of NIBS on MCI patients. Here we aim to critically review such emerging literature. While other reviews have addressed the impact of NIBS in cognitively impaired patients (Freitas et al., 2011; Nardone et al., 2014), here we focus on the key questions and difficulties of setting a treatment protocol. To this end, first we characterize the main features of NIBS. Thereupon, we review available studies examining the impact of NIBS on learning and memory in MCI and SCI. In particular, we describe and discuss in detail the studies' protocols and parameters, highlight the advantages of combining NIBS with cognitive training. Finally, we outline methodological strategies that could promote major developments in this promising research area.

NIBS: FEATURES AND MECHANISMS

With NIBS, researchers can transiently influence behavior by altering neural activity (Miniussi et al., 2013). A considerable body of evidence has been forged through the two most commonly used techniques in humans: repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS). Although they differ in several aspects (**Table 1**), both tools can induce long-term after effects on cortical excitability and neuronal plasticity. Typically, this brings about facilitatory or inhibitory effects (Nitsche and Paulus, 2000), broadly mirroring the workings of long-term potentiation (LTP) and long-term depression (LTD; Dayan et al., 2013). Since such effects can considerably outlast the actual stimulation period, NIBS techniques open valuable avenues for rehabilitation, especially when stimulation protocols are combined with adequate training-based interventions (Zimerman et al., 2013).

TABLE 1 | Summary of the main features of non-invasive brain stimulation (NIBS) techniques.

	TMS	tDCS
Excitatory	High frequency >5 Hz	Anodal stimulation
Inhibitory	Low frequency ~1 Hz	Cathodal stimulation
Mechanism of action	Neuronal depolarization	Membrane modulation
Physiological substrate	NMDA receptor	Voltage-dependent sodium and calcium channels; NMDA receptor
Focality of stimulation	More focal	Less focal
Design of sham-controlled double-blind studies	More difficult	Less difficult
Synchronous application with specific training	More difficult	Less difficult
Discomfort or pain	Mild following sustained application	Mild at the beginning
Adverse effects	Rare, if applied in accordance with safety guidelines	Rare, if applied in accordance with safety guidelines. Sensory discomfort, occasional headaches, no seizures described
Time resolution	Excellent: milliseconds	Poor
Cost	Higher	Lower
Simultaneous combination with EEG	Possible, but with several TMS-related artifacts	Possible. However, brain activity cannot be synchronously recorded from the same channels used for stimulation
Simultaneous combination with fMRI	Possible	Possible, but with electrode artifacts and the remote risk of sudden electrode heating
Portability	Not portable	Portable

Modified from Zimerman and Hummel (2010).

TMS has been recently approved in some countries to treat depression and other disorders, subject to approval by local ethical review boards (Rossi et al., 2009). These reservations largely follow from the suboptimal understanding of the putative mechanisms underlying NIBS-related effects. However, a number of contributing factors have been recently identified. A critical one concerns modulation of neurotransmitter levels (Ridding and Ziemann, 2010). Transcranial stimulation may alter the dynamics of excitatory/inhibitory neurotransmitter systems (e.g., GABA and glutamate) affecting the regulation of cortical neuronal activity. For example, rTMS effects decrease if the subjects consume a drug that interferes with NMDA receptors (Reis et al., 2006). Likewise, post-tDCS effects can be blocked by an NMDA-antagonist, whereas a partial NMDA-agonist (d-cycloserine) can selectively extend the duration of motor cortical excitability induced by anodal tDCS (Nitsche et al., 2004). Moreover, MR spectroscopy research (Stagg et al., 2009) shows that anodal tDCS decreases GABAergic transmission, while cathodal tDCS yields similar effects on glutamate concentrations over the motor cortex (MC).

NIBS may also exert long-lasting plastic changes through gene induction. Research based on rTMS protocols (Fritsch et al., 2010) has shown significant enhancements of brain-derived neurotrophic factor (BDNF) mRNA in the hippocampal, parietal, and piriform cortices. For instance, LTP-like effects in adult mice are abolished for specimens with deletion of the BDNF gene, suggesting that this factor could be a critical mediator of direct stimulation effects (Fritsch et al., 2010). Importantly, as a member of the neurotrophin family, BDNF plays an important role in synaptogenesis and synaptic mechanisms related to learning and memory processes.

Additionally, TMS has been successfully used in AD and MCI to track and modulate abnormal mechanisms, including glutamatergic dysfunctions, disruption of synaptic plasticity, and inhibition of LTP (Nardone et al., 2014). For example, TMS research consistently shows that such patients feature a decreased resting motor threshold, which has been interpreted as the electrophysiological correlate of cortical glutamatergic dysfunctions and as a marker of reduced cortical hyperexcitability in AD (Di Lazzaro et al., 2003, 2004; Inghilleri et al., 2006; Trebbastoni et al., 2016). Furthermore, rTMS reduces facilitation of the motor-evoked potential (MEP) in aMCI patients (Trebbastoni et al., 2016), revealing an initial impairment of the mechanisms that underlie glutamate-induced synaptic potentiation (Trebbastoni et al., 2016). On the other hand, a TMS-MRI study indicates that hyperexcitability of the sensorimotor cortex might represent a protective mechanism counteracting the prominent loss of cortical volume in AD and MCI. However, this supposed protective mechanism was found neither on the precuneus or cuneus of AD patients, nor in an MCI group (Niskanen et al., 2011). Furthermore, an assessment of navigated TMS-evoked EEG responses in AD patients showed prominent changes in functional connectivity and reactivity (Julkunen et al., 2008, 2011). In particular, TMS-evoked responses at 30–50 ms decreased significantly in AD over widespread brain regions, suggesting dysfunctions of a large-scale sensorimotor network. Compatibly, a TMS-EEG co-registration study in AD patients offered direct evidence of MC hyperexcitability, extending to the whole sensorimotor system (Ferreri et al., 2016). Although speculative, these changes could be interpreted as a compensatory mechanism allowing for the preservation of sensorimotor programming and execution over a long period, regardless of the disease's progression. Additional evidence on cortical plasticity in relevant patient samples comes from paired associative stimulation (PAS) and cortical responses to rTMS. Null PAS effects in MCI patients have been reported by Lahr et al. (2016). However, this result should be considered with caution, since the study mixed patients with single- and multiple-domain aMCI which may have clouded effects on a specific subsample. Indeed, previous evidence from Nardone et al. (2012) demonstrated that short-latency afferent inhibition is significantly reduced in patients with multiple-domain amnestic MCI relative to controls, but not in patients with single-domain amnestic MCI or with non-amnestic MCI. Taken together, this incipient evidence suggests that NIBS can directly impinge on the pathological mechanisms underlying some forms of AD and MCI.

Finally, note that the effects of rTMS and tDCS are not restricted to the stimulation site. Depending on stimulation parameters, they can induce changes at distant points through effective modulations of remote, interconnected networks (Plewnia et al., 2003; Hummel and Cohen, 2006). This particular property of NIBS techniques has been assessed in proof-of-principle studies aimed to enhance and decrease excitability of critical brain regions involved in the recovery process (for details see Hummel and Cohen, 2006; Nowak et al., 2010; Schulz et al., 2013). All these tenets illuminate the evidence gathered so far in NIBS studies on MCI patients, which we review below.

DOES NIBS IMPROVE LEARNING AND FORMATION OF NOVEL MEMORIES IN MCI?

Growing evidence indicates that NIBS techniques can significantly modulate various cognitive processes in both elderly neurotypicals and patients with AD. While the impact of NIBS on the latter population has been widely discussed (Hsu et al., 2015), much less attention has been paid to NIBS research on MCI and SCI. Below we offer a joint assessment of this empirical corpus, discussing available results in terms of stimulated brain regions. We deal with TMS studies first, and then consider those employing tDCS (for full details of each study see **Table 2**).

TMS Studies with MCI Patients

Prefrontal Cortex

In a study with SCI patients, Solé-Padullés et al. (2006) reported improvements of face-name associative memory following up-regulation of the bilateral prefrontal cortex (PFC) through high-frequency (5 Hz) rTMS. Specifically, the behavioral

TABLE 2 | Summary of studies using NIBS to influence memory function in MCI patients.

Experiment	Participants	NIBS	Study design	Stimulation protocol	Target area	Stimulated cognitive process and outcome	Control condition	Tests/ follow-up	Results
Anderkova et al. (2015)	8 MCI and 12 AD patients Age: 73 ± 7	rTMS	Crossover design. Each patient received one stimulation session in a random order, over each target area	Three 22-min sessions at 10 Hz. (1/day)	IFG-STG	Sustained attention, psychomotor speed, processing efficacy, set-switching, executive functions, processing speed, attentional and visual processing, encoding and recognition	VTX	Prior and immediately after each session	Enhanced speed processing
Eliasova et al. (2014)	10 aMCI patient Age: 72 ± 8	rTMS	Cross Overdesign. Each patient received one stimulation sessions in a random order, over each target area	Two 22-min sessions at 10 Hz. (1/day)	IFG	Sustained attention, psychomotor speed, efficacy of cognitive processing, set-switching, executive functions, speed cognitive process attentional and visual processing, encoding and recognition	VTX	Prior and immediately after each session.	improvement of attention and psychomotor speed
Cotelli et al. (2012)	1 aMCI patient Age: 69 22 healthy controls Age: 64 ± 4	rTMS	Uncontrolled *pre-post* study	Ten 25-min sessions at 20 Hz (5/week)	IPL	Associative memory, reasoning language, learning, short and long term memory, praxis attention, executive functions	Uncontrolled	2 baselines, immediately after stimulation protocol (2 weeks), 24 weeks	Enhanced associative memory, long term memory
Drumond Marra et al. (2015)	34 MCI patients Age: 60–74	rTMS	Double blind controlled study	Ten 25-min sessions at 10 HZ (5/week)	Left DLPFC	Cognitive processing, everyday memory, logical memory, long-term narrative memory, short-term auditory-verbal memory, rate of learning, learning and retrieval, working memory, executive functions	Sham group with a placebo coil.	Immediately and 30 days after stimulation protocol	Improved everyday memory for at least 1 month
Meinzer et al. (2015)	18 MCI patients Age: 69.56 ± 5.56 18 healthy controls Age: 67.44 ± 7.27	atDCS	Double-blind, cross-over, randomized, sham stimulation. During Cognitive Task and resting state fMRI	One session at 1 mA during 20 min.	Left ventral IFG	Cognitive processing.	Sham group: atDCS turned off after 30 s	During stimulation No follow-up	Improved word retrieval. Reduced activity in the bilateral prefrontal cortex, right middle temporal gyrus, left basal ganglia and thalamus
Sedlackova et al. (2008)	7 MCI-V patients Age: 70.3 ± 8.7	rTMS	Randomized, blind, cross-over study	One 30-min session at 10 HZ (1/day)	Left DLPFC	Executive function, working memory, and psychomotor speed.	MC (control site)	During stimulationNo follow-up	No effects
Turriziani et al. (2012)	8 MCI patients 66.4 ± 5.7 100 healthy controls Age: 20–35	rTMS	Blind, crossover study	One 10-min session at 1 Hz.	Left and right DLPFC	Cognitive processing, non-verbal recognition memory, verbal recognition memory	Sham group: coil held close to the DLPFC but angled away	Immediately after stimulation. No follow-up	Improved the performance on the non-verbal recognition memory test

(Continued)

TABLE 2 | Continued

Experiment	Participants	NIBS	Study design	Stimulation protocol	Target area	Stimulated cognitive process and outcome	Control condition	Tests/ follow-up	Results
Solé-Padullés et al. (2006)	40 adults with SCI Age: 66.95 ± 9.43	rTMS	Pre-post fMRI, randomized double blind sham stimulation	One 5-min session at 5 Hz	PFC	Associative memory	Sham group: coil held tangentially to the head, with its edge resting on the scalp.	Immediately after stimulation during post fMRI	Improvement in associative memory task. Higher activation of the right inferior and middle frontal giry together with the middle and superior occipital gyri.
Manenti et al. (2016)	20 PD patients with MCI Age: 67.1 ± 7.2	atDCS	Double-blind, cross-over, randomized, sham stimulation. During physical therapy.	One session at 2 mA during 25 min per (5/week)	DLPFC	Motor physical therapist for PD. Outcome evaluated: clinical neuropsychological, and motor task,	Sham group: atDCS turned off after 30 s	Prior and immediately after and 3 month follow up	Improvement in motor abilities, reduction of depressive symptoms in sham and atDCS. Improvement in the PD cognitive Rating Scale and verbal fluency test, only in tDCS.

NIBS, Non-invasive brain stimulation; atDCS, anodal transcranial direct current stimulation; rTMS, repetitive transcranial magnetic stimulation; AD, Alzheimer's disease; MCI, mild cognitive impairment; VMCI, vascular mild cognitive impairment; PD, Parkinson's disease; SCI, subjective cognitive impairment; DLPFC, dorsolateral prefrontal cortex; IFG, inferior frontal gyrus; IPL, inferior parietal lobule; MC, motor cortex; PFC, prefrontal cortex; STG, superior temporal gyrus; VTX, vertex.

enhancement positively correlated with the recruitment of the right PFC and posterior bilateral cortices, as shown by fMRI. In another study, Turriziani et al. (2012) found improvements of non-verbal recognition memory in MCI patients after low-frequency (1 Hz) stimulation and subsequent down-regulation of the right but not the left dorsolateral prefrontal cortex (DLPFC). Morever, a double-blind randomized sham-controlled study using 10-Hz rTMS in MCI patients (Drumond Marra et al., 2015) showed that up-regulation of the DLPFC led to improvements in everyday memory. Interestingly, this enhancement remained for at least a month. However, stimulation of prefrontal structures has also yielded null results. In particular, a blind study with a crossover design (Sedlackova et al., 2008) failed to induce positive or negative behavioral effects following either low- or high-frequency rTMSof the DLPFC in seven patients with vascular MCI.

Parietal Cortex

In a single-case study with an aMCI patient, Cotelli et al. (2012) assessed whether up-regulation of the left parietal cortex could lead to long-term improvements in face-name associations. The 10-day protocol consisted in daily 25-min-long stimulation sessions with high-frequency (20 Hz) rTMS. Behavioral enhancements suggested a putative role of the target region in associative memory.

Inferior Frontal Gyrus and Superior Temporal Gyrus

Two related studies Eliasova et al. (2014) and Anderkova et al. (2015) found that rTMS significantly improved Stroop-task performance in aMCI and AD patients. The stimulation protocol comprised three randomized 22-min-long sessions, during which 10-Hz rTMS was applied over the IFG, the right superior temporal gyrus (STG), and the vertex (VTX, a control stimulation site). As compared to the VTX, both IFG and STG simulation improved the patients' behavioral outcome. In particular, through a combination of rTMS and MRI, Anderkova et al. (2015) found that MCI/AD subjects had significant atrophy in the inferior temporal and fusiform gyri, putamen and cerebellum relative to healthy controls. Moreover, the level of atrophy correlated with the change in Stroop-task performance after rTMS of the STG.

Interim Summary

Taken together, these studies indicate that rTMS can have significant effects on cognitive functions in patients with SCI and MCI. Specifically, this form of stimulation appears to enhance face-name association memory, non-verbal recognition memory, attention, psychomotor speed, and everyday memory. However, inconsistencies among comparable studies and null results in some of them cast doubts on the robustness of this technique to restore cognitive function in MCI. A number of caveats can be identified. First, rTMS might not directly stimulate deep brain areas critically compromised in MCI and AD (e.g., the hippocampus). Second, discrepancies in stimulation parameters (frequency, intensity, duration, number of sessions, stimulation site) and experimental design across studies make it difficult to determine which configuration could

systematically yield effective results. Finally, there is only one double-blind randomized sham-controlled rTMS study with MCI patients. Thus, further work and more randomized placebo-controlled studies are needed to establish the optimal stimulation parameters for potential treatments in this population. Critically, a more through characterization is needed of how results are influenced by multiple interrelated factors, such as the patients' clinical profile, specific target areas, stimulation frequency, number of treatment sessions, sessionduration and specific cognitive domains tapped through selected behavioral tasks.

tDCS Studies with MCI Patients

To date, only two studies have explored the potential neurocognitive benefits of anodal tDCS in MCI patients. The target regions in them were the IFG and the PFC.

Inferior Frontal Gyrus

Meinzer et al. (2015) performed a double-blind, crossover, sham-controlled stimulation study with simultaneous task-related and resting-state fMRI recordings. An MRI-compatible stimulator was used to administer a constant direct current (1 mA, for 20 min) over the IFG. During sham stimulation, MCI patients produced fewer correct responses than healthy controls in a semantic fluency task. Interestingly, the degree of correct responses was associated with hyperactivity in bilateral prefrontal regions. Anodal tDCS significantly improved performance in MCI patients to healthy-control levels. Furthermore, such stimulation reduced task-related prefrontal hyperactivity and normalized altered network configurations at during resting state. This study provides evidence that anodal tDCS could ameliorate cognitive dysfunction in MCI patients and temporarily reverse abnormalities in relevant brain activity.

Prefrontal Cortex

A recent study assessing MCI patients with Parkinson's disease (PD; Manenti et al., 2016) evaluated the impact of up-regulating the right or left DLPFC via anodal tDCS combined with physical therapies. For 2 weeks, patients underwent daily administration of 2-mA stimulation during 25 min. Scores on the PD Cognitive Rating Scale and a verbal fluency test increased only in the real tDCS group (compared with the sham group). Notably, the effect remained stable for up to 3 months after the intervention.

Interim Discussion

These extremely preliminary results suggest that anodal tDCS can ameliorate cognitive dysfunction in different subtypes of MCI. In particular, long-term results obtained by combining stimulation with physical training open interesting avenues for further research.

CHALLENGES AND FUTURE DIRECTIONS

So far, NIBS studies seeking to improve cognitive function in MCI/SCI patients have yielded inconclusive findings. Although some studies showed favorable results, statistically significant differences in an experimental setting do not necessarily imply clinical applicability. Consequently, the evidence gathered so far, though promising, must be considered extremely preliminary. No definite answer can yet be provided for the ultimate question underlying the field: can NIBS-induced cognitive improvements persist beyond treatment and translate into daily-life benefits? The methodological considerations listed below emerge as critical milestones to properly address this issue.

Stimulation Parameters

A number of methodological considerations and caveats must be addressed before claiming that a genuine, reliable and replicable effect has been found. TMS stimulation protocols involve several parameters, such as stimulation frequency, intensity, coil shape, pulse and stimulation site. While neuroimaging tools have improved selection of target areas for stimulation, the latter are typically anatomically ill-defined and hugely variable among individual brains. This heterogeneity is reduced by using neuronavigation procedures and factoring individual anatomy into the protocol, thus maximizing the chances that one specific target node is being systematically stimulated within a critical network (Danner et al., 2008). Regarding stimulation frequency and intensity, a general finding is that TMS increases neuronal excitability above a 5-Hz frequency, whereas it yields the opposite effect below 1 Hz (Hallett, 2000; Sparing et al., 2001; Knoch et al., 2006). Stimulation intensity in TMS protocols is usually defined as a percentage of a subject's individual motor threshold, namely, the minimal stimulation intensity that induces a reliable motor evoked potential of minimal amplitude in the targeted muscle (Rossini et al., 2015). This measure is useful to determine the intensity needed to comparably stimulate primary motor regions across subjects. However, the relevance of this parameter remains unknown for the stimulation of other cortical regions. Future research should examine the possibility of designing subject- and area-specific simulation protocols (Klooster et al., 2016), together with region-specific stimulation-tuning.

In the case of tDCS, key parameters include electrode patch positioning, inter-electrode distance, stimulation duration, and stimulation intensity (Klooster et al., 2016). With this technique, anodal and cathodal currents increase and inhibit neural-network activity, respectively. In this sense, one important difference with TMS is that tDCS allows stimulating and inhibiting different target regions at the same time (Ferrucci et al., 2009; Lindenberg et al., 2010; Mahmoudi et al., 2011), which increases the range of methodological decisions on the part of researchers. Another factor known to modulate tDCS results is age. Indeed, montages inducing effects in young adults do not necessary yield the same results in older adults (Zimerman et al., 2013). This highlights the importance of considering information about age-associated brain reorganization when aiming to induce durable neuroplastic effects in aging subjects (Perceval et al., 2016), including MCI patients.

Behavioral Effects of Stimulation in Combination with Cognitive Training

Typically, neuro-rehabilitation protocols aim to stimulate the whole spatially-distributed network subserving a target cognitive function. To guarantee that the right mechanisms are being

stimulated, brain enhancement must be combined with training of key relevant functions. Theoretically, targeting a dysfunctional neural circuit while it is actively engaged by a behavioral task should bring about better therapeutic effects than mere resting-state stimulation of the same area (Miniussi et al., 2013). Given that both cognitive training and NIBS can enhance adaptive plastic mechanisms, their combination could produce synergistic positive effects on behavior (Ditye et al., 2012). This approach offers a chance to stimulate very precise neurocognitive mechanisms, implement experimental designs with strict control of stimulus- and task-related factors and increase protocol viability through short-term longitudinal or pre/post models.

To date, this combined approach has been used in only one study (Park et al., 2014). After 10 daily sessions of tDCS with cognitive training, healthy elderly subjects increased working memory skills for up to 28 days. However, as this study lacked a control group without training, no further conclusions can be drawn regarding the potential benefits of combined NIBS-training interventions. Also relevant is the study protocol proposed by Cheng et al. (2015), aimed to improve working memory in aMCI patients. The design consists of a 4-week, double-blind, randomized controlled trial involving anodal tDCS or sham intervention combined with a working memory (*n*-back) paradigm or a control cognitive training condition. They hypothesize that this joint approach could significantly improve cognitive performance relative to isolated NIBS *or n*-back training. Furthermore, one of the studies reviewed above Manenti et al. (2016) reported cognitive enhancements in PD patients by combining tDCS with physical exercise cognitive enhancements. Taken together, this incipient evidence suggests that significant and long-lasting cognitive improvements could be induced through a combination of NIBS and specific cognitive training in MCI/SCI patients. Future research in the field should test this hypothesis by comparing the impact of joint and single-method interventions in both populations.

The Critical Questions

Where to Stimulate?

Most of the reviewed studies targeted the DLPFC, a region implicated in working memory, everyday memory and automatic processing of learned tasks. These functions, indeed, were enhanced by NIBS in MCI patients and healthy subjects (Turriziani et al., 2012; Iuculano and Cohen Kadosh, 2013; Drumond Marra et al., 2015). Another region targeted within the frontal lobe has been the IFG. Stimulation over this structure significantly improved word retrieval and decreased hyperactivity in the bilateral PFC, the right middle temporal gyrus, the left basal ganglia, and the thalamus (Meinzer et al., 2015). Furthermore, rTMS over the STG has been observed to improve processing speed (Anderkova et al., 2015). Encouraging results have also been obtained through stimulation of the inferior parietal lobe, which enhanced associative and long-term memory in MCI patients (Cotelli et al., 2012). In this sense, note that other portions of the parietal cortex are known to increase blood oxygen level-dependent signals during working memory storage (Linden et al., 2003; Xu and Chun, 2006). The relevance of parietal structures as promising target areas for NIBS in MCI samples is highlighted by its role in cognitive impairments during early stage AD (Reiman et al., 2012; Weintraub et al., 2012).

In sum, available evidence highlights the relevance of specific prefrontal, frontal, temporal, and parietal areas as key targets to develop more robust stimulation protocols in MCI. The main findings emerging from their stimulation in MCI patients are summarized in **Figure 1**.

How to Stimulate?

In general, rTMS protocols follow an offline approach, testing hypothesized cognitive changes roughly 30–60 min after stimulation (Ziemann et al., 2008). The use of patterned rTMS protocols, like theta burst stimulation, allows delivering 600 pulses with sub-threshold intensity in less than a minute. This induces longer-lasting effects in a shorter delivery time (Suppa et al., 2016). The impact of theta burst stimulation has been examined in several psychiatric conditions, like schizophrenia and obsessive-compulsive disorder (Suppa et al., 2016). However, studies in patients with memory impairment are still lacking.

On the other hand, tDCS is a smaller, portable tool which allows designing more ecological paradigms and obtaining online measurements. Thus, tDCS can be used to stimulate a particular neural network during behavioral intervention. Thanks to new developments, tDCS protocols can now employ more than two electrodes, enabling multifocal stimulation of brain networks to increase neurofunctional precision (Alam et al., 2016).

For increased effectiveness, NIBS can be applied in multi-session designs. Cognitive improvement in MCI patients is increased over time by repetitive, daily rTMS sessions (Cotelli et al., 2012; Drumond Marra et al., 2015). An alternative approach could rely on the use of spaced stimulation patterns, with multiple daily sessions. This might potentially lead to prolonged after effects via late-phase LTP/LTD-like neuroplastic mechanisms (Goldsworthy et al., 2015). Regarding tDCS, Manenti et al. (2016) worked with PD patients and found cognitive improvements lasting at least 3 months, after 10 daily sessions (Manenti et al., 2016). The only study employing tDCS in MCI patients employed a single-session protocol (Meinzer et al., 2015), so that no evidence is available on whether multiple-session tDCS protocols can induce long-term beneficial effects in this population.

Another important challenge lies in combining NIBS with daily life activities. This includes home-based training focused on specific cognitive skills. Self-delivered NIBS by the patient in a household environment, ideally according to a patient-specific stimulation protocol, may be a crucial step to hone the applicability of tDCS as a therapeutic tool (Klooster et al., 2016). To be effective, home-based portable devices should incorporate features which guarantee that stimulation is applied over the right brain regions, such as patient-specific cap-positioning tDCS electrodes. This may result in more efficient and less expensive cognitive intervention. In consequence, future research should develop new paradigms and technologies specific to each patient and relevant target areas.

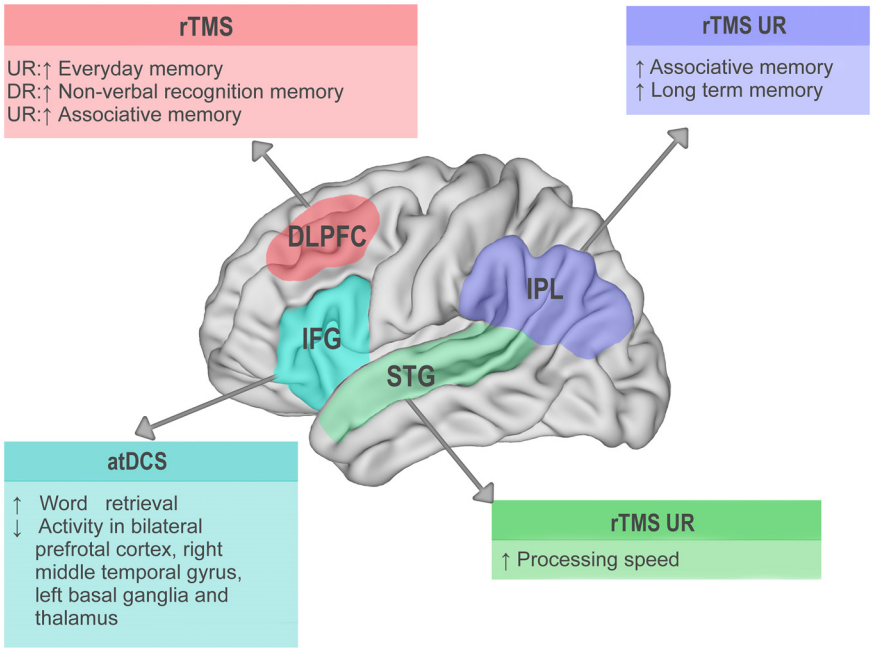


FIGURE 1 | Visual summary of main results from non-invasive brain stimulation (NIBS) studies with mild cognitive impairment (MCI) patients. Lateral view of the left hemisphere. Target areas stimulated in the reviewed studies are highlighted in different colors. DLPFC, dorsolateral prefrontal cortex; IFG, inferior frontal gyrus; STG, superior temporal gyrus; IPL, inferior parietal lobule; atDCS, anodal transcranial direct current stimulation; rTMS, repetitive transcranial magnetic stimulation; ↑ behavioral improvement; ↓ behavioral decrement; UR, up-regulation; DR, down-regulation.

Whom to Stimulate?

The use of NIBS to enhance memory in the elderly represents a promising avenue for both basic and translational research. However, the effects of NIBS likely depend on inter-individual differences in the degree of age-related cognitive impairment and brain reorganization (Craik, 1994; Mungas et al., 2010; Reuter-Lorenz and Park, 2014), under the influence of various genetic and lifestyle factors (Bishop et al., 2010; Grady, 2012). Moreover, different subtypes of MCI may respond in different ways to the same stimulation protocols. This calls for further research on the multiple subject-related variables that can modulate results within and across studies.

Correct diagnosis of MCI subtypes proves critical to discern stimulation effects. Approximately 50% of patients with aMCI convert to AD within 5 years (Gauthier et al., 2006). Also, different genetic influences impact on the development of AD. For instance, the presence of one or two ε4 alleles in the apolipoprotein E (APOE) gene confers risk of AD. An individual who meets the clinical, cognitive and etiologic criteria for MCI, and is also APOE ε4 positive, is more likely to progress to AD dementia within a few years than an individual without this genetic trait (Winblad et al., 2004; Anstey et al., 2008; Albert et al., 2011). Conversely, other familial versions will progress to AD with a 100% of probability (e.g., presenilin 1, PS1). Moreover, despite well-established risk genes (e.g., APOE, SORL1) or causative genes (e.g., APP, PSEN1, PSEN2) of AD, more than 20 loci have been associated with disease risk (Karch et al., 2014). In this sense, the epigenetic approach to clinical phenotypes offers a promissory agenda (Bennett et al., 2015). The need thus arises for more research on the interaction between NIBS and previous genetic risk in the MCI-AD spectrum.

On the other hand, fewer prognostic data are available about patients with non-memory-related impairments, who may develop non-AD dementia (Winblad et al., 2004; Anstey et al., 2008; Albert et al., 2011). Three out of the nine articles reviewed in "Does NIBS Improve Learning and Formation of Novel Memories in MCI?" Section targeted aMCI patients (Cotelli et al., 2012; Eliasova et al., 2014; Anderkova et al., 2015), one focused on vascular MCI (Sedlackova et al., 2008), and another one assessed MCI linked to PD (Manenti et al., 2016). The rest did not specify the MCI subtype. This distinction is crucial to assess the efficacy and extent of the stimulation protocol. For example, no effects were observed in vascular MCI when stimulating the DLPFC with rTMS (Sedlackova et al., 2008). It seems that each subtype of MCI could respond different to stimulation, although it is not clear which ones would profit the most from intervention. In consequence, it is necessary to stratify MCI subtypes to recognize condition-specific stimulation effects and develop more fine-grained protocols for each patient, according to his or her diagnosis.

Finally, given that aMCI patients may be at increased risk of developing AD, future interventions might focus on familial-MCI patients with high probability to progress into

AD. Early-onset AD patients present MCI, rapidly followed by severe deficits in cortical functions (aphasia, apraxia, and agnosia), whereas late-onset AD patients show a slower overall decline and pronounced memory deficits, particularly in the semantic domain (Joubert et al., 2016). Since the progression of early-onset AD is faster than in late-onset AD, the latter variety may profit more from NIBS as a tool to attenuate the rate of cognitive decline. The identification of causative genetic mutations leading to early-onset AD (APP, PSEN1, PSEN2) and susceptibility markers associated with later disease onset (e.g., APOE ε4 carriers) is crucial to determine specific patterns of cognitive impairment in younger patients with a family history of AD or other neurodegenerative diseases (Rocchi et al., 2003; Winblad et al., 2004; Albert et al., 2011), and to better screen candidates for different stimulation protocols targeting specific neurofunctional mechanisms.

A Call for Multicenter Studies

A thorough understanding of potential neurocognitive changes induced by NIBS requires protocols to be complemented with other functional techniques, like electroencephalography (EEG), magnetoelectroencephalography (MEG), positron-emission tomography (PET), fMRI, and magnetic resonance spectroscopy (MRS). Therefore, the direct comparison of neuro-anatomical changes before and after stimulation could shed light on the potentially specific effects of NIBS. Also, the establishment of NIBS techniques as therapeutic tools for MCI requires systematic assessment of multiple subject variables (e.g., cortical thinning, white matter volume and integrity, functional and structural connectivity, genetic variants, learning capacity, age at disease onset and cognitive reserve). In order to gain multidimensional insights into the impact of NIBS in MCI, above and beyond between-subject variability, longer multicenter studies are urgently needed. By revealing common patterns despite differences in diagnosis, sociodemographic factors, neurobiological features, brain recording parameters, measurements, and analyses, such joint protocols will help identify the most consistent changes induced by NIBS, while revealing the most effective stimulation parameters.

In this context, the implementation of normative databases based on neuroscientific techniques might help to recognize abnormal patterns, detect the disease at early stages, and define treatment protocol strategies (Gordon and Konopka, 2005). Available EEG neuromarkers of MCI and AD are critical to such ends (Ibanez and Parra, 2014). Quantitative EEG (qEEG) shows that AD and MCI are characterized by increased theta power, decreased alpha and beta power and decreased coherence in the alpha and theta band in posterior regions (Jelic et al., 2000). These abnormalities are thought to be associated with functional disconnections among cortical areas, loss of cortical neurons, axonal degeneration, and cholinergic deficits. Furthermore, EEG/MEG techniques have been useful to characterize AD and to detect changes in preclinical familial AD and MCI (Jackson and Snyder, 2008; Stam, 2010; Pietto et al., 2016). For example, source EEG functional network disruption in AD and MCI is associated with cognitive decline (Gianotti et al., 2007; Kurimoto et al., 2008; Ishii et al., 2010; Hsiao et al., 2013) and APOE genotype (Canuet et al., 2012). Moreover, relative to normative samples, subjects with SCI present elevated alpha power and increased number of spatiotemporal wave events (Alexander et al., 2006). Furthermore, event-related potential (ERP) measures have shown utility in predicting the conversion to dementia among elderly individuals at risk for AD (Gironell et al., 2005). Beyond its to diagnose functional impairment, EEG is also useful to set stimulation parameters and assess the effect of NIBS in patients (Kropotov, 2016). Combining NIBS with EEG would help to identify stimulus intensity, frequency of the stimulation, location, and duration needed to normalize EEG activity (Jin et al., 2012). This is critical since most rTMS protocols use the same parameters for all patients. With this approach, a customized treatment would be available for each individual based on analysis of resting EEG. In this sense, Marceglia et al. (2016) applied tDCS to change cortical activity as represented by qEEG. Despite working on a small sample, the authors found that the abnormal pattern of EEG activity in AD during memory processing is partially reversed by anodal transcranial direct current stimulation (atDCS) and this reversion correlated with an improvement in word recognition, suggesting that such benefits are supported by the modulation of neuronal cortical activity. In conclusion, normative databases should be systematically considered as a reference to establish the efficacy of individualized stimulation protocols.

CONCLUSIONS

The present review aimed to summarize research on the effects of NIBS in MCI and to outline the approaches that may improve the efficacy of stimulation protocols. Despite major advances over the past few decades, extant MCI/AD treatments are not completely effective. Although NIBS have not yet been massively applied in SCI/MCI patients, available results hold considerable promise. First, TMS and tDCS are well-suited to explore brain plasticity across the lifespan (Heise et al., 2014). Second, they provide valuable physiologic biomarkers of the state of cortical reactivity, brain network connectivity, dynamics, and mechanisms of brain plasticity. Third, NIBS techniques avoid systemic side-effects and allow for temporally and spatially precise neural interventions that escape the possibilities of pharmacological or complementary therapies. Moreover, discrepancies among available studies may be largely due to differences among tasks, designs, and samples, as opposed to limitations inherent to NIBS. However, many issues remain to be solved and large longitudinal studies are needed to more stringently assess the robustness of available positive results. Furthermore, even if NIBS techniques proved irrelevant for clinical purposes, they could remain highly useful for experimental research. While no definitive conclusions on the efficacy and specificity of NIBS can be advanced at this stage, our review delineates the major challenges to be faced in future studies. In this sense, pre-post neuroimaging designs could reveal putative mechanisms underlying NIBS effects. Also, concurrent use of

NIBS techniques with specific tasks targeting impaired domains in individual patients could potentially enhance LTP-like after-effects. In particular, applying NIBS over a dysfunctional neural network while the latter is engaged by a particular behavioral activity could significantly enhance learning- and memory-related effects. Furthermore, the application of NIBS across multiple sessions could render such effects longer lasting. All these considerations should be accompanied by very precise diagnosis of each patient's MCI subtype, so that subject-specific protocols can be designed and assessed with the implementation of normative databases. Moreover, further studies are required to identify the optimal stimulation parameters yielding robust outcomes for different MCI subtypes as well as early- and late-onset AD. Finally, we emphasize the need to conduct longer longitudinal studies to establish the duration of NIBS-induced benefits. By contemplating these issues, future NIBS studies could shed unprecedented light on functional restoration options for MCI patients, paving the way for new treatments aimed to delay (or event prevent) the development of AD and other dementias.

AUTHOR CONTRIBUTIONS

AB did the literature research and wrote the manuscript. AI and AMG provided insightful thoughts to study concepts, and revised the manuscript. LS and JF assisted with the literature review and revised the final version. MZ wrote, revised critically and approved the final version.

ACKNOWLEDGMENTS

This work was partially supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Comisión Nacional de Investigación Científica y Tecnológica (CONICYT)/Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) Regular (1130920), Fondo para la Investigación Científica y Tecnológica (FONCyT-PICT; Grant no. 2012-0412, 2012-1309), Fondo de Financiamiento de Centros de Investigación en Áreas Prioritarias (FONDAP; 15150012) and the Instituto de Neurología Cognitiva (INECO) Foundation.

REFERENCES

Alam, M., Truong, D. Q., Khadka, N., and Bikson, M. (2016). Spatial and polarity precision of concentric high-definition transcranial direct current stimulation (HD-tDCS). *Phys. Med. Biol.* 61, 4506–4521. doi: 10.1088/0031-9155/61/12/4506

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., et al. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the national institute on aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 270–279. doi: 10.1016/j.jalz.2011.03.008

Alexander, D. M., Arns, M. W., Paul, R. H., Rowe, D. L., Cooper, N., Esser, A. H., et al. (2006). EEG markers for cognitive decline in elderly subjects with subjective memory complaints. *J. Integr. Neurosci.* 5, 49–74. doi: 10.1142/s0219635206001021

Anderkova, L., Eliasova, I., Marecek, R., Janousova, E., and Rektorova, I. (2015). Distinct pattern of gray matter atrophy in mild Alzheimer's disease impacts on cognitive outcomes of noninvasive brain stimulation. *J. Alzheimers Dis.* 48, 251–260. doi: 10.3233/JAD-150067

Anstey, K. J., Cherbuin, N., Christensen, H., Burns, R., Reglade-Meslin, C., Salim, A., et al. (2008). Follow-up of mild cognitive impairment and related disorders over four years in adults in their sixties: the PATH through life study. *Dement. Geriatr. Cogn. Disord.* 26, 226–233. doi: 10.1159/000154646

Alzheimer's Association. (2012). 2012 Alzheimer's disease facts and figures. *Alzheimers Dement.* 8, 131–168. doi: 10.1016/j.jalz.2012.02.001

Bennett, D. A., Yu, L., Yang, J., Srivastava, G. P., Aubin, C., and De Jager, P. L. (2015). Epigenomics of Alzheimer's disease. *Transl Res.* 165, 200–220. doi: 10.1016/j.trsl.2014.05.006

Bishop, N. A., Lu, T., and Yankner, B. A. (2010). Neural mechanisms of ageing and cognitive decline. *Nature* 464, 529–535. doi: 10.1038/nature08983

Canuet, L., Tellado, I., Couceiro, V., Fraile, C., Fernandez-Novoa, L., Ishii, R., et al. (2012). Resting-state network disruption and APOE genotype in Alzheimer's disease: a lagged functional connectivity study. *PLoS One* 7:e46289. doi: 10.1371/journal.pone.0046289

Cheng, C. P., Chan, S. S., Mak, A. D., Chan, W. C., Cheng, S. T., Shi, L., et al. (2015). Would transcranial direct current stimulation (tDCS) enhance the effects of working memory training in older adults with mild neurocognitive disorder due to Alzheimer's disease: study protocol for a randomized controlled trial. *Trials* 16:479. doi: 10.1186/s13063-015-0999-0

Cotelli, M., Calabria, M., Manenti, R., Rosini, S., Maioli, C., Zanetti, O., et al. (2012). Brain stimulation improves associative memory in an individual with amnestic mild cognitive impairment. *Neurocase* 18, 217–223. doi: 10.1080/13554794.2011.588176

Craik, F. I. M. (1994). Memory changes in normal aging. *Curr. Dir. Psychol. Sci.* 3, 155–158. doi: 10.1111/1467-8721.ep10770653

Danner, N., Julkunen, P., Könönen, M., Säisänen, L., Nurkkala, J., and Karhu, J. (2008). Navigated transcranial magnetic stimulation and computed electric field strength reduce stimulator-dependent differences in the motor threshold. *J. Neurosci. Methods* 174, 116–122. doi: 10.1016/j.jneumeth.2008.06.032

Dayan, E., Censor, N., Buch, E. R., Sandrini, M., and Cohen, L. G. (2013). Noninvasive brain stimulation: from physiology to network dynamics and back. *Nat. Neurosci.* 16, 838–844. doi: 10.1038/nn.3422

Ditye, T., Jacobson, L., Walsh, V., and Lavidor, M. (2012). Modulating behavioral inhibition by tDCS combined with cognitive training. *Exp. Brain Res.* 219, 363–368. doi: 10.1007/s00221-012-3098-4

Drumond Marra, H. L., Myczkowski, M. L., Maia Memória, C., Arnaut, D., Leite Ribeiro, P., Sardinha Mansur, C. G., et al. (2015). Transcranial magnetic stimulation to address mild cognitive impairment in the elderly: a randomized controlled study. *Behav. Neurol.* 501:287843. doi: 10.1155/2015/287843

Elder, G. J., and Taylor, J. P. (2014). Transcranial magnetic stimulation and transcranial direct current stimulation: treatments for cognitive and neuropsychiatric symptoms in the neurodegenerative dementias? *Alzheimers Res. Ther.* 6:74. doi: 10.1186/s13195-014-0074-1

Eliasova, I., Anderkova, L., Marecek, R., and Rektorova, I. (2014). Non-invasive brain stimulation of the right inferior frontal gyrus may improve attention in early Alzheimer's disease: a pilot study. *J. Neurol. Sci.* 346, 318–322. doi: 10.1016/j.jns.2014.08.036

Ferreri, F., Vecchio, F., Vollero, L., Guerra, A., Petrichella, S., Ponzo, D., et al. (2016). Sensorimotor cortex excitability and connectivity in Alzheimer's disease: a TMS-EEG Co-registration study. *Hum. Brain Mapp.* 37, 2083–2096. doi: 10.1002/hbm.23158

Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., et al. (2006). Global prevalence of dementia: a Delphi consensus study. *Lancet* 366, 2112–2117. doi: 10.1016/S0140-6736(05)67889-0

Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Salvoro, B., Giacopuzzi, M., et al. (2009). Transcranial direct current stimulation in severe, drug-resistant major depression. *J. Affect. Disord.* 118, 215–219. doi: 10.1016/j.jad.2009.02.015

Fregni, F., and Pascual-Leone, A. (2007). Technology insight: noninvasive brain stimulation in neurology—perspectives on the therapeutic potential of rTMS and tDCS. *Nat. Clin. Pract. Neurol.* 3, 383–393. doi: 10.1038/ncpneuro0530

Freitas, C., Mondragón-Llorca, H., and Pascual-Leone, A. (2011). Noninvasive brain stimulation in Alzheimer's disease: systematic review and perspectives for the future. *Exp. Gerontol.* 46, 611–627. doi: 10.1016/j.exger.2011.04.001

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., et al. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* 66, 198–204. doi: 10.1016/j.neuron.2010.03.035

Gauthier, S., Reisberg, B., Zaudig, M., Petersen, R. C., Ritchie, K., Broich, K., et al. (2006). Mild cognitive impairment. *Lancet* 367, 1262–1270. doi: 10.1016/S0140-6736(06)68542-5

Gianotti, L. R., Künig, G., Lehmann, D., Faber, P. L., Pascual-Marqui, R. D., Kochi, K., et al. (2007). Correlation between disease severity and brain electric LORETA tomography in Alzheimer's disease. *Clin. Neurophysiol.* 118, 186–196. doi: 10.1016/j.clinph.2006.09.007

Gironell, A., García-Sánchez, C., Estévez-González, A., Boltes, A., and Kulisevsky, J. (2005). Usefulness of p300 in subjective memory complaints: a prospective study. *J. Clin. Neurophysiol.* 22, 279–284. doi: 10.1097/01.wnp.0000173559.60113.ab

Goldsworthy, M. R., Pitcher, J. B., and Ridding, M. C. (2015). Spaced noninvasive brain stimulation: prospects for inducing long-lasting human cortical plasticity. *Neurorehabil. Neural Repair* 29, 714–721. doi: 10.1177/1545968314562649

Gordon, E., and Konopka, L. M. (2005). Special issue: EEG databases in research and clinical practice: current status and future directions. *Clin. EEG Neurosci.* 36, 53–54. doi: 10.1177/155005940503600203

Grady, C. (2012). The cognitive neuroscience of ageing. *Nat. Rev. Neurosci.* 13, 491–505. doi: 10.1038/nrn3256

Hallett, M. (2000). Transcranial magnetic stimulation and the human brain. *Nature* 406, 147–150. doi: 10.1038/35018000

Heise, K. F., Niehoff, M., Feldheim, J. F., Liuzzi, G., Gerloff, C., and Hummel, F. C. (2014). Differential behavioural and physiological effects of anodal transcranial direct current stimulation in healthy adults of younger and older age. *Front. Aging Neurosci.* 6:146. doi: 10.3389/fnagi.2014.00146

Hsiao, F.-J., Wang, Y. J., Yan, S. H., Chen, W. T., and Lin, Y. Y. (2013). Altered oscillation and synchronization of default-mode network activity in mild Alzheimer's disease compared to mild cognitive impairment: an electrophysiological study. *PLoS One* 8:e68792. doi: 10.1371/journal.pone.0068792

Hsu, W.-Y., Ku, Y., Zanto, T. P., and Gazzaley, A. (2015). Effects of noninvasive brain stimulation on cognitive function in healthy aging and Alzheimer's disease: a systematic review and meta-analysis. *Neurobiol. Aging* 36, 2348–2359. doi: 10.1016/j.neurobiolaging.2015.04.016

Hummel, F. C., and Cohen, L. G. (2006). Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? *Lancet Neurol.* 5, 708–712. doi: 10.1016/s1474-4422(06)70525-7

Ibanez, A., and Parra, M. A. (2014). Mapping memory binding onto the connectome's temporal dynamics: toward a combined biomarker for Alzheimer's disease. *Front. Hum. Neurosci.* 8:237. doi: 10.3389/fnhum.2014.00237

Inghilleri, M., Conte, A., Frasca, V., Scaldaferri, N., Gilio, F., Santini, M., et al. (2006). Altered response to rTMS in patients with Alzheimer's disease. *Clin. Neurophysiol.* 117, 103–109. doi: 10.1016/j.clinph.2005.09.016

Ishii, R., Canuet, L., Kurimoto, R., Ikezawa, K., Aoki, Y., Azechi, M., et al. (2010). Frontal shift of posterior alpha activity is correlated with cognitive impairment in early Alzheimer's disease: a magnetoencephalography-beamformer study. *Psychogeriatrics* 10, 138–143. doi: 10.1111/j.1479-8301.2010.00326.x

Iuculano, T., and Cohen Kadosh, R. (2013). The mental cost of cognitive enhancement. *J. Neurosci.* 33, 4482–4486. doi: 10.1523/JNEUROSCI.4927-12.2013

Jackson, C. E., and Snyder, P. J. (2008). Electroencephalography and event-related potentials as biomarkers of mild cognitive impairment and mild Alzheimer's disease. *Alzheimers Dement.* 4, S137–S143. doi: 10.1016/j.jalz.2007.10.008

Jelic, V., Johansson, S., Almkvist, O., Shigeta, M., Julin, P., Nordberg, A., et al. (2000). Quantitative electroencephalography in mild cognitive impairment: longitudinal changes and possible prediction of Alzheimer's disease. *Neurobiol. Aging* 21, 533–540. doi: 10.1016/s0197-4580(00)00153-6

Jin, Y., Kemp, A. S., Huang, Y., Thai, T. M., Liu, Z., Xu, W., et al. (2012). Alpha EEG guided TMS in schizophrenia. *Brain Stimul.* 5, 560–568. doi: 10.1016/j.brs.2011.09.005

Joubert, S., Gour, N., Guedj, E., Didic, M., Guériot, C., Koric, L., et al. (2016). Early-onset and late-onset Alzheimer's disease are associated with distinct patterns of memory impairment. *Cortex* 74, 217–232. doi: 10.1016/j.cortex.2015.10.014

Julkunen, P., Jauhiainen, A. M., Könönen, M., Pääkkönen, A., Karhu, J., and Soininen, H. (2011). Combining transcranial magnetic stimulation and electroencephalography may contribute to assess the severity of Alzheimer's disease. *Int. J. Alzheimers Dis.* 2011:654794. doi: 10.4061/2011/654794

Julkunen, P., Jauhiainen, A. M., Westerén-Punnonen, S., Pirinen, E., Soininen, H., Könönen, M., et al. (2008). Navigated TMS combined with EEG in mild cognitive impairment and Alzheimer's disease: a pilot study. *J. Neurosci. Methods* 172, 270–276. doi: 10.1016/j.jneumeth.2008.04.021

Karch, C. M., Cruchaga, C., and Goate, A. M. (2014). Alzheimer's disease genetics: from the bench to the clinic. *Neuron* 83, 11–26. doi: 10.1016/j.neuron.2014.05.041

Klooster, D., de Louw, A., Aldenkamp, A. P., Besseling, R. M., Mestrom, R. M., Carrette, S., et al. (2016). Technical aspects of neurostimulation: focus on equipment, electric field modeling and stimulation protocols. *Neurosci. Biobehav. Rev.* 65, 113–141. doi: 10.1016/j.neubiorev.2016.02.016

Knoch, D., Gianotti, L. R., Pascual-Leone, A., Treyer, V., Regard, M., Hohmann, M., et al. (2006). Disruption of right prefrontal cortex by low-frequency repetitive transcranial magnetic stimulation induces risk-taking behavior. *J. Neurosci.* 26, 6469–6472. doi: 10.1523/JNEUROSCI.0804-06.2006

Kropotov, J. D. (2016). *Functional Neuromarkers for Psychiatry: Applications for Diagnosis and Treatment.* Cambridge, MA: Academic Press.

Kurimoto, R., Ishii, R., Canuet, L., Ikezawa, K., Azechi, M., Iwase, M., et al. (2008). Event-related synchronization of alpha activity in early Alzheimer's disease and mild cognitive impairment: an MEG study combining beamformer and group comparison. *Neurosci. Lett.* 443, 86–89. doi: 10.1016/j.neulet.2008.07.015

Lahr, J., Peter, J., Minkova, L., Lauer, E., Reis, J., Heimbach, B., et al. (2016). No difference in paired associative stimulation induced cortical neuroplasticity between patients with mild cognitive impairment and elderly controls. *Clin. Neurophysiol.* 127, 1254–1260. doi: 10.1016/j.clinph.2015.08.010

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Marra, C., et al. (2004). Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 75, 555–559. doi: 10.1136/jnnp.2003.018127

Di Lazzaro, V., Oliviero, A., Tonali, P. A., Mazzone, P., Insola, A., Pilato, F., et al. (2003). Direct demonstration of reduction of the output of the human motor cortex induced by a fatiguing muscle contraction. *Exp. Brain Res.* 149, 535–538. doi: 10.1007/s00221-003-1408-6

Linden, D. E., Bittner, R. A., Muckli, L., Waltz, J. A., Kriegeskorte, N., Goebel, R., et al. (2003). Cortical capacity constraints for visual working memory: dissociation of fMRI load effects in a fronto-parietal network. *Neuroimage* 20, 1518–1530. doi: 10.1016/j.neuroimage.2003.07.021

Lindenberg, R., Renga, V., Zhu, L. L., Nair, D., and Schlaug, G. (2010). Bihemispheric brain stimulation facilitates motor recovery in chronic stroke patients. *Neurology* 75, 2176–2184. doi: 10.1212/WNL.0b013e318202013a

Mahmoudi, H., Borhani Haghighi, A., Petramfar, P., Jahanshahi, S., Salehi, Z., and Fregni, F. (2011). Transcranial direct current stimulation: electrode montage in stroke. *Disabil. Rehabil.* 33, 1383–1388. doi: 10.3109/09638288.2010.532283

Manenti, R., Brambilla, M., Benussi, A., Rosini, S., Cobelli, C., Ferrari, C., et al. (2016). Mild cognitive impairment in Parkinson's disease is improved by transcranial direct current stimulation combined with physical therapy. *Mov. Disord.* 31, 715–724. doi: 10.1002/mds.26561

Manenti, R., Cotelli, M., Robertson, I. H., and Miniussi, C. (2012). Transcranial brain stimulation studies of episodic memory in young adults, elderly adults

and individuals with memory dysfunction: a review. *Brain Stimul.* 5, 103–109. doi: 10.1016/j.brs.2012.03.004

Marceglia, S., Mrakic-Sposta, S., Rosa, M., Ferrucci, R., Mameli, F., Vergari, M., et al. (2016). Transcranial direct current stimulation modulates cortical neuronal activity in Alzheimer's disease. *Front. Neurosci.* 10:134. doi: 10.3389/fnins.2016.00134

Meinzer, M., Lindenberg, R., Phan, M. T., Ulm, L., Volk, C., and Flöel, A. (2015). Transcranial direct current stimulation in mild cognitive impairment: behavioral effects and neural mechanisms. *Alzheimers Dement.* 11, 1032–1040. doi: 10.1016/j.jalz.2014.07.159

Miniussi, C., Harris, J. A., and Ruzzoli, M. (2013). Modelling non-invasive brain stimulation in cognitive neuroscience. *Neurosci. Biobehav. Rev.* 37, 1702–1712. doi: 10.1016/j.neubiorev.2013.06.014

Mungas, D., Beckett, L., Harvey, D., Farias, S. T., Reed, B., Carmichael, O., et al. (2010). Heterogeneity of cognitive trajectories in diverse older persons. *Psychol. Aging* 25, 606–619. doi: 10.1037/a0019502

Nardone, R., Bergmann, J., Christova, M., Caleri, F., Tezzon, F., Ladurner, G., et al. (2012). Short latency afferent inhibition differs among the subtypes of mild cognitive impairment. *J. Neural Transm.* 119, 463–471. doi: 10.1007/s00702-011-0725-3

Nardone, F. T., Tezzon, F., Höller, Y., Golaszewski, S., Trinka, E., and Brigo, F. (2014). Transcranial magnetic stimulation (TMS)/repetitive TMS in mild cognitive impairment and Alzheimer's disease. *Acta Neurol. Scand.* 129, 351–366. doi: 10.1111/ane.12223

Niskanen, E., Könönen, M., Määttä, S., Hallikainen, M., Kivipelto, M., Casarotto, S., et al. (2011). New insights into Alzheimer's disease progression: a combined TMS and structural MRI study. *PLoS One* 6:e26113. doi: 10.1371/journal.pone.0026113

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., et al. (2008). Transcranial direct current stimulation: state of the art 2008. *Brain Stimul.* 1, 206–223. doi: 10.1016/j.brs.2008.06.004

Nitsche, M. A., Jaussi, W., Liebetanz, D., Lang, N., Tergau, F., and Paulus, W. (2004). Consolidation of human motor cortical neuroplasticity by D-cycloserine. *Neuropsychopharmacology* 29, 1573–1578. doi: 10.1038/sj.npp.1300517

Nitsche, M., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nowak, D. A., Bösl, K., Podubeckà, J., and Carey, J. R. (2010). Noninvasive brain stimulation and motor recovery after stroke. *Restor. Neurol. Neurosci.* 28, 531–544. doi: 10.3233/RNN-2010-0552

Park, S.-H., Seo, J.-H., Kim, Y. H., and Ko, M. H. (2014). Long-term effects of transcranial direct current stimulation combined with computer-assisted cognitive training in healthy older adults. *Neuroreport* 25, 122–126. doi: 10.1097/WNR.0000000000000080

Perceval, G., Flöel, A., and Meinzer, M. (2016). Can transcranial direct current stimulation counteract age-associated functional impairment? *Neurosci. Biobehav. Rev.* 65, 157–172. doi: 10.1016/j.neubiorev.2016.03.028

Petersen, R. C., and Negash, S. (2008). Mild cognitive impairment: an overview. *CNS Spectr.* 13, 45–53. doi: 10.1017/s1092852900016151

Pietto, M., Parra, M. A., Trujillo, N., Flores, F., García, A. M., Bustin, J., et al. (2016). Behavioral and electrophysiological correlates of memory binding deficits in patients at different risk levels for Alzheimer's disease. *J. Alzheimers Dis.* 53, 1325–1340. doi: 10.3233/JAD-160056

Plewnia, C., Lotze, M., and Gerloff, C. (2003). Disinhibition of the contralateral motor cortex by low-frequency rTMS. *Neuroreport* 14, 609–612. doi: 10.1097/00001756-200303240-00017

Reiman, E. M., Quiroz, Y. T., Fleisher, A. S., Chen, K., Velez-Pardo, C., Jimenez-Del-Rio, M., et al. (2012). Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study. *Lancet Neurol.* 11, 1048–1056. doi: 10.1016/S1474-4422(12)70228-4

Reis, J., John, D., Heimeroth, A., Mueller, H. H., Oertel, W. H., Arndt, T., et al. (2006). Modulation of human motor cortex excitability by single doses of amantadine. *Neuropsychopharmacology* 31, 2758–2766. doi: 10.1038/sj.npp.1301122

Reisberg, B., and Gauthier, S. (2008). Current evidence for subjective cognitive impairment (SCI) as the pre-mild cognitive impairment (MCI) stage of subsequently manifest Alzheimer's disease. *Int. Psychogeriatr.* 20, 1–16. doi: 10.1017/S1041610207006412

Reuter-Lorenz, P. A., and Park, D. C. (2014). How does it STAC up? Revisiting the scaffolding theory of aging and cognition. *Neuropsychol. Rev.* 24, 355–370. doi: 10.1007/s11065-014-9270-9

Ridding, M. C., and Ziemann, U. (2010). Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J. Physiol.* 588, 2291–2304. doi: 10.1113/jphysiol.2010.190314

Rocchi, A., Pellegrini, S., Siciliano, G., and Murri, L. (2003). Causative and susceptibility genes for Alzheimer's disease: a review. *Brain Res. Bull.* 61, 1–24. doi: 10.1016/s0361-9230(03)00067-4

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., and Safety of TMS Consensus Group. (2009). Safety, ethical considerations and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* 120, 2008–2039. doi: 10.1016/j.clinph.2009.08.016

Rossini, P., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., et al. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an IFCN Committee. *Clin. Neurophysiol.* 126, 1071–1107. doi: 10.1016/j.clinph.2015.02.001

Schulz, R., Gerloff, C., and Hummel, F. C. (2013). Non-invasive brain stimulation in neurological diseases. *Neuropharmacology* 64, 579–587. doi: 10.1016/j.neuropharm.2012.05.016

Sedlackova, S., Rektorova, I., Fanfrdlova, Z., and Rektor, I. (2008). Neurocognitive effects of repetitive transcranial magnetic stimulation in patients with cerebrovascular disease without dementia. *J. Psychophysiol.* 22, 14–19. doi: 10.1027/0269-8803.22.1.14

Solé-Padullés, C., Bartrés-Faz, D., Junqué, C., Clemente, I. C., Molinuevo, J. L., Bargalló, N., et al. (2006). Repetitive transcranial magnetic stimulation effects on brain function and cognition among elders with memory dysfunction. A randomized sham-controlled study. *Cereb. Cortex* 16, 1487–1493. doi: 10.1093/cercor/bhj083

Sparing, R., Mottaghy, F. M., Hungs, M., Brügmann, M., Foltys, H., Huber, W., et al. (2001). Repetitive transcranial magnetic stimulation effects on language function depend on the stimulation parameters. *J. Clin. Neurophysiol.* 18, 326–330. doi: 10.1097/00004691-200107000-00004

Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kincses, Z. T., et al. (2009). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *J. Neurosci.* 29, 5202–5206. doi: 10.1523/JNEUROSCI.4432-08.2009

Stam, C. J. (2010). Use of magnetoencephalography (MEG) to study functional brain networks in neurodegenerative disorders. *J. Neurol. Sci.* 289, 128–134. doi: 10.1016/j.jns.2009.08.028

Suppa, A., Huang, Y.-Z., Funke, K., Ridding, M. C., Cheeran, B., Di Lazzaro, V., et al. (2016). Ten years of theta burst stimulation in humans: established knowledge, unknowns and prospects. *Brain Stimul.* 9, 323–335. doi: 10.1016/j.brs.2016.01.006

Trebbastoni, A., Pichiorri, F., D'Antonio, F., Campanelli, A., Onesti, E., Ceccanti, M., et al. (2016). Altered cortical synaptic plasticity in response to 5-Hz repetitive transcranial magnetic stimulation as a new electrophysiological finding in amnestic mild cognitive impairment converting to Alzheimer's disease: results from a 4-year prospective cohort study. *Front. Aging Neurosci.* 7:253. doi: 10.3389/fnagi.2015.00253

Turriziani, P., Smirni, D., Zappalà, G., Mangano, G. R., Oliveri, M., and Cipolotti, L. (2012). Enhancing memory performance with rTMS in healthy subjects and individuals with Mild Cognitive Impairment: the role of the right dorsolateral prefrontal cortex. *Front. Hum. Neurosci.* 6:62. doi: 10.3389/fnhum.2012.00062

Weintraub, D., Dietz, N., Duda, J. E., Wolk, D. A., Doshi, J., Xie, S. X., et al. (2012). Alzheimer's disease pattern of brain atrophy predicts cognitive decline in Parkinson's disease. *Brain* 135, 170–180. doi: 10.1093/brain/awr277

Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L. O., et al. (2004). Mild cognitive impairment–beyond controversies, towards a consensus: report of the international working group on mild cognitive impairment. *J. Intern. Med.* 256, 240–246. doi: 10.1111/j.1365-2796.2004.01380.x

Xu, Y., and Chun, M. M. (2006). Dissociable neural mechanisms supporting visual short-term memory for objects. *Nature* 440, 91–95. doi: 10.1038/nature04262

Ziemann, U., Paulus, W., Nitsche, M. A., Pascual-Leone, A., Byblow, W. D., Berardelli, A., et al. (2008). Consensus: motor cortex plasticity protocols. *Brain Stimul.* 1, 164–182. doi: 10.1016/j.brs.2008.06.006

Zimerman, M., and Hummel, F. C. (2010). Non-invasive brain stimulation: enhancing motor and cognitive functions in healthy old subjects. *Front. Aging Neurosci.* 2:149. doi: 10.3389/fnagi.2010.00149

Zimerman, M., Nitsch, M., Giraux, P., Gerloff, C., Cohen, L. G., and Hummel, F. C. (2013). Neuroenhancement of the aging brain: restoring skill acquisition in old subjects. *Ann. Neurol.* 73, 10–15. doi: 10.1002/ana.23761

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

CASE REPORT
published: 08 June 2017
doi: 10.3389/fnagi.2017.00178

Enhanced Working Memory Binding by Direct Electrical Stimulation of the Parietal Cortex

*Agustina Birba[1,2], Eugenia Hesse[1,2,3], Lucas Sedeño[1,2], Ezequiel P. Mikulan[1,2], María del C. García[4], Juan Ávalos[4], Federico Adolfi[1,2], Agustina Legaz[1], Tristán A. Bekinschtein[5], Máximo Zimerman[1], Mario Parra[6,7,8,9], Adolfo M. García[1,2,10] and Agustín Ibáñez[1,2,9,11,12]**

[1]*Laboratory of Experimental Psychology and Neuroscience (LPEN), Institute of Cognitive and Translational Neuroscience (INCyT), INECO Foundation, Favaloro University, Buenos Aires, Argentina,* [2]*National Scientific and Technical Research Council (CONICET), Buenos Aires, Argentina,* [3]*Instituto de Ingeniería Biomédica, Facultad de Ingeniería, Universidad de Buenos Aires, Buenos Aires, Argentina,* [4]*Hospital Italiano de Buenos Aires, Buenos Aires, Argentina,* [5]*Consciousness and Cognition Laboratory, Department of Psychology, University of Cambridge, Cambridge, United Kingdom,* [6]*Department of Psychology, School of Social Sciences, Heriot-Watt University, Edinburgh, United Kingdom,* [7]*Human Cognitive Neuroscience, Centre for Cognitive Ageing and Cognitive Epidemiology, Alzheimer Scotland Dementia Research Centre, Department of Psychology, University of Edinburgh, Edinburgh, United Kingdom,* [8]*Neuroprogressive and Dementia Network, NHS Research Scotland, Edinburgh, United Kingdom,* [9]*Facultad de Psicología, Universidad Autónoma del Caribe, Barranquilla, Colombia,* [10]*Faculty of Education, National University of Cuyo (UNCuyo), Mendoza, Argentina,* [11]*Center for Social and Cognitive Neuroscience (CSCN), School of Psychology, Universidad Adolfo Ibañez, Santiago, Chile,* [12]*Centre of Excellence in Cognition and its Disorders, Australian Research Council (ARC), Sydney, NSW, Australia*

Recent works evince the critical role of visual short-term memory (STM) binding deficits as a clinical and preclinical marker of Alzheimer's disease (AD). These studies suggest a potential role of posterior brain regions in both the neurocognitive deficits of Alzheimer's patients and STM binding in general. Thereupon, we surmised that stimulation of the posterior parietal cortex (PPC) might be a successful approach to tackle working memory deficits in this condition, especially at early stages. To date, no causal evidence exists of the role of the parietal cortex in STM binding. A unique approach to assess this issue is afforded by single-subject direct intracranial electrical stimulation of specific brain regions during a relevant cognitive task. Electrical stimulation has been used both for clinical purposes and to causally probe brain mechanisms. Previous evidence of electrical currents spreading through white matter along well defined functional circuits indicates that visual working memory mechanisms are subserved by a specific widely distributed network. Here, we stimulated the parietal cortex of a subject with intracranial electrodes as he performed the visual STM task. We compared the ensuing results to those from a non-stimulated condition and to the performance of a matched control group. In brief, direct stimulation of the parietal cortex induced a selective improvement in STM. These results, together with previous studies, provide very preliminary but promising ground to examine behavioral changes upon parietal stimulation in AD. We discuss our results regarding: (a) the usefulness of the task to target prodromal stages of AD; (b) the role of a posterior network in STM binding and in AD; and (c) the potential opportunity to improve STM binding through brain stimulation.

Keywords: working memory binding, Alzhimer's disease, direct electrical stimulation, short term memory, single case study

OPEN ACCESS

Edited by:
Alessio Avenanti, Università di Bologna, Italy

Reviewed by:
Lucia Serenella De Federicis, National Institute of Social Welfare, Italy
John Fredy Ochoa, University of Antioquia, Colombia

****Correspondence:***
Agustín Ibáñez
aibanez@ineco.org.ar

Received: *27 March 2017*
Accepted: *19 May 2017*
Published: *08 June 2017*

Citation:
Birba A, Hesse E, Sedeño L, Mikulan EP, García MdC, Ávalos J, Adolfi F, Legaz A, Bekinschtein TA, Zimerman M, Parra M, García AM and Ibáñez A (2017) Enhanced Working Memory Binding by Direct Electrical Stimulation of the Parietal Cortex. Front. Aging Neurosci. 9:178. doi: 10.3389/fnagi.2017.00178

WORKING MEMORY BINDING: ANTECEDENTS AND CASE REPORT

Recent works (Parra et al., 2009, 2010, 2015) have evidenced the critical role of visual short-term memory (STM) binding deficits as a clinical and preclinical marker of Alzheimer's disease (AD). These studies (and other related reports, see below) show that, in AD, working memory is selectively impaired for tasks requiring binding of multiple elements, but preserved for processing isolated features. Moreover, they suggest a potential role of parieto-temporo-posterior regions in both the neurocognitive deficits of AD patients and STM binding at large. Here we show that direct electrical stimulation of the parietal cortex through invasive electrodes selectively enhances working memory binding, with no effects on feature-level working memory processes.

Memory binding is the function that allows integrating multiple elements of complex events into unified wholes (von der Malsburg, 1999; Baddeley, 2000; Tulving, 2002; Zimmer et al., 2006). STM or working memory binding underpins the temporary retention of arrays of features (e.g., shapes with colors) as integrated complex objects (Treisman and Zhang, 2006). Parra et al. (2010) visual working memory task discriminates between patients with familial AD and preclinical carriers of the causative E280A mutation in the presenilin-1 gene (Lemere et al., 1996) from non-carriers of such a mutation. Notably, despite the impairment in STM binding, asymptomatic carriers and healthy controls did not differ in tasks assessing general memory, attention and executive functions. Impairments in this function are associated with lower white matter integrity in familial Alzheimer disease (Parra et al., 2015). Furthermore, this STM binding deficit is absent in non-AD dementias, such as frontotemporal, vascular, or Lewy body dementias (Della Sala et al., 2012). STM binding seems to place minimal demands on executive functions and appears to be subserved by components of the memory network impaired in AD, but not in other dementias (Della Sala et al., 2012). On the other hand, working memory deficits have been reported in epilepsy patients. For example, temporal lobe epilepsy typically has disabling effects on verbal memory functions. Furthermore, juvenile myoclonic epilepsy and benign epilepsy in children are associated with impaired performance on visual working memory tasks (Elger et al., 2004).

STM binding yields specific activation increases across neural generators that collectively support temporary visual memory for isolated and integrated features. Within the left hemisphere, binding of object features mainly engages the superior and inferior parietal cortex, the fusiform gyrus and the dorsal premotor cortex (Parra et al., 2014). Moreover, research on the temporal dynamics of STM-relevant networks suggest an early role of posterior regions in binding processes (Smith et al., 2017).

Following this evidence, we inferred that stimulation of the posterior parietal cortex (PPC) might offer new opportunities to approach working memory deficits in AD, especially at early stages. However, no study has yet demonstrated a causal role of such a region in STM binding. A unique approach to define necessary hubs in brain networks and infer reliable mechanisms in cognitive neuroscience consists in applying direct intracranial electrical stimulation to single subjects (epilepsy patients implanted with depth electrodes) in specific brain regions to causally modulated cognitive task performance. Here, as part of an ongoing program of research (Chennu et al., 2013; Canales-Johnson et al., 2015; Hesse et al., 2016), we profited from the unique opportunity to produce such causal evidence via single-subject direct intracranial electrical stimulation (Parvizi et al., 2012; Mégevand et al., 2014). Specifically, we stimulated the left parietal cortex (precuneus) during the visual STM task in a patient implanted with intracranial electrodes, and compared the results with a non-stimulated condition and to the performance of a matched control group (for details about the participant and the controls, see "Participants" Section; intracranial recording specifications are offered in "Subject's Intracranial Recording" Section). The patient presented a specific pattern of working memory deficits (with impairments in the binding condition but not in the shape condition) resembling the typical performance of AD patients on this task (Parra et al., 2009, 2010). This allowed us to test the selective and specific effect observed in the binding condition alone, as a relevant model mirroring the pattern which characterizes AD.

We used the visual working memory task developed by Parra and colleagues (Parra et al., 2010, 2015; Pietto et al., 2016). This task involves two sets of stimuli, one with black shapes, used to evaluate memory for shapes; and a second one with colored shapes, assessing shape-color binding in working memory (see **Figure 1**). The subject performed the task with and without stimulation. During task performance we directly stimulated the left precuneus with 1 mA at 50 Hz in 2-s intervals (stimulation block). In the control condition (sham block) the patient continued the task under a simulated stimulation (for details see "Experimental Design and Stimuli" and "Stimulation Protocol" Sections, respectively). The subject was not able to distinguish between stimulation and sham blocks.

Direct stimulation of the parietal cortex induced a selective improvement in STM binding. Relative to controls ("Behavioral Analysis" Section), the subject only reached normal STM binding performance upon stimulation (Crawford's $t = 0.20$, $p = 0.84$, $Z_{ccc} = 0.21$; **Figure 2A**). In addition, compared with the SHAM condition, electrical stimulation during the binding condition significantly enhanced performance (Crawford's $t = -2.93$, $p = 0.02$, $Z_{ccc} = -3.31$; **Figure 2A**). Moreover, the subject's performance in the shapes condition did not significantly differ from that of controls, in any of the treatments (PPC: Crawford's $t = 1.33$, $p = 0.23$, $Z_{ccc} = -1.42$; SHAM: Crawford's $t = 2$, $p = 0.08$, $Z_{ccc} = 2$; **Figure 2A**). To assess the impact and spread of the electrical stimulation, we compared intracranial event-related activity in all the recording electrodes between stimulated and SHAM trials (**Figure 2B**; for data pre-processing and processing see "Signal Preprocessing and Data Quality" Section, respectively). Propagation

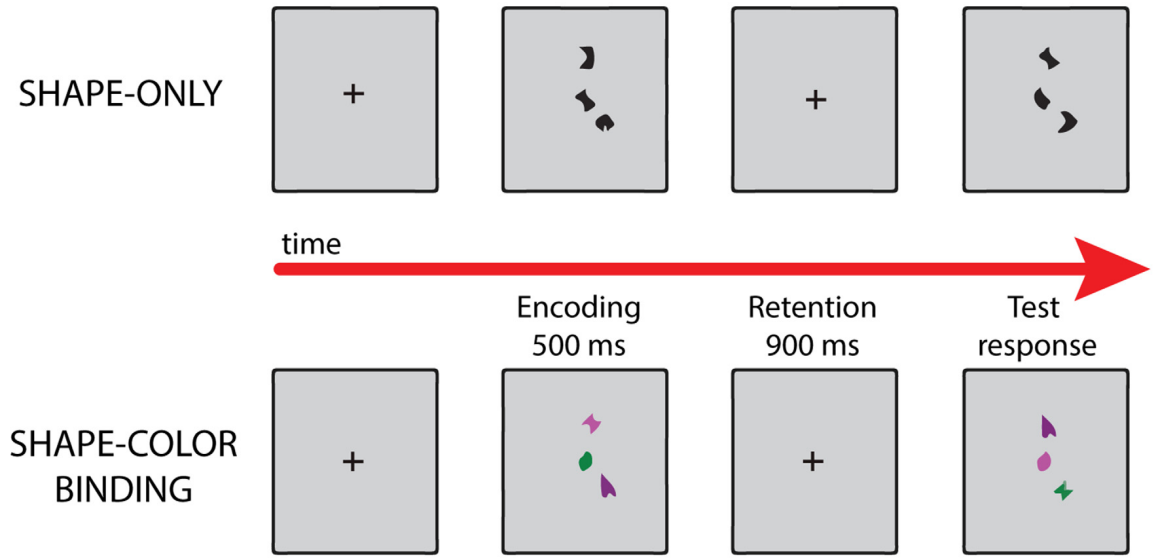


FIGURE 1 | Examples of different trials in each condition of the visual working memory task. For details, see "Experimental Design and Stimuli" Section.

of the excitation was widely distributed across the left hemisphere. The intracranial event related potentials (iERP) showed maximal effects in parietal regions, followed by the cuneus, the posterior cingulate, the postcentral gyrus, the middle frontal gyrus, and the hippocampus, in a constant decreasing fashion (all *ps* < 0.005, permutation test with bootstrapping and false discovery rate correction; **Figure 2C**).

Meta-analytic evidence suggests an early compromise of parietal networks in AD (Jacobs et al., 2013). Here, using a causal stimulation-based method, we offer the first evidence of a selective and causal involvement of the parietal cortex in working memory binding. Such selectivity is reinforced by the null effect yielded by the same stimulation on STM processing of individual features (shapes only, control of task). Although there is a tendency to decrease in shapes accuracy with the precuneus' stimulation, we are not able to explain this with our present data. However, we could speculate that this diminution might reflect resource distribution effect, such increased allocation of resources to "binding" processes would deplete resources available for the "encoding" process. Furthermore, the improvement of STM binding was triggered by stimulation of the precuneus, which in turn induced a spreading of activation throughout a frontoparietal and hippocampal network including the posterior network previously related to visual memory binding (Parra et al., 2014). These regions are also part of the top-down attentional control network (Gazzaley and Nobre, 2012). Importantly, this binding-specific improvement is not due to a task-learning effect, since real stimulation was performed before the SHAM condition (no-stimulation control). Together with previous findings, these results provide preliminary but promising findings for a new agenda aiming at evaluating behavioral changes upon parietal stimulation in AD through TMS or tDCS. The next step will be to perform systematic stimulation studies targeting various specific and unspecific posterior hubs to assess how critical the parietal cortex is for STM binding. In this sense, we hypothesize that the stimulation of the left PPC would specifically improve performance on this task. Future study designs should take into account the method, type, duration and number of sessions of the stimulation protocol to modulate STM binding processes. In particular, future non-invasive stimulation protocols should be extended in their length and their number of trials. Furthermore, it would be of great interest to assess physiological changes related to the stimulation protocol, so as to better identify the mechanisms underlying STM binding. Furthermore, tDCS is a safe, non-invasive method that could be used to improve memory impairments or diminish seizure frequency in in drug-resistant epilepsy. In patients with intractable lateral frontal lobe epilepsy, Karvigh et al. (2017) found that cathodal HD-tDCS of the epileptogenic zone significantly improved attention and working memory immediately and 1 month after stimulation. Moreover, Tekturk et al. (2016) applied modulated cathodal stimulation (2 mA for 30 min on three consecutive days) to patients diagnosed with mesial temporal lobe epilepsy with hippocampal sclerosis, and found that more than at least 8 out of 10 patients had more than 50% decrease in seizure frequency. Therefore, non-invasive brain stimulation might be a promising tool to attenuate or delay memory deficits in AD and may be used as an additional treatment option for refractory epilepsies. However, further studies are necessary to assess these approaches.

Intracranial recordings are exceptional in humans and provide a unique opportunity to obtain causal stimulation-based evidence with high spatiotemporal resolution, they have,

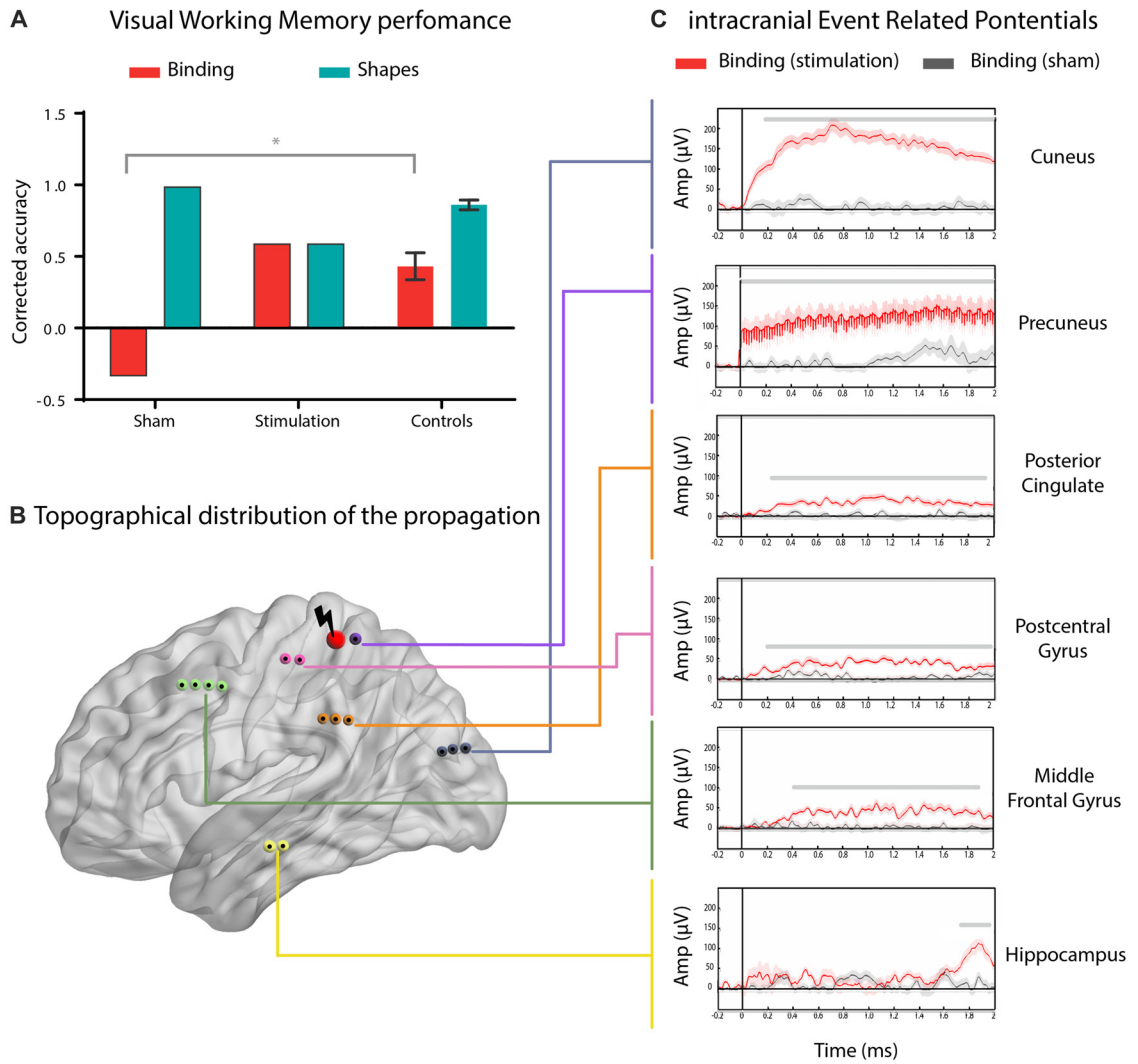


FIGURE 2 | Enhanced working memory binding by direct electrical stimulation of the parietal cortex. **(A)** Mean performance of the subject during the real (posterior parietal cortex, PPC) or sham (sham) stimulation in the shape-only (shapes) and shape-color binding (binding) conditions, compared to the performance of seven matched control participants. Error bars represent standard deviations (SD) from the mean. **(B)** Schematic brain localization of the reported contact sites. Red nodes represent the stimulated site and black nodes show the ROIs were stimulation propagation was detected. **(C)** Intracranial event related potentials (iERP) activity from significant ROIs comparing real and SHAM stimulation in the shape-color binding condition. Shadowed bars around potentials indicate SEM. For visualization purposes, the signal was renormalized, filtered and smoothed. $p < 0.005$, permutation test with bootstrapping and false discovery rate correction, minimum length of windows with significant differences: 100 ms.

however, important limitations. While we have accounted for the known caveats of intracranial EEG recordings by adopting several measures (see "Signal Preprocessing and Data Quality" Section), future studies could further test our conclusions while circumventing method-specific limitations.

As reported in the pioneering work (Penfield and Boldrey, 1937), electrical stimulation has been used both for clinical purposes and to causally probe brain mechanisms. The evidence for electrical currents to spread through white matter along functionally well-defined brain networks (Duffau et al., 2005; Tolias et al., 2005) supports our result that a wide network underlies specific visual working memory mechanisms. Direct cortical stimulation becomes much more than a blunt tool to modulate a simple area, and turns into an unique causal instrument to probe into the process of the investigated function.

Finally, given the usefulness of the STM binding task as a marker of prodromal AD, and alongside the potential to improve STM binding by stimulation, our results open a new area of research centered in the non-invasive brain stimulation of the PPC in clinical and preclinical populations. Future studies with this approach may shed light on functional restoration options for AD patients and

subjects at risk for the disease (mild cognitive impairment, MCI), paving the way for new treatments to delay the development of neurocognitive deficits associated with this pathology.

MATERIALS AND METHODS

Data Acquisition and Protocol Design

Participants

As part of an ongoing protocol (Chennu et al., 2013; Canales-Johnson et al., 2015; Hesse et al., 2016), we recruited one patient with intractable epilepsy who was offered surgical intervention to alleviate his condition. The subject was an 18-year-old, right-handed male who had completed high school and suffered from drug-resistant epilepsy since the age of six. He was attentive and cooperative throughout the task. In addition, we recruited a control group comprised of seven healthy male participants matched with the patient for age (M = 18.57, SD = 1.27; t = −0.35, p = 0.70) and years of education (M = 12.71, SD = 1.25; t = −0.53, p = 0.61). None of these subjects reported a history of psychiatric or neurological disease. This study was carried out in accordance with the recommendations of the Declaration of Helsinki, as well as the Guidelines of the Ethics Committee of INECO (approved protocol number FONCyT-PICT 2012-0412 and 2012-1309). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Subject's Intracranial Recording

Direct cortical recordings were obtained from semi-rigid, multi-lead electrodes implanted in the patient's brain. The electrodes were 0.8 mm in diameter and consisted of 5, 10 or 15 2-mm wide contact leads placed 1.5 mm apart from each other (DIXI Medical Instruments). We used a Micromed video-SEEG monitoring system which records as many as 128 depth-EEG electrode sites simultaneously. Recordings were obtained from 127 sites and sampled at 512 Hz. The data were collected from the precuneus, the cuneus, the hippocampus, the posterior cingulate and the postcentral gyrus. The recordings obtained were distal to the epileptogenic foci, and no single recording site presented epileptogenic activity (see below). We also obtained post-implantation MRI and CT scans were obtained from each patient. Both volumetric images were affine registered and normalized on the SPM8 MATLAB toolbox. Using MRIcron, we established the coordinates of each contact site and their respective Brodmann areas.

Experimental Design and Stimuli

The task assessed memory for shapes and combinations of shapes and colors. Stimuli were randomly selected from a set of eight shapes and eight colors and presented as individual features or as features combined into integrated objects. Each type of stimulus was presented in a separate condition. Two experimental conditions were used, each consisting of 32 test trials, leading to a total of 64 test trials. Trials were fully randomized and, for the control participants, conditions were delivered in a counter-balanced order. In the "Shapes" condition, arrays of shapes were presented in the study display. In the test display for the "different" trials, two new shapes from the study array were replaced with two new shapes (**Figure 1**). Hence, in these conditions, participants were required to detect changes in individual features. In the "Shape-color binding" condition, the study display showed combinations of shapes and colors. In the test display for "different" trials, two shapes swapped the colors in which they had been shown in the study display (**Figure 1**). Hence, detection of this change relied on shape-color bindings. No shape or color was repeated within a given array. Fifty percent of the test trials were "same" trials (the study and test displays presented identical items) and 50% were "different" trials.

Stimulation Protocol

Electrical stimulation was delivered in bipolar square waves between two adjacent electrode contacts in the left precuneus, 54 (−4 −60 56, MNI coordinates) 55 (−2 −60 56, MNI coordinates). Stimulation occurred at 1 mA for the real stimulation condition and at 0 mA for the control SHAM condition using a 200 ms pulse width at a frequency of 50 Hz, during 2 s. Each condition involved 10 trials under real stimulation and another 10 under SHAM stimulation. The stimulation began at the onset of the study display and continued through the test display. First we performed the real and SHAM stimulation (in that order) for the shape-color binding condition and then for the shape-only condition. EEG signals were simultaneously monitored before and after discharges. Electrodes and trials compromised by seizures or leading to epileptic activity were excluded. The subject was asked to describe any perceptual or physical changes he experienced during or after each stimulation trial.

Data Analysis

Behavioral Analysis

As in previous studies (Parra et al., 2010, 2015; Pietto et al., 2016), recognition during the visual working memory task was calculated by subtracting the proportion of false alarms from the hits. The subject's indexes were compared to those of a control sample through a modified two-tailed t test (Crawford and Garthwaite, 2002; Crawford et al., 2009, 2011). This methodology allows the assessment of significance by comparing test scores of one or several individuals with norms derived from small samples. This modified test is robust for non-normal distributions, presents low values of type I error, and has already been reported in single case studies (Straube et al., 2010). The alpha level was set at $p < 0.05$.

Signal Preprocessing and Data Quality

Several measures were adopted to circumvent the caveats of data obtained from an epileptic patient. We excluded channels in epileptogenic foci, used stringent inclusion criteria for the remaining channels, and ensured the

absence of neuroanatomical abnormalities or major cognitive deficits in the subject. We discarded the contact sites that presented pathological waveforms. Electrodes with epileptic activity were discarded upon visual identification by two professional neurologists (MCG and JA). Moreover, we discarded channels whose values exceeded five times the signal's mean and/or consecutive signal samples exceeding five standard deviations (SD) from the gradient's mean (Chennu et al., 2013; Hesse et al., 2016). A total of 83 contact sites remained after applying these criteria, and all of them were processed. Then, the data were referenced to the mean value of the non-stimulated sites (averages of such sites were subtracted from each recording). Finally, the data were segmented into 2000 ms epochs, including a −200 to 0 ms pre-stimulus baseline period. The epochs were baseline corrected.

AUTHOR CONTRIBUTIONS

AB and AI designed the study. AB, EH, LS, EPM, MdCG, JA, FA, AL, TAB, MZ, MP, AMG and AI carried out and analyzed the experiments. AB and AI wrote the article. AI and AB conceived the study and wrote the final article, together with the other authors. All authors have approved the manuscript.

ACKNOWLEDGMENTS

Partial support was obtained from grants by CONICET; CONICYT/FONDECYT Regular (1170010); FONCyT-PICT (2012-0412 and 2012-1309), FONDAP (15150012); and the INECO Foundation. MAP work is supported by Alzheimer's Society, Grant ASSF-14-008.

REFERENCES

Baddeley, A. (2000). The episodic buffer: a new component of working memory? *Trends Cogn. Sci.* 4, 417–423. doi: 10.1016/s1364-6613(00)01538-2

Canales-Johnson, A., Silva, C., Huepe, D., Rivera-Rei, Á., Noreika, V., Garcia Mdel, C., et al. (2015). Auditory feedback differentially modulates behavioral and neural markers of objective and subjective performance when tapping to your heartbeat. *Cereb. Cortex* 25, 4490–4503. doi: 10.1093/cercor/bhv076

Chennu, S., Noreika, V., Gueorguiev, D., Blenkmann, A., Kochen, S., Ibáñez, A., et al. (2013). Expectation and attention in hierarchical auditory prediction. *J. Neurosci.* 33, 11194–11205. doi: 10.1523/JNEUROSCI.0114-13.2013

Crawford, J. R., and Garthwaite, P. H. (2002). Investigation of the single case in neuropsychology: confidence limits on the abnormality of test scores and test score differences. *Neuropsychologia* 40, 1196–1208. doi: 10.1016/s0028-3932(01)00224-x

Crawford, J. R., Garthwaite, P. H., and Howell, D. C. (2009). On comparing a single case with a control sample: an alternative perspective. *Neuropsychologia* 47, 2690–2695. doi: 10.1016/j.neuropsychologia.2009.04.011

Crawford, J. R., Garthwaite, P. H., and Ryan, K. (2011). Comparing a single case to a control sample: testing for neuropsychological deficits and dissociations in the presence of covariates. *Cortex* 47, 1166–1178. doi: 10.1016/j.cortex.2011.02.017

Della Sala, S., Parra, M. A., Fabi, K., Luzzi, S., and Abrahams, S. (2012). Short-term memory binding is impaired in AD but not in non-AD dementias. *Neuropsychologia* 50, 833–840. doi: 10.1016/j.neuropsychologia.2012.01.018

Duffau, H., Gatignol, P., Mandonnet, E., Peruzzi, P., Tzourio-Mazoyer, N., and Capelle, L. (2005). New insights into the anatomo-functional connectivity of the semantic system: a study using cortico-subcortical electrostimulations. *Brain* 128, 797–810. doi: 10.1093/brain/awh423

Elger, C. E., Helmstaedter, C., and Kurthen, M. (2004). Chronic epilepsy and cognition. *Lancet Neurol.* 3, 663–672. doi: 10.1016/s1474-4422(04)00906-8

Gazzaley, A., and Nobre, A. C. (2012). Top-down modulation: bridging selective attention and working memory. *Trends Cogn. Sci.* 16, 129–135. doi: 10.1016/j.tics.2011.11.014

Hesse, E., Mikulan, E., Decety, J., Sigman, M., Garcia Mdel, C., Silva, W., et al. (2016). Early detection of intentional harm in the human amygdala. *Brain* 139, 54–61. doi: 10.1093/brain/awv336

Jacobs, H. I., Radua, J., Lückmann, H. C., and Sack, A. T. (2013). Meta-analysis of functional network alterations in Alzheimer's disease: toward a network biomarker. *Neurosci. Biobehav. Rev.* 37, 753–765. doi: 10.1016/j.neubiorev.2013.03.009

Karvigh, S. A., Motamedi, M., Arzani, M., and Roshan, J. H. N. (2017). HD-tDCS in refractory lateral frontal lobe epilepsy patients. *Seizure* 47, 74–80. doi: 10.1016/j.seizure.2017.03.005

Lemere, C. A., Lopera, F., Kosik, K., Lendon, C., Ossa, J., Saido, T., et al. (1996). The E280A presenilin 1 Alzheimer mutation produces increased *Ab42* deposition and severe cerebellar pathology. *Nat. Med.* 2, 1146–1150.

Mégevand, P., Groppe, D. M., Goldfinger, M. S., Hwang, S. T., Kingsley, P. B., Davidesco, I., et al. (2014). Seeing scenes: topographic visual hallucinations evoked by direct electrical stimulation of the parahippocampal place area. *J. Neurosci.* 34, 5399–5405. doi: 10.1523/JNEUROSCI.5202-13.2014

Parra, M. A., Abrahams, S., Fabi, K., Logie, R., Luzzi, S., and Della Sala, S. (2009). Short-term memory binding deficits in Alzheimer's disease. *Brain* 132, 1057–1066. doi: 10.1093/brain/awp036

Parra, M. A., Abrahams, S., Logie, R. H., Méndez, L. G., Lopera, F., and Della Sala, S. (2010). Visual short-term memory binding deficits in familial Alzheimer's disease. *Brain* 133, 2702–2713. doi: 10.1093/brain/awQ158

Parra, M. A., Della Sala, S., Logie, R. H., and Morcom, A. M. (2014). Neural correlates of shape-color binding in visual working memory. *Neuropsychologia* 52, 27–36. doi: 10.1016/j.neuropsychologia.2013.09.036

Parra, M. A., Saarimäki, H., Bastin, M. E., Londoño, A. C., Pettit, L., Lopera, F., et al. (2015). Memory binding and white matter integrity in familial Alzheimer's disease. *Brain* 138, 1355–1369. doi: 10.1093/brain/awv048

Parvizi, J., Jacques, C., Foster, B. L., Withoft, N., Rangarajan, V., Weiner, K. S., et al. (2012). Electrical stimulation of human fusiform face-selective regions distorts face perception. *J. Neurosci.* 32, 14915–14920. doi: 10.1523/JNEUROSCI.2609-12.2012

Penfield, W., and Boldrey, E. (1937). Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain* 60, 389–443. doi: 10.1093/brain/60.4.389

Pietto, M., Parra, M. A., Trujillo, N., Flores, F., García, A. M., Bustin, J., et al. (2016). Behavioral and electrophysiological correlates of memory binding deficits in patients at different risk levels for Alzheimer's disease. *J. Alzheimers Dis.* 53, 1325–1340. doi: 10.3233/JAD-160056

Smith, K., Ricaud, B., Shahid, N., Rhodes, S., Starr, J. M., Ibáñez, A., et al. (2017). Locating temporal functional dynamics of visual short-term memory binding using graph modular dirichlet energy. *Sci. Rep.* 7:42013. doi: 10.1038/srep42013

Straube, T., Weisbrod, A., Schmidt, S., Raschdorf, C., Preul, C., Mentzel, H.-J., et al. (2010). No impairment of recognition and experience of disgust in a patient with a right-hemispheric lesion of the insula and basal ganglia. *Neuropsychologia* 48, 1735–1741. doi: 10.1016/j.neuropsychologia.2010.02.022

Tekturk, P., Erdogan, E. T., Kurt, A., Vanli-yavuz, E. N., Ekizoglu, E., Kocagoncu, E., et al. (2016). The effect of transcranial direct current stimulation on seizure frequency of patients with mesial temporal lobe epilepsy with hippocampal sclerosis. *Clin. Neurol. Neurosurg.* 149, 27–32. doi: 10.1016/j.clineuro.2016.07.014

Tolias, A. S., Sultan, F., Augath, M., Oeltermann, A., Tehovnik, E. J., Schiller, P. H., et al. (2005). Mapping cortical activity elicited with electrical microstimulation using FMRI in the macaque. *Neuron* 48, 901–911. doi: 10.1016/j.neuron.2005.11.034

Treisman, A., and Zhang, W. (2006). Location and binding in visual working memory. *Mem. Cognit.* 34, 1704–1719. doi: 10.3758/bf03195932

Tulving, E. (2002). Episodic memory: from mind to brain. *Annu. Rev. Psychol.* 53, 1–25. doi: 10.1146/annurev.psych.53.100901.135114

von der Malsburg, C. (1999). The what and why of binding: the modeler's perspective. *Neuron* 24, 95–104. doi: 10.1016/S0896-6273(00)80825-9

Zimmer, H. D., Mecklinger, A., and Lindenberger, U. (Eds) (2006). "Levels of binding: types, mechanisms, and functions of binding in remembering," in *Handbook of Binding and Memory: Perspectives from Cognitive Neuroscience*, (Oxford: Oxford University Press), 3–22.

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

DATA REPORT
published: 20 March 2017
doi: 10.3389/fnagi.2017.00064

Brain Transcriptome Sequencing of a Natural Model of Alzheimer's Disease

Francisco Altimiras[1,2†], Barbara Uszczynska-Ratajczak[3,4†], Francisco Camara[3,4], Anna Vlasova[3,4], Emilio Palumbo[3,4], Stephen Newhouse[5], Robert M. J. Deacon[6,7], Leandro A. E. Farias[1], Michael J. Hurley[8], David E. Loyola[9], Rodrigo A. Vásquez[10], Richard Dobson[5], Roderic Guigó[3,4*] and Patricia Cogram[6,7*]*

[1] Faculty of Engineering and Sciences, Universidad Adolfo Ibañez, Santiago, Chile, [2] Telefonica Research and Development, Santiago, Chile, [3] Centre for Genomic Regulation, Barcelona Institute of Science and Technology, Barcelona, Spain, [4] Universitat Pompeu Fabra, Barcelona, Spain, [5] Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK, [6] Laboratory of Molecular Neuropsychiatry, Institute of Cognitive and Translational Neuroscience (INCyT), INECO Foundation, Favaloro University, National Scientific and Technical Research Council, Buenos Aires, Argentina, [7] GeN.DDI Ltd, London, UK, [8] Division of Brain Sciences, Centre for Neuroinflammation and Neurodegeneration, Imperial College, London, UK, [9] National Center for Genomics and Bioinformatics, Santiago, Chile, [10] Faculty of Sciences, Institute of Ecology and Biodiversity, Universidad de Chile, Santiago, Chile

Keywords: Alzheimer's disease, *Octodon degus*, RNA-seq, gene expression, animal models

OPEN ACCESS

Edited by:
George E. Barreto, Pontifical Xavierian University, Colombia

Reviewed by:
Ángel M. Carrión, Pablo de Olavide University, USA
Odete A. B. Da Cruz E. Silva, University of Aveiro, Portugal

***Correspondence:**
Francisco Altimiras
fjaltimiras@gmail.com
Roderic Guigó
roderic.guigo@crg.eu
Patricia Cogram
patricia.cogram@gmail.com

† Shared first authorship.

Received: *03 November 2016*
Accepted: *01 March 2017*
Published: *20 March 2017*

Citation:
Altimiras F, Uszczynska-Ratajczak B, Camara F, Vlasova A, Palumbo E, Newhouse S, Deacon RMJ, Farias LAE, Hurley MJ, Loyola DE, Vásquez RA, Dobson R, Guigó R and Cogram P (2017) Brain Transcriptome Sequencing of a Natural Model of Alzheimer's Disease. Front. Aging Neurosci. 9:64. doi: 10.3389/fnagi.2017.00064

INTRODUCTION

Alzheimer's disease (AD) is a slowly progressive disease characterized by impairment of memory and eventually by disturbances in reasoning, planning, language, and perception. Ageing is the greatest risk factor for its development but mutations in amyloid precursor protein (APP), apolipoprotein E (APOE), microtubule-associated protein tau (MAPT) among others, are also a major factor (Blasko et al., 2004). The symptoms of AD result from neurofibrillary tangles that are composed of aggregates of hyper-phosphorylated tau protein and an increase in the production of amyloid-beta (Aβ) protein in the brain that leads to deposits of senile plaques. As such, there is a worldwide effort to find an effective disease-modifying treatment that can reverse symptoms and/or delay onset of the disease. Transgenic mouse models exist that mimic a range of AD–related pathologies, although none of the models fully replicate all pathological features of the human disease (Birch et al., 2014). Drugs developed using these mouse models have failed in phase III clinical trials (Mangialasche et al., 2010; Braidy et al., 2012; Saraceno et al., 2013). These failures question not only our accurate understanding of the disease (Castellani and Perry, 2012) but also the validity of the animal models upon which the drug discovery efforts are rooted (Windisch, 2014; Nazem et al., 2015).

Animal models have contributed significantly to our understanding of the underlying mechanisms of AD. To date, however, these findings have not resulted in target validation in humans and successful translation to disease-modifying therapies. The *Octodon degus (O. degus)* is a model that naturally integrates multiple AD pathological hallmarks like tau fibrilary tangles and β-amyloid deposits (Inestrosa et al., 2005, 2015; Deacon et al., 2015). The Aβ peptide sequence in *O. degus* is 97.5% homologous to the human Aβ peptide sequence (Inestrosa et al., 2005). The species presents acetylcholine (AChE)-rich pyramidal neurons in their forebrain, which decline in numbers during the progression to an AD-like behavioral state, similar to that seen in AD patients (Ardiles et al., 2012). Affected *O. degus* also present the characteristic medical signs and symptoms surrounding AD like macular degeneration, diabetes and circadian rhythm dysfunction (Laurijssens et al., 2013). Behavioral experiments have shown that the *O. degus* can also present behavioral deficits and neural alterations in the frontal cortex and aggression similar to those seen in patients with AD (Tarragon et al., 2013). Most importantly, the *O. degus* shows a correlation of expression with human AD- related genes making this model a powerful tool to characterize the effects of novel treatments for AD and identify new therapeutic targets. Our findings advance the use of the *O. degus* as an effective tool for AD research.

MATERIALS AND METHODS

Animals

In this study *O. degus* (Rodentia: Octodontidae) were captured from a natural population in central Chile, 30 km west of Santiago (Rinconada de Maipú, RM). In this environment, degus typically breed once per year in late autumn (May-June), with conceptions in late winter to early spring (September-October). The animals were captured as juveniles during early austral summer (November), when degus are 2–3 months old, with mesh made traps with a Sherman-trap type mechanism. Juveniles corresponded to individuals weighing 70–130 g (females) or 70–140 g (males), and hence it is possible to differentiate them categorically from adults, which weight above 130 g for females, 140 g for males (Ebensperger and Hurtado, 2005; Correa et al., 2016).

They were housed in standard metal cages 50 × 40 × 35 cm with a layer of wood shaving as bedding and containing a small metallic box (25 × 15 × 10 cm with a single entrance), under natural photoperiod (12 h light/dark; starting 7 a.m.) and in an air-conditioned animal facility at the Faculty of Sciences, University of Chile (Santiago, Chile). They were fed with a commercial rodent diet (Prolab RMH 3000, Lab diet). Water and food were provided *ad libitum* during the entire experimental period. Behavioral assessment of a population ($N = 84$) of 3-years old *O. degus* was performed using the burrowing test in an earlier work (Deacon et al., 2015), to address behavioral dysfunctions in activities of daily living (ADL) (Deacon, 2006, 2014). The population was divided in terms of burrowing performance into two groups: poor-burrowers (PB) and good-burrowers (GB). Brain extract samples were obtained from 8 females (four per each group). Brain soluble $A\beta_{1-42}$peptide was determined for these 8 animals by MALDI-TOF MS as described in earlier work (Deacon et al., 2015). Samples were classified according to burrowing performance and brain soluble $A\beta_{1-42}$ levels. Samples from four PB animals, with an increased level of soluble brain $A\beta_{1-42}$ (AD-like group), and three samples from GB animals (with soluble levels of $A\beta_{1-42}$ used as control group) were used for further analysis. Brain tissues were frozen directly in RNAlater solution (Ambion) and stored at −80°C until use. All procedures of capture, transportation, maintenance and experimentation followed the recommendations of the ethics committee of the Faculty of Sciences of the University of Chile, and complied with Chilean regulations (SAG-Chilean Agriculture and Livestock Service) as well as recommendations by the Animal Behavior Society.

Total RNA and mRNA Isolation

For total RNA extraction, PureLinkTM RNA Mini kit (Ambion, Life Technologies) was used according to the manufacturer's instructions. For genomic DNA digestion, RNase-Free DNase I (Ambion) was used. Total RNA samples were quantified in a Nanodrop 2000 spectrophotometer and integrity was evaluated by agarose gels electrophoresis with a standard protocol. For mRNA isolation from total RNA samples, MicroPoly(A) Purist kit was used according to the manufacturer's instructions. For sizing, quantitation and quality control of mRNA Bioanalyzer system (Agilent) was used, discarding samples with an RIN < 7.

RNA-sequencing

RNA-seq was performed at the National Center for Genomics and Bioinformatics (Santiago, Chile) including library preparation (using SOLiD Total RNA-seq kit), fragmentation and PCR enrichment of target mRNA according to strand-specific protocols. Two batches of paired-end (75 × 35 bp) library sequencing were done in a SOLiD 5,500 × l system (Applied Biosystems).

Gene Prediction

We combined both *ab initio* and evidence-based approaches by using the Program to Assemble Spliced Alignments (PASA) and Evidence Modeler (EVM) to *de novo* annotate protein coding genes in *O. degus* genome assembly (v1.0) (Haas, 2003). In total 25,621 transcript models were generated by PASA from an initial set of 1,767,640 sequences including (1) 26,240 *O. degus* mRNA transcripts and (2) 1,741,759 ESTs and mRNA sequences from four species of rodents: mouse, guinea pig, Chinese hamster and rat. Transcript sequences for all five species were derived from the GenBank NCBI databases. We also used >30 million UniRef90 protein clusters and 26,259 highly curated rodent-specific Swiss-Prot proteins that were split-mapped to the *O. degus* genome by using SPALN2 (Iwata and Gotoh, 2012) and EXONERATE (Slater and Birney, 2005) with mouse-specific parameters. The protein-coding gene predictions were obtained on the latest repeat-masked reference assembly of the *O. degus* genome by using the existing mammalian-specific parameter files of four *ab initio* gene prediction programs: Augustus, Geneid, SGP2, and SNA (Guigó et al., 1992; Guigo et al., 2003; Parra et al., 2003; Stanke et al., 2006a,b). For SGP2, TBLASTX (Altschul, 1997) alignments between human (hg38) and *O. degus* were additionally used to improve the accuracy of gene predictions (Table S1). We also used external evidence for Geneid including PASA-derived introns, while for Augustus we used both PASA-derived intron and exon hints. Performance of all programs with and without external evidence was evaluated for accuracy on an artificial scaffold made up of 238 concatenated *O. degus* transcripts taken from the NCBI reference annotation. The alignments (transcript and protein) and *ab initio* gene models were combined into consensus CDS models using Evidence Modeler (Haas et al., 2008). The initial gene set was filtered to remove reference gene models supported exclusively by SNAP *ab initio* predictions. The single-exon consensus gene models derived from predictions solely supported by Geneid, SGP2 or AUGUSTUS with length <300 nucleotides and an EVM score <5 were also excluded. The weights of each given source were chosen empirically and based on suggestions contained in the EVM documentation. The highest weights were given to transcript alignments, followed by protein alignments and finally *ab initio* predictions (Augustus, Geneid, SGP2, and SNAP, Table S2). The consensus gene models were loaded into the PASA database and passed through five rounds of annotation updates to add UTRs and alternative splicing variants (Table S3).

Functional Annotation

Predicted protein-coding genes in the *O. degus* genome were further functionally annotated using an in-house developed, automatic pipeline. For each protein sequence we assigned protein signatures, orthology groups, as well as annotated metabolic pathways and reactions using an orthology-based approach. In this analysis we used InterProScan v.5 (Zdobnov and Apweiler, 2001) to scan though all available InterPro databases, including PANTHER, Pfam, TIGRFAM, HAMAP, and SUPERFAMILY and to specify different protein coding signatures in predicted protein coding genes. The protein signatures (protein families, regions, domains, repeats and sites) were further employed to investigate the classification and to assign biological functions to predicted proteins. Blast2GO (Götz et al., 2008) analysis was used to identify GO terms for the predicted proteins, while KEGG Automatic Annotation Server (KAAS) (Moriya et al., 2007) was employed to compare protein sequences against KEGG (Kanehisa et al., 2012, 2014) orthology (KO). In this analysis, KASS applied its bi-directional best hit (BBH) method in homology search against a representative gene set from 33 different species, including *Mus musculus* and *Cricetulus griseus*. KO identifiers were then used to retrieve the KEGG relevant functional annotation, such as metabolic pathways and external database references. In addition, we assigned the NCBI gene names from the *O. degus* reference NCBI annotation (ref_OctDeg1.0) to the predicted genes. This was done by taking NCBI gene names from the corresponding, NCBI annotated proteins, showing full sequence similarity. The sequence similarity was measured by assigning SHA1 checksum to each protein in both proteomes, followed by comparing those sums.

Mapping *O. degus* Brain RNA-Seq Samples

The *O. degus* RNA Sequences were aligned to a reference transcriptome obtained from the masked primary genome assembly version 1.0 (WGS Project: AJSA01) and the EVM-based genome annotation. The transcriptome sequence was prepared using RSEM version 1.2.12 (Li and Dewey, 2011) and projected from base space to SOLiD color space using SHRiMP version 2.2.3 (Rumble et al., 2009). The latter program was also employed to align SOLiD 75 × 35 bp pair-end sequenced reads to the reference transcriptome according to the following non-default parameters: -h 80% -o 10 -p opp-in –no-half-paired.

Mapping Human Brain RNA-Seq Samples

The RNA-seq data set consisting of 6 human brain samples (3 AD subjects and 3 controls, University of Kentucky brain bank) was derived from the National Center for Biotechnology Information Sequence Read Archive database (ncbi.nlm.nih.gov/sra, accession no. SRA060572). Next, pair-end reads were mapped to the human reference transcriptome using our in-house pipeline that combines STARv.2.4.0.1 (Dobin et al., 2013) and RSEM version 1.2.12 (Table S4). The reference transcriptome was obtained from human reference genome hg38 and GENCODE v21 (Harrow et al., 2012) as a reference annotation.

Expression Profiling of Human and *O. degus* Brain Samples

The number of reads mapped to each gene was calculated using SAMtools (Li et al., 2009). The statistical significance of expression profile for each gene between two groups was determined using edgeR, an open source R/Bioconductor package (Robinson et al., 2010). In this study, to estimate the significance of gene expression difference between AD or AD-like (PB) subjects and human controls or *O. degus* (GB) controls, the absolute value of log2 Ratio (logFC ≥ 1), log read count per million reads (logCPM > 1) and FDR < 0.05 were used as a criterion. Raw gene reads counts were also normalized to RPKM values (reads per kilobase per million mapped reads) using the RPKM formula described by Mortazavi et al. (2008).

GO Enrichment Analysis for Differentially Expressed Genes

Functional enrichment analysis for *O. degus* was performed using Fisher's exact test implemented in R programming language. The GO enrichment analysis for human was also done using R. The GOstats package was used to detect the significantly enriched GO terms for DEGs, which were compared to the full GENCODE 21 gene set. For both species, we further analyzed top 20 GO terms ranked by p-value with $p < 0.05$.

RESULTS

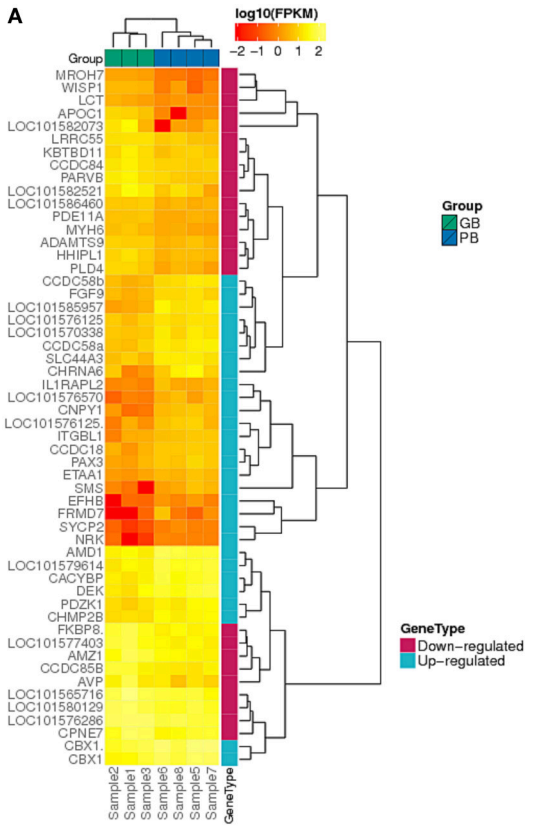
Gene Prediction and Functional Annotation

With the aim to enrich the current version of *O. degus* genome annotation with the new data obtained in this study, we performed *de novo* protein coding gene annotation. Our Evidence-Modeler (EVM) based annotation of repeat-mask *O. degus* genome (v1.0) resulted in prediction of 31,739 protein-coding genes, corresponding to 36,866 predicted transcripts and 36,575 proteins. The comparison of EVM-based and NCBI reference annotation revealed that the number of protein coding genes increased by 1.52-fold from 20,779 (NCBI) to 31,739 (EVM-based). Similar 1.40-fold change was reported for protein coding transcripts (26,248 for NCBI and 36,866 for EVM-based annotation) (Table S3).

Each protein sequence was functionally annotated using the in house automated pipeline. Annotation features were assigned to a total of 35,618 (97%) proteins, 30,661 (97%) genes), of these 29,847 (82%) of the proteins were assigned some GO terms (Tables S5, S6, Figures S1, S2). This functional annotation, including GO terms and KEGG orthology groups, allowed us to perform enrichment analysis for genes of interest.

The Brain Transcriptome of AD-like *O. degus*

After mapping the reads to the *O. degus* genome, we performed pairwise comparisons to measure the gene expression level differences between AD-like subjects and controls. As a result 54 DEGs were identified between those two groups. Statistical analysis revealed 29 genes to be up- and 25 to be down-regulated



B

KEEG PATHWAY	DESCRIPTION	KEEG PATHWAY	DESCRIPTION
UP-REGULATED GENES		DOWN-REGULATED GENES	
Ko04015	Rap1 signaling pathway	Ko01100	Metabolic pathways
Ko04111	Cell cycle - yeast	Ko04260	Cardiac muscle contraction
Ko04810	Regulation of actin cytoskeleton	Ko00190	Oxidative phosphorylation
Ko04010	MAPK signaling pathway	Ko05032	Morphine addiction
Ko04014	Ras signaling pathway	Ko05010	Alzheimer's disease
Ko05231	Choline metabolism in cancer	Ko00140	Steroid hormone biosynthesis
Ko04725	Cholinergic synapse	Ko04962	Vasopresin-regulated water reabsorption
Ko05218	Melanoma	Ko04726	Serotonergic synapse
Ko05200	Pathways in cancer	Ko04932	Non-aclholic fatty liver disease
Ko04144	Endocytosis	Ko00230	Purine metabolism
Ko05033	Nicotine addiction	Ko04973	Carbohydrate digestion and absorbtion
Ko04080	Neuroactive ligand-receptor interaction	Ko04510	Focal adhesion
Ko04151	PI3K-Akt signaling pathway	Ko05012	Parkinson's disease
		Ko00052	Galactose metabolism
		Ko04261	Adrenergic signaling in cardiomyocytes
		Ko05416	Viral myocarditis
		Ko04530	Tight junction
		Ko05016	Huntington's disease
		Ko03010	Ribosome
		Ko00564	Glycerophospholipid metabolism
		Ko00565	Ether lipid metabolism

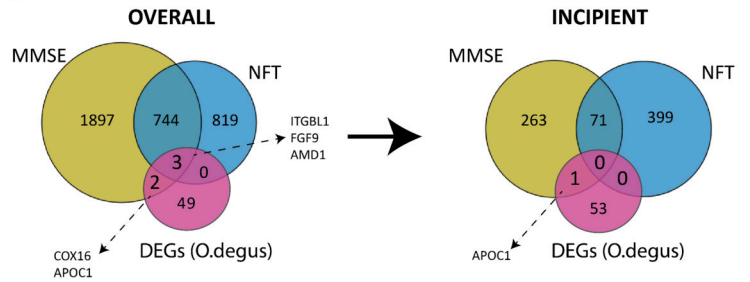


FIGURE 1 | (A) Expression (FPKM values) analysis of 54 differentially expressed genes in PB group compared to GB group with two-way clustering applied. **(B)** Complete list of KEGG pathways corresponding to 29 up- and 25 down-regulated genes between PB and GB. Columns A and C: KEGG entry for up- and down-regulated genes, Columns B and D: Pathway names for up- and down-regulated genes. **(C)** Comparison of differentially expressed genes in PB vs. GB and AD-related human genes. Overall AD-correlated genes and AD-correlated genes with incipient AD stage.

in our study (Table S10). The relative change in gene expression levels across AD-like and control samples is shown in **Figure 1A**.

To determine the function of DEGs, a Gene Ontology (GO) enrichment analysis was performed (Table S7). The up-regulated genes were enriched notably in chromosome related processes, which include chromosome condensation and chromosome organization. GO results showed also that up-regulated genes were largely involved in amine biosynthesis, in particular biosynthesis of polyamines. In contrast, most down-regulated genes were mainly engaged in ion homeostasis related processes, such as sodium-independent organic anion transport, hyperosmotic salinity response or regulation of cellular pH reduction. To fully determine which pathways could be directly affected in AD, DEGs were analyzed using KEGG PATHWAY Database (Braidy et al., 2012). The up-regulated genes were observed to be mainly part of signaling pathways including MAPK, Rap1, Ras and neurotransmission (cholinergic synapse), while down-regulated genes were part of pathways related to AD, Parkinson's disease and Huntington's disease (**Figure 1B**). Specifically down-regulation of *COX8A* gene. The *COX8A* encodes subunit VIIIA of cytochrome c oxidase, a crucial element for the formation of complex IV, which is a part of electron transport chain (ETC) in mitochondria. This is consistent with previously published data, as AD has been already linked to low activity of brain cytochrome c oxidase (Alleyne et al., 2011; Readnower et al., 2011). A substantial number of other down-regulated genes were also found to participate in different metabolic pathways.

Comparison of Human and *O. degus* AD-like Brain Transcriptomes

To gain insights to how *O. degus* could be employed as a natural model for studying the pathogenesis of AD, a human AD brain transcriptome was analyzed using RNA-seq data derived from hippocampus tissue of AD subjects and controls (Bai et al., 2013). Analysis of DEGs resulted in identification of 1,572 up and 1,391 down-regulated genes, which were further compared to DEGs identified for *O. degus* (Table S8). This comparison revealed overlap between seven genes, including *FGF9*, *WISP1* and *CPNE7* that are known to be linked with AD. Both *FGF9* and *WISP1* were observed to show the opposite expression changes for human and *O. degus*, while the altered expression of *CPNE7* was consistent between both species. Other common

DEGs included one up-regulated gene: *ITGBL1*, and two down-regulated genes: *C1orf95*, *LRRC55*, as well as *IL1RAPL2*, which was down-regulated in *O. degus* brain and up-regulated in human hippocampus. To further compare human and *O. degus* AD-like brain transcriptomes, GO enrichment was employed. GO analysis revealed that that up-regulated genes for human were mainly involved in system and tissue development, in particular circulatory system and vasculature development (Table S9). This is to some extend consistent with biological processes described for *O. degus* up-regulated genes, as majority of enriched terms were biosynthesis related. Down-regulated genes were significantly enriched in neurological processes including signal transduction and neurogenesis. Similar to *O. degus*, AD affects glutamate secretion and glutamate receptor signaling pathways in human brain. Interestingly, genes involved in regulation of ion transport was also affected, supporting the relationship between cellular stress signals and AD.

Comparison of *O. degus* DEGs and Human AD-related Genes

To investigate the potential role of *O. degus* DEGs in human AD progression, we compared our results to a set of AD-related genes, generated using Affymetrix expression microarrays across different stages of human AD: incipient, moderate and severe, as well as control samples (Blalock et al., 2011). AD-related genes were defined as genes, whose expression was positively or negatively correlated with results of MiniMental Status Examination (MMSE) and neurofibrillary tangles (NFT) counts across three AD categories (Blalock et al., 2004). Interestingly, five *O. degus* DEGs could be linked to AD-related genes in all subjects (overall correlation) this included *COX16*, *APOC1*, *ITGBL1*, *FGF9,* and *AMD1*. All five DEGs correlated with MMSE scores, while three: *ITGBL1*, *FGF9,* and *AMD1* were both MMSE and NFT-correlated. However, only *AMD1* showed a similar correlation with incipient AD (**Figure 1C**).

DISCUSSION

The purpose of the present study was to characterize the brain transcriptome of the *O. degus* to support its use as a natural model of AD, by comparing the transcriptomes of AD-like and healthy *O. degus*. This study is, to the best of our knowledge, the first report of *O. degus* brain whole transcriptome sequencing (RNA-seq). Our results revealed in *O. degus* a number of genes previously implicated in AD and related disorders indicating that this model is a suitable alternative to transgenic mice models for AD research. Because the AD-pathology can vary widely with animal age we carefully matched control and AD-like groups. Animals aged 3 years have optimal AD-like pathology development namely, the presence of beta-amyloid deposits as has been previously reported (Inestrosa et al., 2005; Ardiles et al., 2012; Deacon et al., 2015). This allowed for an acceptable signal dynamic for the experimental analysis. The sample size used for this study was calculated on the magnitude of the biological effect and the inherent variability of the target being measured, in this case the presence or absence of beta-amyloid deposits. We also considered the variability in the behavioral measures, which as previously reported (Deacon et al., 2015) showed clustering in two cohort of animals, AD-like and controls in the tests of daily living.

This work has identified up-regulated genes involved in AD including the CHRNA6 gene, which encodes a sub-type of neuronal nicotinic acetylcholine receptor widely associated to AD, which are mostly linked to Aβ aggregation theory and that was found previously affected in AD patients (Lombardo and Maskos, 2015). Another DEG AMD1 (which encodes an intermediate enzyme relevant to the polyamine biosynthesis) has an altered activity in AD and has also been implicated in schizophrenia and mood disorders, and was found up-regulated in affected AD-like *O. degus* (Morrison et al., 1993; Fiori and Turecki, 2008). The down-regulated genes found in the brains of AD-like *O. degus* included WNT1 inducible signaling pathway protein 1 (WISP1) (Varela-Nallar and Inestrosa, 2013). This gene previously implicated in neurodegeneration by regulation of mitochondrial signaling and apoptosis is a downstream target in the Wnt1 signaling pathway (Wang et al., 2012). Wnt signaling that involves Wnt1 and WISP1 is becoming recognized as a vital neuroprotective component during AD. WISP1 stops p53 mediated DNA damage and apoptosis offering neuronal protection by blocking cytochrome c release (Su et al., 2008; Wang et al., 2012). Mitochondrial-related genes were also found to be differentially expressed in AD-like *O. degus* brains, including the Cytochrome C Oxidase Subunit 8A (COX8A) that has been associated with the reduction of neuronal energy metabolism and mitochondrial dysfunction observed in AD patients by the inhibition of COX activity as a result of binding to Aβ-subunits (Lustbader et al., 2004; Liang et al., 2008).

The comparison of human and *O. degus* AD-like brain transcriptomes, revealed seven common genes that were deregulated. This included fibroblast growth factor 9 (*FGF9*), *WISP1* and Copine VII (*CPNE7*). Interestingly, the *FGF9* gene is known to be related to the MAPK pathway affected in AD patients (Antonell et al., 2013), and is one of the therapeutic targets currently used in AD treatment (Zhang et al., 2014).

Retinal abnormalities have also been reported to be associated to AD (Berisha et al., 2007). *O. degus* naturally develops cataracts, an eye dysfunction linked to neurodegeneration (Inestrosa et al., 2005, 2015; Braidy et al., 2015; Szabadfi et al., 2015). Our study revealed deregulated expression of two genes: *FRMD7* and *ADAMTS9,* which were previously associated to retinal abnormalities. The *FRMD7* gene plays role in retinal and neurite development, as well as in the neuronal outgrowth. It was also connected to X-linked infantile nystagmus, a disease characterized by abnormal eye function (Betts-Henderson et al., 2010). The *ADAMTS9* gene is also known to be involved in abnormal eye development. Extracellular matrix is a key mediator in the pathogenesis of age related macular degeneration and includes *ADAMTS9*. *ADAMTS9* activates *AKT* promoting photoreceptor degeneration and consequently membrane thickening and damage of retinal pigmentary cells. Moreover regulation of mTOR by ADAMTS9 leads to angiogenesis via VEGF A and TSP-1 this way regulating angiogenesis in the eye (Wight, 2005; Parry et al., 2009). Moreover, the activity disorders of ADAMTS9 regulation of the AKT/mTOR pathway leads to glycolysis and glucose

uptake by increasing hypoxia-inducible factor (HIF)-1α (HIF1A) also been linked to AD (Avramovich-Tirosh et al., 2010). In humans, an ADAMTS9 gene variant is associated with type 2 diabetes (T2DM) is most frequently linked with aging, cognitive impairment, AD-associated neuronal APP-Aβ deposits (de la Monte and Wands, 2005). In relation to these results, the *O. degus* has insulin resistance and naturally develops type 2 diabetes and associated cataracts when fed with a diet high in glucose (Datiles and Fukui, 1989).

We have also identified *APOC-I* (apolipoprotein C-I) to be deregulated in AD-like *O. degus* brain samples. Notably, *APOC-I* is part of the APOE/C-I/C-IV/C-II gene cluster on the chromosome 19 (Smit et al., 1988) and APOE modulates its expression. This gene encodes a small apolipoprotein that is associated with Aβ plaques (Kamino et al., 1996). APOC-I plays a critical role in CNS homeostasis, since altered expression impairs memory (Abildayeva et al., 2008). Moreover, is to be highlighted that APOC-I co-localizes with Aβ plaques in the brain in AD (Abildayeva et al., 2008).

The pathogenic mechanism resulting in the onset and progression of neurodegenerative diseases like AD is often associated with genetic variants and mutations and interactions with environmental impacts, lifestyle risk factors, and slowly evolving molecular changes due to aging (Jicha and Carr, 2010; Duchen, 2012). Thus, a number of AD research avenues could be investigated in this model other than RNA analysis. For example post-translationally through modification of protein residues like phosphorylation.

In this study we focused in whole brain, so studies of additional brain regions are needed and may point to different genes and additional pathways. The RNA-seq uncovered multiple DE transcripts, several of these are novel and some have been previously implicated in AD like WISP1 and APOC1. Being the present work a pilot study we have probably uncovered only a fraction of what these data may reveal about this model and its link with AD supporting further work with this species. Future work is underway in our laboratory to elucidate the characterization of global gene expression of young and old *O. degus* by analysing tissue from different brain areas. To our knowledge this is the first *O. degus* brain transcriptome study. Salazar et al. (2016), compare the sequences of genes in relation to AD using published data from *O. degus*, human and other rodents. They analyzed tau, APP, Apolipoprotein E (APOE), Presenilin 1 (Psen1), and Aβ-peptide sequences in correlation with specific human variants associated with AD. Their findings revealed that *O. degus* have the arginine substitution present in the ApoE4 pathogenic human allele; Psen1 gene showed a greater relatedness between the isoforms of human, degus, guinea pig, and mole rat, compared to those of others rodents such the rat, and also described that *O. degus* Aβ-peptide sequence presents high homology with the human protein, differing in only one amino acid (Salazar et al., 2016). Furthermore, Deacon et al. (2015) shows that AD-like *O. degus* have high levels of Aβ-peptide aggregates, APP, TNF-a, IL-6, IFN-a, and the oxidative stress marker NFE2L2 when compared to healthy control *O. degus*, suggesting that AD-like *O. degus* present increased inflammation as is observed in human AD patients (Deacon et al., 2015). Overall the study of others and the present explorative transcriptome analysis revealed that the AD-like *O. degus* shares common affected genes with those have been implicated in human AD and therefore provides further characterization of the *O. degus* as a relevant natural model. *O. degus* shows a correlation of expression with human AD-related genes making this model a powerful preclinical tool to characterize target effects of novel therapeutics in AD. This study provides strong evidence to support the *O. degus* as a valuable natural model for preclinical work in AD research.

ETHICS STATEMENT

This study was carried out under the approval of the ethics committee of the Faculty of Sciences, Universidad de Chile, directed by Dr. Marco Méndez and integrated by:-Dr. Eduardo Friedman-Dr. Victoria Guixé-Dr. Madeleine Lamborot-Dr. Roberto Morales-Dr. Aurelio San Martín-Dr. Cecilia Vergara.

DATA AVAILABILITY

All the *Octodon degus* transcriptome data is available through the NCBI using accession PRJNA326273.

AUTHOR CONTRIBUTIONS

FA and BU contributed to the experimental procedures, data analysis and the manuscript preparation. FC, AV, EP, SN, and DL contributed to created the bioinformatic pipeline for sequencing data analysis. LF contributed to the experimental procedures and data analysis. RMJD, MH, and RD contributed to comprehensive analysis of the results and to the manuscript preparation. RV, RG, and PC contributed to the funding of the project, the experimental design and the manuscript preparation.

ACKNOWLEDGMENTS

This research was funded by GeN.DDI Ltd (London, UK) and by the Chilean National Commission for Scientific and Technological Research Grants 1090794, 1140548, ICM-P05-002 and PFB-23-CONICYT (to the Institute of Ecology and Biodiversity). Research was conducted under permit No. 5193 issued by the Servicio Agrícola y Ganadero, Chile, with the supervision of the Ethics Committee of the Faculty of Sciences, Universidad de Chile. We acknowledge support of the Spanish Ministry of Economy and Competitiveness, "Centro de Excelencia Severo Ochoa 2013–2017," SEV-2012-0208 and Project PT13/0001/0021 (ISCIII - Subdirección General de Evaluación y Fomento de la Investigación/FEDER "Una Manera de hacer Europa").

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2017.00064/full#supplementary-material

REFERENCES

Abildayeva, K., Berbée, J. F. P., Blokland, A., Jansen, P. J., Hoek, F. J., Meijer, O., et al. (2008). Human apolipoprotein C-I expression in mice impairs learning and memory functions. *J. Lipid Res*. 49, 856–869. doi: 10.1194/jlr.M700518-JLR200

Alleyne, T., Mohan, N., Joseph, J., and Adogwa, A. (2011). Unraveling the role of metal ions and low catalytic activity of cytochrome C oxidase in Alzheimer's disease. *J. Mol. Neurosci*. 43, 284–289. doi: 10.1007/s12031-010-9436-8

Altschul, S. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402. doi: 10.1093/nar/25.17.3389

Antonell, A., Lladó, A., Altirriba, J., Botta-Orfila, T., Balasa, M., Fernández, M., et al. (2013). A preliminary study of the whole-genome expression profile of sporadic and monogenic early-onset Alzheimer's disease. *Neurobiol. Aging* 34, 1772–1778. doi: 10.1016/j.neurobiolaging.2012.12.026

Ardiles, A. O., Tapia-Rojas, C. C., Mandal, M., Alexandre, F., Kirkwood, A., Inestrosa, N. C., et al. (2012). Postsynaptic dysfunction is associated with spatial and object recognition memory loss in a natural model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A*. 109, 13835–13840. doi: 10.1073/pnas.1201209109

Avramovich-Tirosh, Y., Bar-Am, O., Amit, T., Youdim, M. B. H., and Weinreb, O. (2010). Up-regulation of hypoxia-inducible factor (HIF)-1α and HIF-target genes in cortical neurons by the novel multifunctional iron chelator anti-Alzheimer drug, M30. *Curr. Alzheimer Res.* 7, 300–306. doi: 10.2174/156720510791162403

Bai, B., Hales, C. M., Chen, P.-C., Gozal, Y., Dammer, E. B., Fritz, J. J., et al. (2013). U1 small nuclear ribonucleoprotein complex and RNA splicing alterations in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A*. 110, 16562–16567. doi: 10.1073/pnas.1310249110

Berisha, F., Feke, G. T., Trempe, C. L., McMeel, J. W., and Schepens, C. L. (2007). Retinal abnormalities in early alzheimer's disease. investig opthalmology *Vis. Sci*. 48, 2285. doi: 10.1167/iovs.06-1029

Betts-Henderson, J., Bartesaghi, S., Crosier, M., Lindsay, S., Chen, H.-L., Salomoni, P., et al. (2010). The nystagmus-associated FRMD7 gene regulates neuronal outgrowth and development. *Hum. Mol. Genet*. 19, 342–351. doi: 10.1093/hmg/ddp500

Birch, A. M., Katsouri, L., and Sastre, M. (2014). Modulation of inflammation in transgenic models of Alzheimer's disease. *J. Neuroinflam.* 11:25. doi: 10.1186/1742-2094-11-25

Blalock, E. M., Buechel, H. M., Popovic, J., Geddes, J. W., and Landfield, P. W. (2011). Microarray analyses of laser-captured hippocampus reveal distinct gray and white matter signatures associated with incipient Alzheimer's disease. *J. Chem. Neuroanat*. 42, 118–126. doi: 10.1016/j.jchemneu.2011.06.007

Blalock, E. M., Geddes, J. W., Chen, K. C., Porter, N. M., Markesbery, W. R., and Landfield, P. W. (2004). Incipient Alzheimer's disease: microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc. Natl. Acad. Sci. U.S.A*. 101, 2173–2178. doi: 10.1073/pnas.0308512100

Blasko, I., Stampfer-Kountchev, M., Robatscher, P., Veerhuis, R., Eikelenboom, P., and Grubeck-Loebenstein, B. (2004). How chronic inflammation can affect the brain and support the development of Alzheimer's disease in old age: the role of microglia and astrocytes. *Aging Cell.* 3, 169–176. doi: 10.1111/j.1474-9728.2004.00101.x

Braidy, N., Muñoz, P., Palacios, A. G., Castellano-Gonzalez, G., Inestrosa, N. C., Chung, R. S., et al. (2012). Recent rodent models for Alzheimer's disease: clinical implications and basic research. *J. Neural Transm*. 119, 173–195. doi: 10.1007/s00702-011-0731-5

Braidy, N., Poljak, A., Jayasena, T., Mansour, H., Inestrosa, N. C., and Sachdev, P. S. (2015). Accelerating Alzheimer's research through "natural"animal models. *Curr. Opin. Psychiatry* 28, 155–164. doi: 10.1097/YCO.0000000000000137

Castellani, R. J., and Perry, G. (2012). Pathogenesis and disease-modifying therapy in Alzheimer's disease: the flat line of progress. *Arch. Med. Res*. 43, 694–698. doi: 10.1016/j.arcmed.2012.09.009

Correa, L. A., León, C., Ramírez-Estrada, J., Soto-Gamboa, M., Sepúlveda, R. D., and Ebensperger, L. A. (2016). Masculinized females produce heavier offspring in a group living rodent. *J. Anim. Ecol.* 85, 1552–1562. doi: 10.1111/1365-2656.12588

Datiles, M. B. III, and Fukui, H. (1989). Cataract prevention in diabetic Octodon degus with Pfizer's sorbinil. *Curr. Eye Res.* 8, 233–237.

Deacon, R. M. J. (2014). A novel approach to discovering treatments for alzheimer's disease. *J. Alzheimer Dis. Park*. 4:142. doi: 10.4172/2161-0460.1000142

Deacon, R. M. J. (2006). Burrowing in rodents: a sensitive method for detecting behavioral dysfunction. *Nat. Protoc*. 1, 118–121. doi: 10.1038/nprot.2006.19

Deacon, R. M. J., Altimiras, F. J., Bazan-Leon, E. A., Pyarasani, R. D., Nachtigall, F. M., Santos, L. S., et al. (2015). Natural AD-Like neuropathology in *Octodon degus*: impaired burrowing and neuroinflammation. *Curr. Alzheimer Res*. 12, 314–322. doi: 10.2174/1567205012666150324181652

de la Monte, S. M., and Wands, J. R. (2005). Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: relevance to Alzheimer's disease. *J. Alzheimer's Dis*. 7, 45–61. Available online at: http://content.iospress.com/articles/journal-of-alzheimers-disease/jad00401

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., et al. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21. doi: 10.1093/bioinformatics/bts635

Duchen, M. R. (2012). Mitochondria, calcium-dependent neuronal death and neurodegenerative disease. *Pflügers Arch*. 464. 111–121. doi: 10.1007/s00424-012-1112-0

Ebensperger, L., and Hurtado, M. J. (2005). Seasonal changes in the time budget of degus, *Octodon degus*. *Behaviour* 142, 91–112. doi: 10.1163/1568539053627703

Fiori, L. M., and Turecki, G. (2008). Implication of the polyamine system in mental disorders. *J. Psychiatry Neurosci*. 33, 102–110. Available online at: http://jpn.ca/vol33-issue2/33-2-102/

Götz, S., García-Gómez, J. M., Terol, J., Williams, T. D., Nagaraj, S. H., Nueda, M. J., et al. (2008). High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Res.* 36, 3420–3435. doi: 10.1093/nar/gkn176

Guigo, R., Dermitzakis, E. T., Agarwal, P., Ponting, C. P., Parra, G., Reymond, A., et al. (2003). Comparison of mouse and human genomes followed by experimental verification yields an estimated 1,019 additional genes. *Proc. Natl. Acad. Sci. U.S.A*. 100, 1140–1145. doi: 10.1073/pnas.0337561100

Guigó, R., Knudsen, S., Drake, N., and Smith, T. (1992). Prediction of gene structure. *J. Mol. Biol.* 226, 141–157. doi: 10.1016/0022-2836(92)90130-C

Haas, B. J. (2003). Improving the Arabidopsis genome annotation using maximal transcript alignment assemblies. *Nucleic Acids Res.* 31, 5654–5666. doi: 10.1093/nar/gkg770

Haas, B. J., Salzberg, S. L., Zhu, W., Pertea, M., Allen, J. E., Orvis, J., et al. (2008). Automated eukaryotic gene structure annotation using Evidencemodeler and the program to assemble spliced alignments. *Genome Biol*. 9:R7. doi: 10.1186/gb-2008-9-1-r7

Harrow, J., Frankish, A., Gonzalez, J. M., Tapanari, E., Diekhans, M., Kokocinski, F., et al. (2012). GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Res*. 22, 1760–1774. doi: 10.1101/gr.135350.111

Inestrosa, N. C., Reyes, A. E., Chacón, M. A., Cerpa, W., Villalón, A., Montiel, J., et al. (2005). Human-like rodent amyloid-beta-peptide determines Alzheimer pathology in aged wild-type *Octodon degu*. *Neurobiol. Aging* 26, 1023–1028. doi: 10.1016/j.neurobiolaging.2004.09.016

Inestrosa, N. C., Ríos, J. A., Cisternas, P., Tapia-Rojas, C., Rivera, D. S., Braidy, N., et al. (2015). Age progression of neuropathological markers in the brain of the chilean rodent *Octodon degus*, a natural model of Alzheimer's disease. *Brain Pathol*. 25, 679–691. doi: 10.1111/bpa.12226

Iwata, H., and Gotoh, O. (2012). Benchmarking spliced alignment programs including Spaln2, an extended version of Spaln that incorporates additional species-specific features. *Nucleic Acids Res*. 40, e161. doi: 10.1093/nar/gks708

Jicha, G. A., and Carr, S. A. (2010). Conceptual evolution in Alzheimer's disease: implications for understanding the clinical phenotype of progressive neurodegenerative disease. *J. Alzheimer's Dis.* 19, 253–272. doi: 10.3233/JAD-2010-1237

Kamino, K., Yoshiiwa, A., Nishiwaki, Y., Nagano, K., Yamamoto, H., Kobayashi, T., et al. (1996). Genetic association study between senile dementia of Alzheimer's Type and APOE/C1/C2 gene cluster. *Gerontology* 42(Suppl. 1), 12–19. doi: 10.1159/000213820

Kanehisa, M., Goto, S., Sato, Y., Furumichi, M., and Tanabe, M. (2012). KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res*. 40(Database issue), D109–D114. doi: 10.1093/nar/gkr988

Kanehisa, M., Goto, S., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M. (2014). Data, information, knowledge and principle: back to

metabolism in KEGG. *Nucleic Acids Res.* 42(Database issue), D199–D205. doi: 10.1093/nar/gkt1076

Laurijssens, B., Aujard, F., and Rahman, A. (2013). Animal models of Alzheimer's disease and drug development. *Drug Discov. Today Technol.* 10, e319–e327. doi: 10.1016/j.ddtec.2012.04.001

Li, B., and Dewey, C. N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 12:323. doi: 10.1186/1471-2105-12-323

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*. 25, 2078–2079. doi: 10.1093/bioinformatics/btp352

Liang, W. S., Reiman, E. M., Valla, J., Dunckley, T., Beach, T. G., Grover, A., et al. (2008). Alzheimer's disease is associated with reduced expression of energy metabolism genes in posterior cingulate neurons. *Proc. Natl. Acad. Sci. U.S.A.* 105, 4441–4446. doi: 10.1073/pnas.0709259105

Lombardo, S., and Maskos, U. (2015). Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. *Neuropharmacology* 96, 255–262. doi: 10.1016/j.neuropharm.2014.11.018

Lustbader, J. W., Cirilli, M., Lin, C., Xu, H. W., Takuma, K., Wang, N., et al. (2004). ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science* 304, 448–452. doi: 10.1126/science.1091230

Mangialasche, F., Solomon, A., Winblad, B., Mecocci, P., and Kivipelto, M. (2010). Alzheimer's disease: clinical trials and drug development. *Lancet Neurol.* 9, 702–716. doi: 10.1016/S1474-4422(10)70119-8

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., and Kanehisa, M. (2007). KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res.* 35, W182–W185. doi: 10.1093/nar/gkm321

Morrison, L. D., Bergeron, C., and Kish, S. J. (1993). Brain S-adenosylmethionine decarboxylase activity is increased in Alzheimer's disease. *Neurosci Lett.* 154, 141–144. doi: 10.1016/0304-3940(93)90191-M

Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L., and Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods* 5, 621–628. doi: 10.1038/nmeth.1226

Nazem, A., Sankowski, R., Bacher, M., and Al-Abed, Y. (2015). Rodent models of neuroinflammation for Alzheimer's disease. *J. Neuroinflam.* 12, 74. doi: 10.1186/s12974-015-0291-y

Parra, G., Agarwal, P., Abril, J. F., Wiehe, T., Fickett, J. W., and Guigó, R. (2003). Comparative gene prediction in human and mouse. *Genome Res.* 13, 108–117. doi: 10.1101/gr.871403

Parry, D. A., Toomes, C., Bida, L., Danciger, M., Towns, K. V., McKibbin, M., et al. (2009). Loss of the metalloprotease ADAM9 leads to cone-rod dystrophy in humans and retinal degeneration in mice. *Am. J. Hum. Genet.* 84, 683–691. doi: 10.1016/j.ajhg.2009.04.005

Readnower, R. D., Sauerbeck, A. D., and Sullivan, P. G. (2011). Mitochondria, Amyloid β, and Alzheimer's disease. *Int. J. Alzheimers Dis.* 2011, 1–5. doi: 10.4061/2011/104545

Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 26, 139–140. doi: 10.1093/bioinformatics/btp616

Rumble, S. M., Lacroute, P., Dalca, A. V., Fiume, M., Sidow, A., and Brudno, M. (2009). SHRiMP: accurate mapping of short color-space reads. *PLoS Comput. Biol.* 5:e1000386. doi: 10.1371/journal.pcbi.1000386

Salazar, C., Valdivia, G., Ardiles, Á. O., Ewer, J., and Palacios, A. G. (2016). Genetic variants associated with neurodegenerative Alzheimer disease in natural models. *Biol. Res.* 49, 14. doi: 10.1186/s40659-016-0072-9

Saraceno, C., Musardo, S., Marcello, E., Pelucchi, S., and Luca, M. D. (2013). Modeling Alzheimer's disease: from past to future. *Front. Pharmacol.* 4:77. doi: 10.3389/fphar.2013.00077

Slater, G. S. C., and Birney, E. (2005). Automated generation of heuristics for biological sequence comparison. *BMC Bioinformatics*. 6:31. doi: 10.1186/1471-2105-6-31

Smit, M., van der Kooij-Meijs, E., Frants, R. R., Havekes, L., and Klasen, E. C. (1988). Apolipoprotein gene cluster on chromosome 19. *Hum. Genet.* 78, 90–93.

Stanke, M., Keller, O., Gunduz, I., Hayes, A., Waack, S., and Morgenstern, B. (2006a). AUGUSTUS: ab initio prediction of alternative transcripts. *Nucleic Acids Res.* 34(Web Server issue), W435–W439. doi: 10.1093/nar/gkl200

Stanke, M., Schöffmann, O., Morgenstern, B., and Waack, S. (2006b). Gene prediction in eukaryotes with a generalized hidden Markov model that uses hints from external sources. *BMC Bioinformatics* 7:62. doi: 10.1186/1471-2105-7-62

Su, Y. A., Wu, J., Zhang, L., Zhang, Q., Su, D. M., He, P., et al. (2008). Dysregulated mitochondrial genes and networks with drug targets in postmortem brain of patients with posttraumatic stress disorder (PTSD) revealed by human mitochondria-focused cDNA microarrays. *Int. J. Biol. Sci.* 4, 223–235. doi: 10.7150/ijbs.4.223

Szabadfi, K., Estrada, C., Fernandez-Villalba, E., Tarragon, E., Setalo, G., Izura, V., et al. (2015). Retinal aging in the diurnal Chilean rodent (*Octodon degus*): histological, ultrastructural and neurochemical alterations of the vertical information processing pathway. *Front. Cell Neurosci.* 9:126. doi: 10.3389/fncel.2015.00126

Tarragon, E., Lopez, D., Estrada, C., Ana, G.-C., Schenker, E., Pifferi, F., et al. (2013). *Octodon degus*: a model for the cognitive impairment associated with Alzheimaer's disease. *CNS Neurosci Ther.* 19, 643–648. doi: 10.1111/cns.12125

Varela-Nallar, L., and Inestrosa, N. C. (2013). Wnt signaling in the regulation of adult hippocampal neurogenesis. *Front. Cell Neurosci.* 7:100. doi: 10.3389/fncel.2013.00100

Wang, S., Chong, Z. Z., Shang, Y. C., and Maiese, K. (2012). Wnt1 inducible signaling pathway protein 1 (WISP1) blocks neurodegeneration through phosphoinositide 3 kinase/Akt1 and apoptotic mitochondrial signaling involving Bad, Bax, Bim, and Bcl-xL. *Curr. Neurovasc. Res.* 9, 20–31. doi: 10.2174/156720212799297137

Wight, T. N. (2005). The ADAMTS proteases, extracellular matrix, and vascular disease: waking the sleeping giant(s)! *Arterioscler. Thromb. Vasc. Biol.* 25, 12–14. doi: 10.1161/01.ATV.0000150043.43083.aa

Windisch, M. (2014). We can treat Alzheimer's disease successfully in mice but not in men: failure in translation? A perspective. *Neurodegener Dis.* 13, 147–150. doi: 10.1159/000357568

Zdobnov, E. M., and Apweiler, R. (2001). InterProScan–an integration platform for the signature-recognition methods in InterPro. *Bioinformatics* 17, 847–848. doi: 10.1093/bioinformatics/17.9.847

Zhang, C., Chen, J., Feng, C., Shao, X., Liu, Q., Zhang, Q., et al. (2014). Intranasal nanoparticles of basic fibroblast growth factor for brain delivery to treat Alzheimer's disease. *Int. J. Pharm.* 461, 192–202. doi: 10.1016/j.ijpharm.2013.11.049

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: 29 March 2017
doi: 10.3389/fnagi.2017.00066

Identification of Cerebral Metal Ion Imbalance in the Brain of Aging *Octodon degus*

*Nady Braidy[1], Anne Poljak[1,2,3], Chris Marjo[2], Helen Rutlidge[2], Anne Rich[2], Bat-Erdene Jugder[4], Tharusha Jayasena[1], Nibaldo C. Inestrosa[1,5] and Perminder S. Sachdev[1,6]**

[1] Centre for Healthy Brain Ageing, School of Psychiatry, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia, [2] Mark Wainwright Analytical Centre, University of New South Wales, Sydney, NSW, Australia, [3] School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia, [4] School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Sydney, NSW, Australia, [5] Centre for Ageing and Regeneration, Faculty of Biological Sciences, Pontifical Catholic University of Chile, Santiago, Chile, [6] Neuropsychiatric Institute, Euroa Centre, Prince of Wales Hospital, Sydney, NSW, Australia

The accumulation of redox-active transition metals in the brain and metal dyshomeostasis are thought to be associated with the etiology and pathogenesis of several neurodegenerative diseases, and Alzheimer's disease (AD) in particular. As well, distinct biometal imaging and role of metal uptake transporters are central to understanding AD pathogenesis and aging but remain elusive, due inappropriate detection methods. We therefore hypothesized that *Octodon degus* develop neuropathological abnormalities in the distribution of redox active biometals, and this effect may be due to alterations in the expression of lysosomal protein, major Fe/Cu transporters, and selected Zn transporters (ZnTs and ZIPs). Herein, we report the distribution profile of biometals in the aged brain of the endemic Chilean rodent *O. degus*—a natural model to investigate the role of metals on the onset and progression of AD. Using laser ablation inductively coupled plasma mass spectrometry, our quantitative images of biometals (Fe, Ca, Zn, Cu, and Al) appear significantly elevated in the aged *O. degus* and show an age-dependent rise. The metals Fe, Ca, Zn, and Cu were specifically enriched in the cortex and hippocampus, which are the regions where amyloid plaques, tau phosphorylation and glial alterations are most commonly reported, whilst Al was enriched in the hippocampus alone. Using whole brain extracts, age-related deregulation of metal trafficking pathways was also observed in *O. degus*. More specifically, we observed impaired lysosomal function, demonstrated by increased cathepsin D protein expression. An age-related reduction in the expression of subunit B2 of V-ATPase, and significant increases in amyloid beta peptide 42 (Aβ42), and the metal transporter ATP13a2 were also observed. Although the protein expression levels of the zinc transporters, ZnT (1,3,4,6, and 7), and ZIP7,8 and ZIP14 increased in the brain of aged *O. degus*, ZnT10, decreased. Although no significant age-related change was observed for the major iron/copper regulator IRP2, we did find a significant

OPEN ACCESS

Edited by:
Agustin Ibanez, Institute of Cognitive and Translational Neuroscience, Argentina

Reviewed by:
Jingwen Niu, Temple University, USA
Indira García Cordero, INECO, Spain

***Correspondence:**
Perminder S. Sachdev
p.sachdev@unsw.edu.au

Received: *02 May 2016*
Accepted: *03 March 2017*
Published: *29 March 2017*

Citation:
Braidy N, Poljak A, Marjo C, Rutlidge H, Rich A, Jugder B-E, Jayasena T, Inestrosa NC and Sachdev PS (2017) Identification of Cerebral Metal Ion Imbalance in the Brain of Aging Octodon degus. Front. Aging Neurosci. 9:66. doi: 10.3389/fnagi.2017.00066

increase in the expression of DMT1, a major transporter of divalent metal species, 5′-aminolevulinate synthase 2 (ALAS2), and the proto-oncogene, FOS. Collectively, our data indicate that transition metals may be enriched with age in the brains of *O. degus*, and metal dyshomeostasis in specific brain regions is age-related.

Keywords: LA-ICPMS, metals, Alzheimer's disease, bioimaging, *Octodon degus*

INTRODUCTION

Alzheimer's disease (AD) is the most common progressive age-related neurodegenerative disorder, characterized by debilitating effects on brain function, such as memory loss and decline in cognitive abilities ultimately resulting in loss of independent functioning (Teri et al., 1989; Baddeley et al., 1991; Terry et al., 1991). The two major pathological hallmarks of AD are extracellular amyloid plaques composed of insoluble amyloid beta (Aβ) protein, and intra-neuronal neurofibrillary tangles (NFTs) containing hyperphosphorylated tau (Khachaturian, 1985; Joachim et al., 1987; Selkoe et al., 1987; Mirra et al., 1991; Brun and Englund, 2002). While a substantial body of evidence has implicated neuroinflammation, oxidative stress, excitotoxicity, and oligomeric Aβ toxicity as AD contributing factors, the precise mechanism of AD etiopathology remains unclear (Halliwell, 1989). Several studies have shown that dysregulated transition metal metabolism, particularly Cu, Fe, and Zn may be casually linked to the neuropathology of AD, and may enhance Aβ aggregation and toxicity (Bush, 2003; Bush et al., 2003; Finefrock et al., 2003).

Over the last two decades, several studies using clinical diagnosis and post-mortem AD brain tissue have shown that the levels of Cu, Fe, and Zn accumulate in large concentrations within Aβ plaques (Lovell et al., 1998). Moreover, it has been demonstrated that Cu, Fe, and Zn are associated with the metabolism and functional roles of Aβ and amyloid precursor protein (APP; Smith et al., 2007). This has led to the hypothesis that abnormal biometal deposition may play an important role in the pathobiology of AD, and metal chelation may represent an important therapeutic strategy to prevent the onset or slow down the progression of AD (Bush, 2003; Faux et al., 2010; Bonda et al., 2011; Braidy et al., 2014). Levels of Cu and Fe are upregulated in the brain with age, suggesting that an age-dependent increase in bioactive transition metals may contribute to AD pathology (Maynard et al., 2002). However, the anatomical distribution of these metals within the brain remains unclear. The latter is important for further etiological exploration and the development of agents for the treatment and management of AD.

Apart from the involvement of Cu and Fe, Zn may also play a critical role in the pathogenesis of AD, although the exact mechanism remains unclear (Graham et al., 2014; Hancock et al., 2014; McCord and Aizenman, 2014; Rembach et al., 2014; Yuan et al., 2014). The transcription of APP is mediated by Zn-dependent transcription factors, Sp1 and NF-κB (Borchardt et al., 2000). Zn has also been shown to facilitate the oligomerization of Aβ (Taddeo et al., 2014). Furthermore, cleavage of APP by the protease α-secretase is inhibited by Zn (Parvathy et al., 2000). Therefore, it is highly likely that dysregulation of Zn may be involved in AD (Watt et al., 2014). The levels of Zn in brain tissue are modulated by two main families of Zn transporters; ZnTs (zinc transporters) and ZIPs (Zrt/Irt-like protein, SLC39). Briefly, the intracellular uptake of Zn by neurons is mediated by ZIP protein, and through activated Ca^{2+} voltage gated ion channels, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, and *N*-methyl-D-aspartate (NMDA) receptors (Sensi et al., 2009). The export of zinc extracellularly, or from the cytosol to the lumen of intracellular organelles is mediated by ZnTs. Although several Zn transporters have previously been investigated in several AD models, a more comprehensive study is warranted (Sensi et al., 2009), especially in natural models which are likely more representative of late onset AD.

Several *in vitro* and *in vivo* models have been developed, the most commonly used being transgenic mice models (Braidy et al., 2012). However, due to the high complexity of AD pathology, transgenic mice models recapitulate many, but not all, of the major features of AD, and their validity for the sporadic form of AD is questionable (Braidy et al., 2012). In this context, the establishment and validation of a wild-type "natural" animal model of AD, which recapitulates the neuronal, neuropathological, and behavioral abnormalities of sporadic or late-onset AD, and is small and easy to handle, would be of great benefit.

Previous studies from our laboratory have identified the *Octodon degus*, a South American rodent endemic to Central Chile, as a "natural" rodent model for AD (Inestrosa et al., 2005). The *O. degus* is a diurnal, visual, and highly social rodent that "naturally" develops several symptoms that can be linked to the neuropathology of AD. This caviomorph rodent lives up to an average of 7 years in captivity, making it an interesting model for use in longitudinal studies, including those related to the neurobiology of AD (Colonnello et al., 2011; Uekita and Okanoya, 2011). The amino acid sequence of wild-type *O. degus* Aβ shares a high degree (97.5%) of genetic homology with humans (Inestrosa et al., 2005; Braidy et al., 2012). It was recently reported that age-relate changes in Aβ oligomers and *tau* phosphorylation in *O. degus* correlated with a decrease in both spatial and object recognition memory, and impaired postsynaptic and neural plasticity (Ardiles et al., 2012). However, changes in the levels and spatial distribution of Cu, Zn, and Fe, and other redox-active metals in the aged *O. degus* brain tissue are of particular interest.

In this study, we aim to further examine the homology between Alzheimer-type pathology in the South American rodent *O. degus* and sporadic AD in humans by studying the neuropathological abnormalities in the animal to determine if it is indeed a unique "natural" model for the study of

the pathobiology of AD. We hypothesized that: (1) *O. degus* develop neuropathological abnormalities in the distribution of redox active biometals, such as Fe, Ca, Zn, Cu, and Al; (2) lysosomal markers are affected in the "natural" model, similar to human AD; (3) altered biometal trafficking pathways, including major Fe/Cu regulatory genes, selected ZnTs, and ZIPs may represent a potential mechanism for altered distribution of these biometals in brain sections across the entire life-cycle of the *O. degus*. To address these hypotheses, we quantitatively imaged the anatomical distribution of Cu, Fe, Zn, Ca, and Al, in the brains of aged *O. degus* by a laser ablation inductively coupled plasma (ICP) system using mass spectrometry (LA-ICPMS). We have delineated regions of interest (ROIs) and determined the biometal concentrations in the *O. degus* brain with advanced age. Using western blotting, we also investigated potential mechanisms for these changes by examining the expression of biometal trafficking pathways, including lysosomal function, major Fe/Cu regulatory genes, and selected ZnTs and ZIPs.

MATERIALS AND METHODS

Animals

Octodon degus were obtained from a breeding colony at the animal facility of the University of Valparaiso and maintained in a controlled temperature room (23 ± 1°C), under a 12:12 h light/dark cycle, with water and food provided *ad libitum*. At the time of this study, 16 male *O. degus* were grouped by age, from 12 to 36 months of age ($n = 8$ per group). Ages were selected to represent the development of AD-like pathology (36 months). All efforts were made to minimize animal discomfort and stress while also limiting the number of animals used. Aged animals were anesthetized with Equitesin (2.5 ml/kg, i.p.) and injected with heparin (4 USP/kg, i.p.). Afterward, brains were surgically removed from their skulls and frozen in isopentane at −78.5°C. All procedures were conducted according to animal protocols approved by the Institutional Animal Care and Use Committee at the University of Valparaiso and Pontifical Catholic University of Chile.

Sample Preparation for LA-ICPMS

Cryosections of brains of 10 μm thickness were mounted on silanized slides. Neighboring sections were stained with hematoxylin and eosin staining.

LA-ICPMS Instrumentation

LA-ICPMS was performed using a NewWave NWR213 (New Wave UP 266, New Wave, Fremont, CA, USA) laser ablation pulsed laser sampling accessory connected to a PerkinElmer Nexion ICPMS. The material was ablated using a focused Nd:YAG laser and then transported by argon as carrier gas into the ICP plume. Following ion formation in the ICP ion source, the positively charged ions were extracted from the argon plasma (at ≈100 kPa) via the differentially pumped interface (at ≈130 Pa) between the sampler and skimmer cones into the high vacuum of the quadrupole mass analyzer, and were separated with respect to their mass-to-charge ratios and detected by the ion detector. The experimental parameters of LA-ICPMS were optimized with respect to the maximum ion intensity of $^{63}Cu^{+}$ using a well-homogenized synthetic laboratory standard. Tissue was ablated using a laser setting of 25% of maximum power, with a repetition rate of 20 Hz, a laser spot size of 50 μm, and a scan speed of 25. The gas flows in the LA-sample chamber were 0.85 l/m for He, and 0.65 l/m for Ar. Elemental analysis on the ICPMS was performed using peak hopping scan mode, with a dwell time of 90.7 ms per amu, and an integration time of 24,048 ms. All mass spectrometric measurements were performed with established protocols in routine mode in Becker's BrainMet Laboratory (BrainMet–Bioimaging of Metals in Brain and Metallomics) at Forschungszentrum Jülich. Optimized experimental parameters have been previously described (Matusch et al., 2010). Instrument response was calibrated using the NIST 614 and NIST 612 standards to produce images of the change in elemental distribution across the tissue. Image data was processed and calibrated using the IOLITE software, then rendered as color images using MATLAB.

Hematoxylin and Eosin Staining

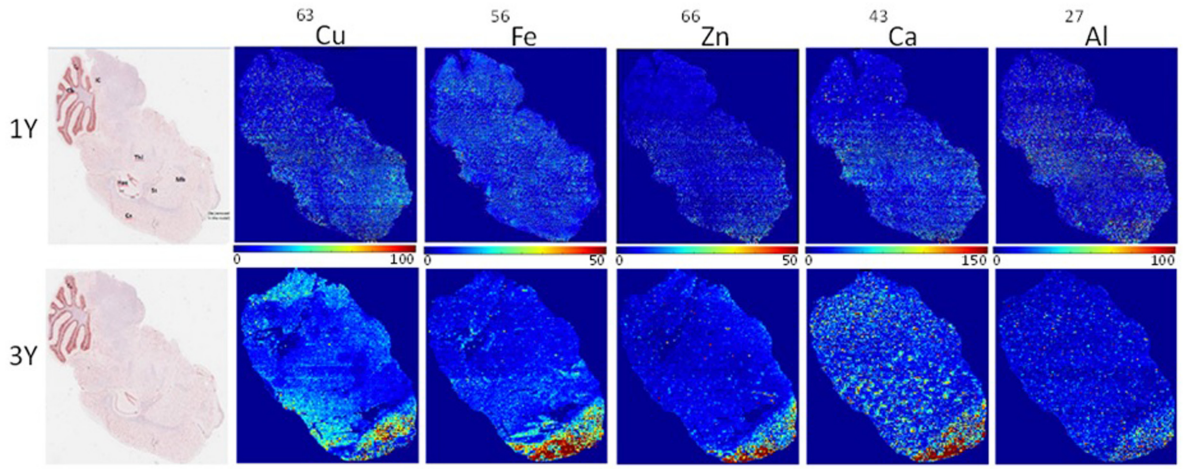
Slides were over-stained with hematoxylin for 10 min and excess stain was removed by running under tap water for 2 min. Afterward, they were differentiated and destained by submerging in acid alcohol for a few seconds until sections appeared red then rinsed briefly under tap water to remove acid. Slides were blued in bicarbonate until nuclei stood out significantly then rinsed under tap water for 5 min. Afterward, the slides were submerged in 70% ethanol for 3 min, and then in eosin for 2 min. The slides were then taken through three changes of 95% ethanol, for 5 min each. Finally, slides were rinsed in 100% ethanol, coverslipped and mounted.

Western Blotting

Octodon degus brains were dissected on ice and immediately processed. Briefly, cortical tissue was homogenized in radioimmunoprecipitation assay buffer (RIPA buffer) [50 mM, Tris–Cl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 1% sodium dodecyl sulfate (SDS)], supplemented with a protease inhibitor cocktail (Sigma-Aldrich P8340) and phosphatase inhibitors (50 mM NaF, 1 mM Na_3VO_4, and 30 μM $Na_4P_20_7$), using a Potter homogenizer and then passed sequentially through different caliber syringes. Protein samples were centrifuged at 14,000 rpm at 4°C twice for 15 min. Total protein concentration was determined using the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). Hippocampal samples (20 μg) were resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane The incubation with a primary antibody; then a secondary anti-goat peroxidase conjugated antibody (Pierce) was used and developed using an ECL kit (Western Lightning Plus ECL, PerkinElmer) following the manufacturer's instructions.

Statistical Analysis

To validate the metal ion images (**Figure 1**) and quantify the levels of Cu, Fe, Zn, Ca, and Al in the hippocampus



CSImageSettings (used for Iolite images – values in $\mu g.g^{-1}$)

C13_CPS	YellowHot	0	10000
Cu_ppm_m63	YellowHot	0	100
Fe_ppm_m56	YellowHot	0	50
Zn_ppm_m66	YellowHot	0	50
Ca_ppm_m43	YellowHot	0	150
Al_ppm_m27	YellowHot	0	100

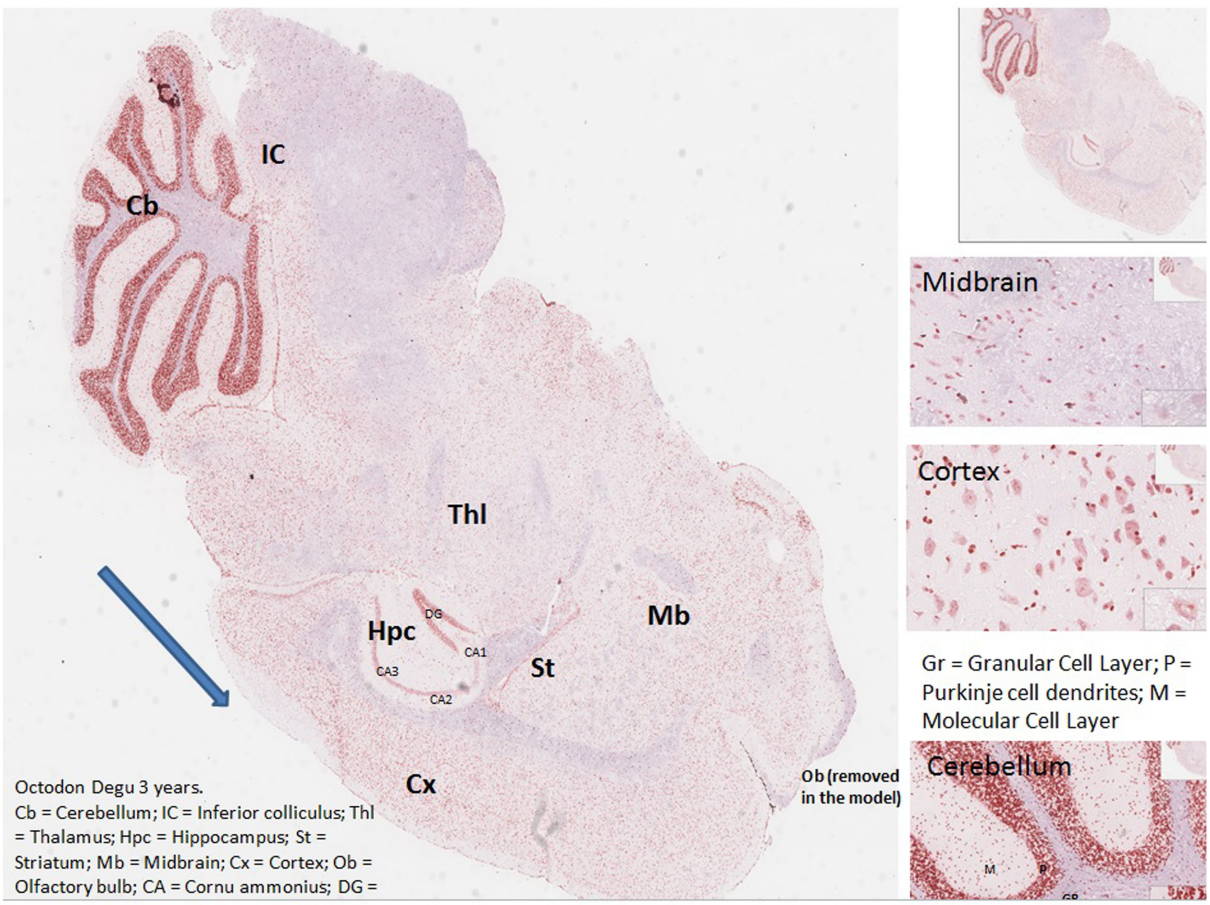
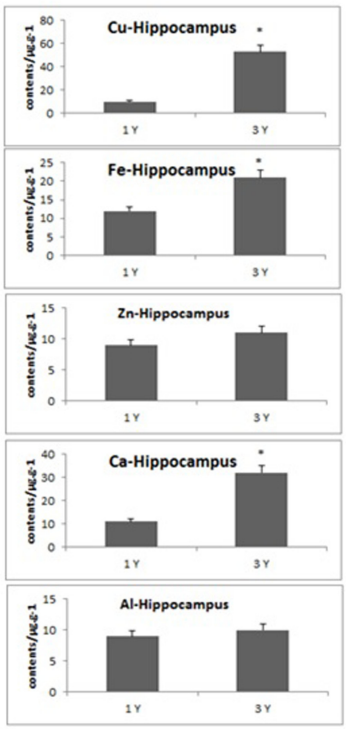


FIGURE 1 | LA-ICPMS imaging of Cu, Fe, Zn, Ca, and Al, in whole brain, sagittal sections of *O. degus* with age. H&E stained histological sections are shown on the far left, and neighboring brain sections were used for LA-ICPMS of each of five metals.

A



B

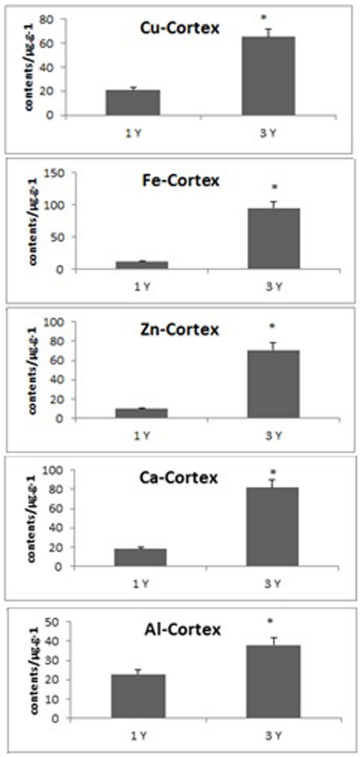
Element	1 years (contents/µg.g-1, mean ±SEM)	3 years (contents/µg.g-1, mean ±SEM)	T value	p value
Cu	10±1.2	53±4.8	5.1	0.02
Fe	12±1.5	21±2.3	2.5	0.02
Zn	9±0.8	11±0.9	0.3	0.1
Ca	11±0.9	32±3.2	3.4	0.02
Al	9±0.9	10±1.1	0.5	0.26

FIGURE 2 | (A) Quantitative alteration of Cu, Fe, Zn, Ca, and Al in the hippocampus of *O. degus* with age. Significant difference between 1- and 3-year-old *O. degus*. $^*p < 0.05$. **(B)** Table of absolute values mean ± SEM.

(**Figure 2**) and cortex (**Figure 3**) of *O. degus* with age, two-dimensional images were first acquired as previously described by da Silva and Arruda (2013). The raw data that was collected from the IOLITE software were exported as Microsoft Excel files, and each line that was formed by ablation was exported to a different worksheet. A total of 60 lines were required for mapping the entire slide surface. Therefore, 60 IOLITE files were generated, and each one was exported to a new worksheet, culminating in 60 Excel worksheets. Each worksheet contained the results of the analyte values on the entire slide surface. Blanks were added to account for the argon atmosphere where there was no ablation, and the blank signal was subtracted from the sample signal. The results were normalized using ^{13}C as an internal standard. The average net ^{13}C ion intensity was used as a surrogate of slice thickness, and calculated from histograms of pixel values (Becker et al., 2012). Afterward, excel files were converted into a text file to generate an image using MATLAB. The MATLAB image was generated using (x, y) coordinates. The x data set were collected from the LA-ICPMS, and the y data set was based on the number of lines. The z value representing the relative intensity at each point was correlated with the x and y matrix plane. For the x-axis, the ICP-MS acquisition time was considered, and LA system scan speed were used. For the y-axis, the spacing among the lines was used. Finally, data were converted into a matrix plane (x, y) in MATLAB. For data acquisition, a transpose function ($w = z'$) was used to adjust the image, and maintain relative intensity at each point. MATLAB images were saved as 8 bit grayscale TIFFs and were corrected for linear drift (factor of 1 was used at the end of the sample next to the standard, and the correction factor progressively increased or decreased line by line toward the other end) using IMAGENA (Osterholt et al., 2011). The hippocampus and cortex, including glass background and entire section trace metal concentrations were calculated from ion intensities averaged from freely drawn ROIs (representing the location of the hippocampus and cortex, respectively) within ion intensity images using PMOD version 3.0[1]. This was repeated four times per sample to ensure greater reliability and accuracy of data. Microphotographs obtained prior to ablation and the RB4 Watson Paxinos

[1]www.pmod.com

A



B

Element	1 years (contents/µg.g-1, mean ±SEM)	3 years (contents/µg.g-1, mean ±SEM)	T value	p value
Cu	21±1.8	65±6.2	5.9	0.004
Fe	12±1.0	95±9.0	9.2	0.001
Zn	10±1.3	71±7.3	7.5	0.001
Ca	18±1.6	82±7.8	8.3	0.001
Al	23±2.5	38±3.5	0.7	0.03

FIGURE 3 | (A) Quantitative alteration of Cu, Fe, Zn, Ca, and Al in the frontal cortex of *O. degus* with age. Significant difference between 1- and 3-year-old *O. degus*. $^*p < 0.05$. **(B)** Table of absolute values mean ± SEM.

Atlas[2] were used to neuroanatomically define ROIs. Standard measurements were processed using Microsoft Excel algorithm calculating slope of the calibration curve. Data were analyzed based on concentration = (maximum counts − minimum counts) × (ion intensity-background)/(255 × slope). Results were expressed as mean ± standard error over the 5 vs 4 individual averages of regional element concentrations using Prism software (GraphPad Software Inc.). The classical Bonferroni threshold for 4 regions × 5 elements was $p = 0.008$. For the Holmes modified Bonferroni correction, a p-value of $\leq$0.05 was considered to be statistically significant for the first region, for the second 0.05/2, for the third 0.05/3 and so on.

Western blots (**Figures 4–7**), were quantified using ImageJ 1.47v (NIH; Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA[3], 1997–2010). Statistical analysis was performed using Prism software (GraphPad Software Inc.). Data were analyzed using the Student's t-test, and the *post hoc* Tukey's multiple comparison test was applied. A p-value of $\leq$0.05 was considered to be statistically significant.

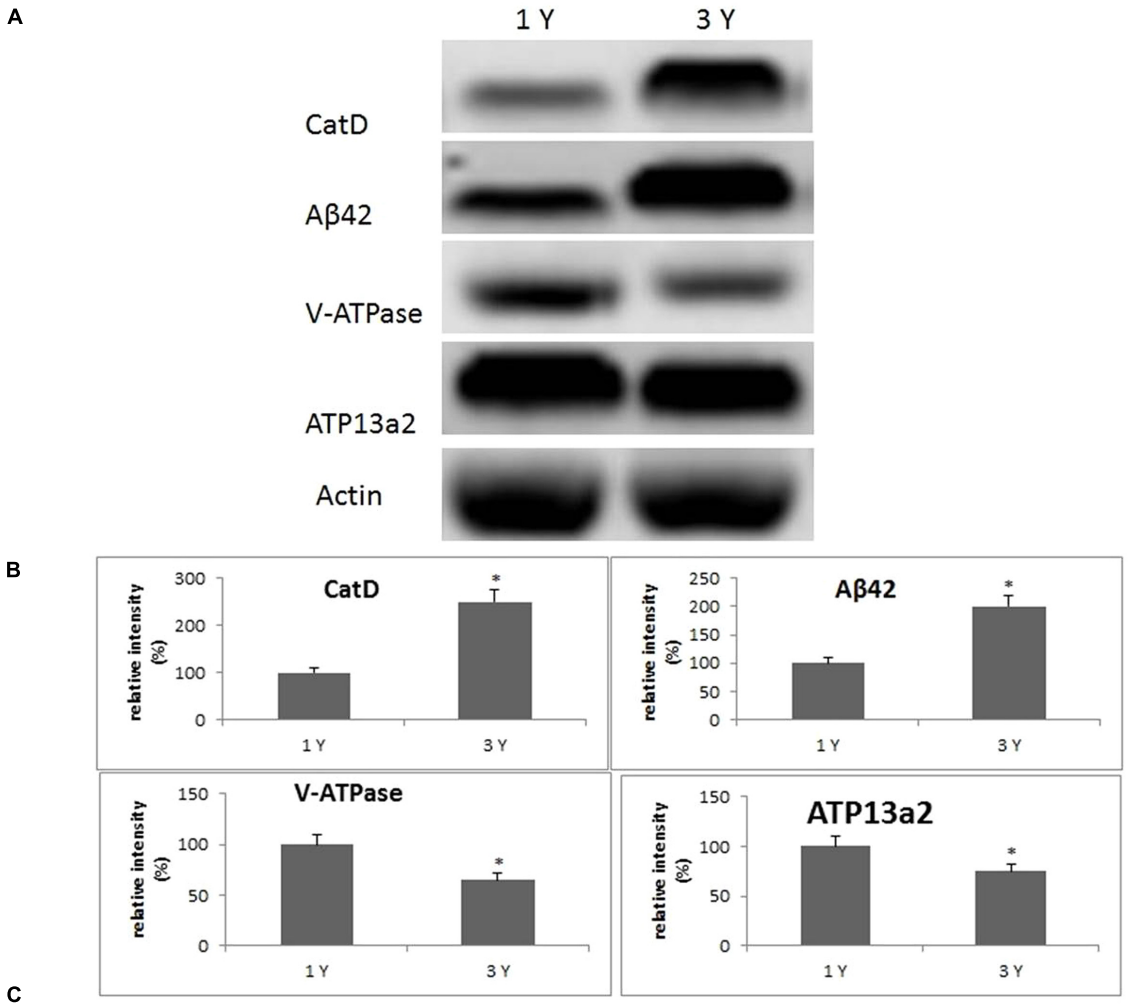
RESULTS

Microdistribution of Cu, Fe, Zn, Ca, and Al, in Whole Brain Sections of *O. degus* as a Natural Model of Aging and AD Progression

Biometal accumulation can only be examined in humans using post-mortem brain sections collected at the end stage of the disease, and the distribution of redox-active brain-metals during the pathobiology of AD remains unclear. By using the *O. degus*—a natural model for AD—dyshomeostasis in Cu, Fe, Zn, Ca, and Al distribution can be examined in various brain regions with age (from 1 to 3 years old) and AD progression (from early stage

[2]http://www.callisto-science.org/NSI/Neuroscience_Image_Database/RUN_RBSC.PDF

[3]http://imagej.nih.gov/ij



Lysosomal related Protein	1 years (relative intensity, %)	3 years (relative intensity, %)	T value	p value
CatD	100±10.0	250±21.8	5.9	0.005
Aβ42	100±10.0	200±19.3	5.5	0.006
V-ATPase	100±10.0	65±5.5	3.2	0.007
ATP13a2	100±10.0	75±6.9	2.9	0.009

FIGURE 4 | Evidence for lysosomal aberrations in the frontal cortex with age. (A) Altered levels of cathepsin D, V-ATPase, Aβ42, ATP13a2 were observed in the brain at 3 years of age compared to 1-year-old *O. degus*. The blots shown are representative data from an experiment repeated eight times. **(B)** Graphs are mean ± SEM brains from brains of eight *O. degus* for each age group. Significantly different $^{*}p < 0.01$ compared to 1-year-old *O. degus*. **(C)** Table of absolute values mean ± SEM.

to late stage). Brain section elemental images are presented in **Figure 1**. These images were used for metals quantification in the brain regions of hippocampus and cortex (**Figures 2**, **3**).

Several studies have shown that Cu is highly abundant in plaques but is present at lower than the limits of detection, in the whole brain of AD (Burdo and Connor, 2003; Bush, 2003; Feaga et al., 2011). Our Cu images in **Figure 1** show that the amount of Cu in the brain of *O. degus* increases with age. These increases are focused in the hippocampal and cortical regions of *O. degus*. **Figure 2** shows that Cu in the hippocampus of *O. degus* significantly increased with age, from 10.1 $\mu g\ g^{-1}$ at 1 year of age to 52.1 $\mu g\ g^{-1}$ at 3 years of age. Comparatively, an increase in the Cu level (21–65 $\mu g\ g^{-1}$) was found in cortex from 1 to 3 years of age. Our imaging data suggests that Cu is enriched in the aging brain, and to a greater extent in the AD brain.

Similarly, Fe levels are increased with age in the brain of *O. degus*, indicating that, like Cu, Fe is an age-dependent enriched element in the brain (**Figure 1**). Fe was found to be increased in the hippocampus and cortex. The content of Fe in the hippocampus of 3 years old *O. degus* was in the range of 15–25

$\mu g\ g^{-1}$, which was significantly higher than young *O. degus* (10–15 $\mu g\ g^{-1}$). **Figure 3** also shows an obvious Fe increase in the cortex of *O. degus* with age. The Fe content in the cortex of 3-year-old *O. degus* was about 90–100 $\mu g\ g^{-1}$, which was considerably higher than that of the 1-year-old *O. degus* (10–15 $\mu g\ g^{-1}$).

The dysregulation of Zn in AD pathology is also of major interest (Watt et al., 2014). Our quantitative images of Zn in this study show the cortex and hippocampus are Zn-rich regions in brain **Figure 1**. The average content of Zn in the hippocampus was 10–15 $\mu g\ g^{-1}$ and did not significantly increase with age. However, in the cortex, the content of Zn increased significantly with age, from ∼10 $\mu g\ g^{-1}$ in 1-year-old *O. degus*, to ∼80 $\mu g\ g^{-1}$ in 3-year-old *O. degus* (**Figures 1**, **2**).

Ca represents another important metal that plays a fundamental role in normal brain cell function (Chen and Nguyen, 2014). The level of Ca was found to increase with age throughout the brain (**Figure 1**), and in the hippocampus and cortex in particular, supporting the hypothesis of the imbalance of calcium homeostasis and disturbed Ca flux in brain components in the development of AD.

A link between Al and AD has also been the subject of scientific debate for several decades (Andrasi et al., 1995; De Sole et al., 2013). No significant increase in the levels of Al was observed in the hippocampus in our study. However, Al levels increased significantly in the cortex of aged *O. degus*, from ∼20 $\mu g\ g^{-1}$ in 1-year-old *O. degus*, to ∼40 $\mu g\ g^{-1}$ in 3-year-old *O. degus*.

Impaired Lysosomal Function in *O. degus*

Lysosomal dysfunction, has previously been reported in AD and several transgenic rodent models (Armstrong et al., 2014; Coffey et al., 2014; Xue et al., 2014). In frontal cortex, we found an age-dependent increase in cathepsin D activity from 1 to 3 years of age (**Figure 4**). An age-dependent reduction of subunit B2 of V-ATPase was also observed in the frontal cortex, the site of neuropathological changes in AD, suggestive of lysosomal impairment. We also examined the levels of Aβ peptide Aβ42, and the metal transporter, ATP13a2. Significantly increased concentrations of Aβ42, and decreased ATP13a2 levels were also observed after onset of neurological disease (**Figure 4**), providing further support for a relationship between AD and lysosomal aberrations.

IRP2, DMT1, ALAS2, and FOS expression in *O. degus*

To evaluate the biological significance of increased Cu and Fe with age, we measured the protein expression of IRP2 and DMT1 in the frontal cortex. DMT1 is a major transporter of divalent metal species and exists in two isoforms—one that contains an iron responsive element in the untranslated region and would be expected to be regulated by IRP2 (DMT1-IRE) and a second that lacks an IRE and should be regulated independently of IRP2 levels (DMT1-non-IRE) (Wilkinson and Pantopoulos, 2014). We found a significant increase in expression of DMT1-non-IRE in 3-year-old *O. degus* compared to 1-year-old *O. degus* (**Figure 5**). However, IRP2 protein expression remained unchanged (**Figure 5**).

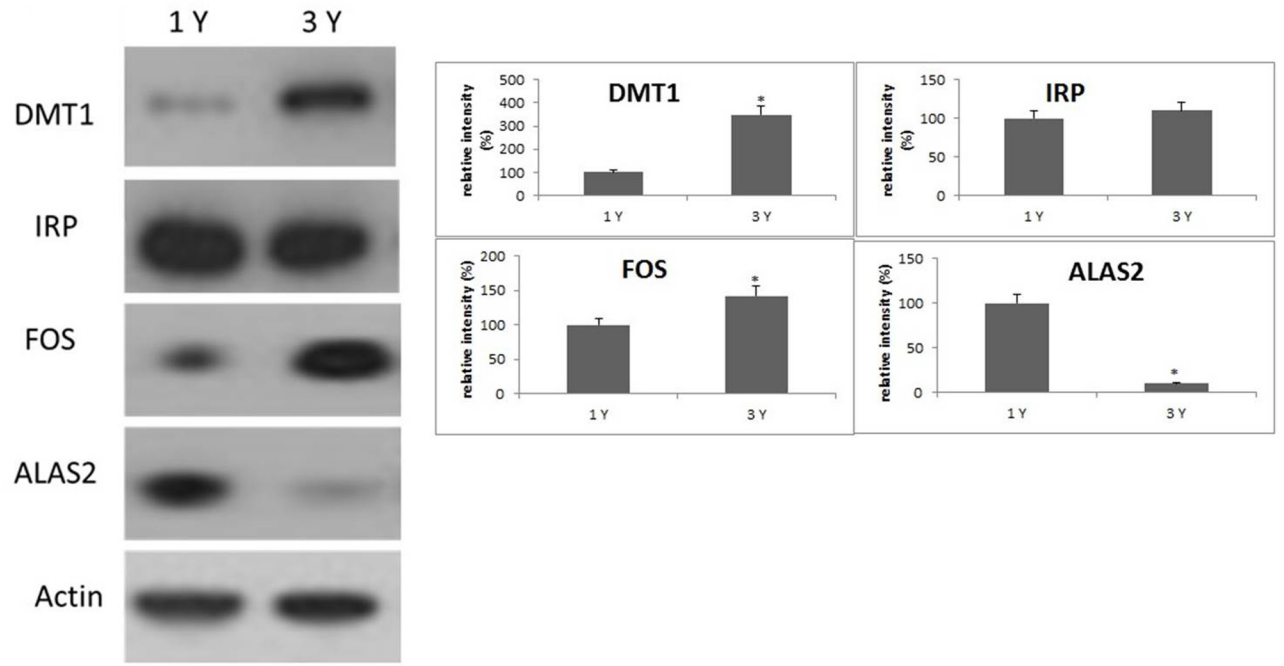
We also assessed the expression of additional proteins related to Cu and Fe metabolism. The proteins of interest were proto-oncogene c-Fos (FOS), and 5-aminolevulinate synthase (ALAS2) (**Figure 5**). We found a significant increase in expression of FOS in 3-year-old *O. degus* compared to 1-year-old *O. degus* (**Figure 5**). However, the levels of ALAS2 significantly declined with age in 3-year-old *O. degus* compared to 1-year-old *O. degus* (**Figure 5**). This suggests that alterations in the ratio of Cu and Fe significantly alter intracellular Cu/Fe pathways.

Age-Specific Alterations to Zn Transport Pathways

Western blotting revealed age related changes in the protein expression of Zip and ZnT metal transporter proteins. The expression of the ER/Golgi-resident transporter, Zip7, significantly reduced in the frontal cortex in an age-dependent manner in 3-year-old *O. degus*. Similarly, significantly reduced expression of Zip8, and Zip14 were observed in 3-year-old *O. degus* (**Figure 6**). On the contrary, the protein expression of ZnT1, ZnT3, ZnT4, ZnT6, and ZnT7 were significantly increased with age in the frontal cortex of 3-year-old *O. degus* (**Figure 7**). However, the expression of ZnT10 was significantly reduced. Therefore, dysregulated expression of ZIP and ZnT family may be associated with the progression and neuropathology of AD.

DISCUSSION

Natural animal models which closely resemble the etiopathogenesis of AD are needed for research on disease mechanisms and for drug development. No reliable "natural" model of AD is available, and current AD research largely uses transgenic models, which have a number of limitations, including the fact that they do not recapitulate the morphological and temporal patterns observed in clinical AD in humans (Braidy et al., 2012, 2015). Therefore, data on molecular mechanisms and drug efficacy testing using these genetically modified models should be interpreted with care, as many previous discoveries made in transgenic models have been lost in human translation (Braidy et al., 2012, 2015). Spontaneous age-related neurodegeneration reported in *O. degus* mimics many of the cellular and molecular events that have been observed extensively in experimental models and clinical AD (Inestrosa et al., 2005, 2015; Du et al., 2015). An interesting feature of the AD-like changes observed in *O. degus* brains is the fact that they occur in aged animals and so far, have never been detected in young animals (1-year-old; Inestrosa et al., 2015). In one study, 3-year-old wild-type *O. degus* have been shown to develop significant Aβ peptide deposition in blood vessel walls of the brain (van Groen et al., 2011). Capillary Aβ deposition in the cerebral cortex has been recently reported in a population-based study of 601 individuals aged greater than 85 years, and was associated with the severity of dementia and AD-type pathology (Makela et al., 2016). Moreover, hippocampal tau deposits



C

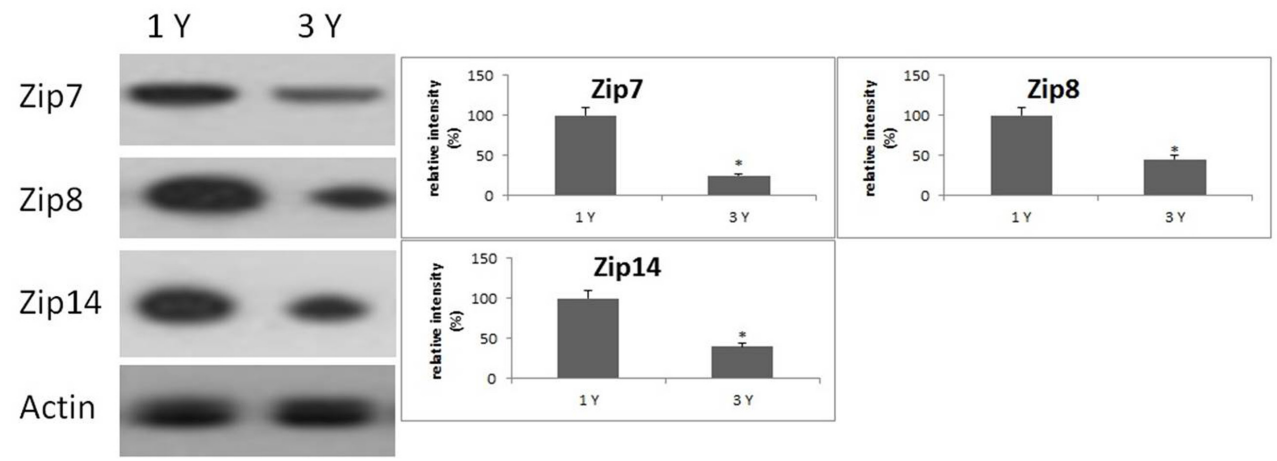
Cu/Fe metabolic Protein	1 years (relative intensity, %)	3 years (relative intensity, %)	T value	p value
DMT1	100±10.0	350±32.5	3.5	0.008
IRP	100±10.0	110±12.1	0.4	0.14
FOS	100±10.0	142±17.2	2.1	0.03
ALAS2	100±10.0	10±0.4	5.2	0.002

FIGURE 5 | Alterations in the expression of Cu/Fe metabolic protein in the frontal cortex with age. (A) Altered levels of IRP2, DMT1, ALAS2, and FOS were reported in the brain at 3 years of age compared to 1-year-old *O. degus*. The blots shown are representative data from an experiment repeated eight times. **(B)** Graphs are mean ± SEM brains from brains of eight *O. degus* for each age group. Significantly different in 3-year-old $^*p < 0.01$ compared to 1-year-old *O. degus*. **(C)** Table of absolute values mean ± SEM.

were shown to occur parallel to loss of myelin, indicative of significant white matter degeneration in that same study (van Groen et al., 2011). Hippocampal tau deposits have also been detected in post-mortem AD brain tissue using THK5117, a radiotracer for positron emission tomography (PET) scan imaging of tau deposits (Lemoine et al., 2015). Moreover, white matter abnormalities have been shown to not only represent an early pathological event in AD, but may also be involved in Aβ aggregation and tau hyperphosphorylation (Sachdev et al., 2013). Taken together, we can postulate that amyloid and tau deposition are age-dependent processes in *O. degus*, as also occurs in clinical AD patients.

Abnormal metal deposition have also been reported in the APP/V717I transgenic mouse model and wild-type age-matched mice as controls using LA-ICPMS (Wang et al., 2012). However, very few studies using LA-ICPMS as a tool for biometal analysis, have been performed in the brain of "physiologically" aged rodent models. One study performed demonstrated a significant increase in Fe levels in the substantia nigra, the thalamus, and the CA1 region of the hippocampus of 14-month-old mice compared to 2-month-old mice (Becker et al., 2010). As iron is well known to form free radicals due to its high redox active properties, increased cerebral Fe levels may potentiate neurodegeneration. The same study showed that the levels of Zn remained unchanged with age. The latter supports the essential role of Zn as a major neurotransmitter that can be stored in vesicules. Another study showed that the global cerebral content of Cu was increased in 14-month-old mice compared to 2-month-old mice. However, reduced Cu uptake was noted in the striatum and ventral cortex in aged mice, which were associated with the highest decline in cerebral superoxide dismutase-1 (SOD-1). This further suggests that dysregulated Cu homeostasis may contribute to oxidative stress and neurodegeneration in the aging brain (Wang et al., 2010).

However, the current study is the first to identify specific age-related changes in biometal trafficking and transition metal distribution in *O. degus*, a potential "natural" model for AD. It is well established that deposition of the redox elements Fe and Cu



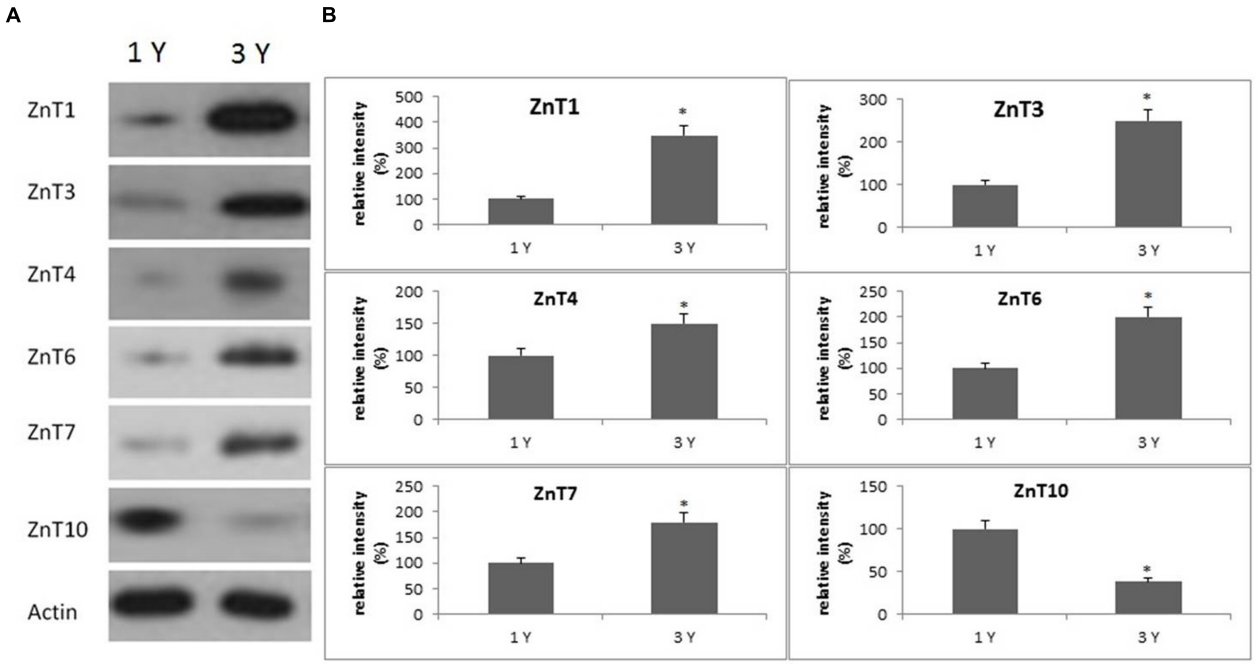
C

ZIP Protein	1 years (relative intensity, %)	3 years (relative intensity, %)	T value	p value
Zip7	100±10.0	25±3.1	3.5	0.008
Zip8	100±10.0	45±5.2	2.6	0.009
Zip14	100±10.0	40±3.9	3.1	0.008

FIGURE 6 | Changes in the expression of ZIP protein in the frontal cortex with age. (A) Altered levels of ZIP7,8 and ZIP14 were reported in the brain after 3 years of age compared to 1-year-old *O. degus*. The blots shown are representative data from an experiment repeated eight times. **(B)** Graphs are mean ± SEM brains from brains of eight *O. degus* for each age group. Significantly different in 3-year-old $^*p < 0.01$ compared to 1-year-old *O. degus*. **(C)** Table of absolute values mean ± SEM.

contribute to Aβ-induced toxicity in the AD brain (Lovell et al., 1998; Bush, 2003; Bush et al., 2003; Matusch et al., 2010). Evidence shows the redox cycling of Cu contributes to the oxidative stress of AD (Burdo and Connor, 2003; Chen and Nguyen, 2014; Hancock et al., 2014; McCord and Aizenman, 2014). Although evidence for altered Cu levels in serum are conflicting, Cu has been found to be significantly dysregulated in patients with mild cognitive impairment (MCI) who subsequently progressed to dementia (Mueller et al., 2012). Furthermore, the levels of Cu have been shown to be increased with age in the hippocampus and cortex of APP/V717I transgenic mice (Wang et al., 2012). Moreover, the ratio of Cu/Fe as well as Cu levels may be a useful biomarker to distinguish between progressive and cognitively stable MCI patients (Mueller et al., 2012). This ratio is consistent with a previous study which examined the ratio of ceruloplasmin and transferrin (the major protein carriers of Cu and Fe, respectively) in serum from early AD cases: ceruloplasmin was increased while transferrin was decreased (Squitti et al., 2002). However, an earlier study supported an increase in serum ceruloplasmin, but challenged the decrease in serum transferrin in AD (Molaschi et al., 1996).

We also observed a significant increase in the levels of Fe in aged *O. degus*. This is in line with previous findings in the APP/V717I transgenic mouse model, and age-matched mice as controls using LA-ICPMS (Wang et al., 2012), suggesting that Fe is age-dependently enriched in the brain. The increased Fe level is likely due to the constant transportation of Fe to the brain mediated by capillary endothelial cells, and increased release of Fe into the blood *via* the blood–brain barrier (Burdo and Connor, 2003; Burdo et al., 2003). While Fe was found mainly enriched in the substantia nigra and corpus callosum of APP mice (Wang et al., 2012), Fe was primarily found in the substantia nigra and cortical area of age-matched controls. However, several studies have shown that Fe is enriched in plaques, and the hippocampus and frontal cortex are the main plaque deposition regions (Burdo and Connor, 2003; Leskovjan et al., 2009). Therefore, increased Fe content in the hippocampal and cortical areas of the *O. degus* is suggestive of defective metal transport in some micro-regions of the AD brain. Quantitative results from a previous study using LA-ICPMS show an increased tendency for Fe in the hippocampi of both APP and age-matched controls, suggesting regional variations in Fe reactions in the brain (Wang et al., 2012). The increased level of Fe in the brain can be attributed to the presence of iron-binding proteins. Ferritin and transferrin, represent the two-most important Fe carriers, and are commonly found in both the white and gray matter (Burdo et al., 1999; Thomas and Jankovic, 2004). Therefore, accumulation of Fe may be due to the functional role of ferritin in the brain of *O. degus*. Additionally, serum levels of Fe in AD patients are inconsistent, and no study has specifically evaluated the levels of non-heme (chelatable) iron (Mueller et al., 2012).



C

ZnT Protein	1 years (relative intensity, %)	3 years (relative intensity, %)	T value	p value
Znt1	100±10.0	350±32.1	3.5	0.008
Znt3	100±10.0	250±23.5	2.8	0.006
Znt4	100±10.0	150±15.0	2.1	0.009
Znt6	100±10.0	200±18.6	2.4	0.009
Znt7	100±10.0	180±16.5	2.5	0.008
Znt10	100±10.0	38±3.5	2.9	0.008

FIGURE 7 | Changes in the expression of ZnT protein in the frontal cortex with age. (A) Altered levels of ZnT1,3,4,6,7, and 10 were reported in the brain after 3-years of age compared to 1-year-old *O. degus*. The blots shown are representative data from an experiment repeated eight times. **(B)** Graphs are mean ± SEM for brains of eight *O. degus* for each age group. Significance $^*p < 0.01$ compared to 1-year-old *O. degus*. **(C)** Table of absolute values mean ± SEM.

As well, Zn is an important redox-active metal that plays an important role in Aβ aggregation and extracellular plaque formation (Watt et al., 2014). Therefore, determining the mechanism(s) of Zn dysregulation in AD and aging is of particular interest. The microdistribution of zinc in aged *O. degus* is remarkably similar to a previous study in the APP/V717I transgenic mouse model (Wang et al., 2012). We observed a significant age-related increase in Zn levels in the cortex and hippocampus, which present as Zn-enriched regions.

Additionally, Ca represents another important metal that plays an important role in normal brain cell function (Lisek et al., 2015). It is well established that increased Aβ can reduce the membrane potential, thus enhancing Ca^{2+} influx, leading to an accumulation of Ca^{2+} in cells, leading to excitotoxicity and cell death via apoptotic processes (Greenamyre, 1991; Harkany et al., 2000; Barger, 2004; Hynd et al., 2004; Tsai et al., 2005; Koutsilieri and Riederer, 2007). Increased oxidative stress may further interfere with Ca immobilization, leading to cell death via additional cytotoxic pathways (Esposito et al., 2013; Ong et al., 2013). It has been previously reported that Ca^{2+} overload can modulate APP metabolism, and accelerate APP hydrolysis to generate more Aβ (Wang et al., 1994; Ye et al., 2010). Our study and others have shown that Ca increases with age in the brain, and provides further evidence supporting the hypothesis of the imbalance of calcium homeostasis and disturbed Ca flux in the brain can enhance AD progression (Peterson et al., 1985, 1989; Deary and Hendrickson, 1986; Martyn et al., 1989; Kelliher et al., 1999; O'Day and Myre, 2004; Attems et al., 2008).

Numerous studies have shown that Al may also contribute to the pathology of AD (Martyn, 1992; Copestake, 1993; Doll, 1993; Kruck, 1993; Rifat, 1993; Taylor et al., 1995). A collective body of published work indicates that: (1) Al accumulates in microtubules and induces cortical atrophy *in vivo* (Garruto and Brown, 1994), (2) Al may be involved in the formation of extracellular plaques and NFTs (Mera, 1991; McLachlan et al., 1992; Perl, 2006); (3) Increased incidence of AD has been observed in populations where there are high levels of Al in drinking water supplies (French et al., 1989; Martyn, 1992; Harrington et al., 1994; Polizzi et al., 2002; Gupta et al., 2005; Perl, 2006); (4) Al chelators have shown positive responses for the treatment of AD in transgenic rodent models (Guy et al., 1991; Justin Thenmozhi et al., 2015); and (5) Increased Al deposition is also associated with

the pathogenesis of other neurodegenerative diseases including Parkinson's disease and amyotrophic lateral sclerosis (Bharathi et al., 2008; Singla and Dhawan, 2014). While our study shows that Al is increased with age in the cortex, no other study has reported changes in Al distribution in the brain in other AD models, and it is still unclear whether Al levels are elevated in human AD patients. Thus, further studies are warranted to elucidate the association between Al levels and AD.

The expression of cellular trafficking systems plays a critical role in metal-induced toxicity. Abnormalities in the lysosomal pathway have been previously reported in AD pathology, prior to the development of NFTs or Aβ plaques (Cataldo and Nixon, 1990; Cataldo et al., 1994). Since proteolytic processing of APP is necessary for the formation of Aβ, lysosomal proteases have been linked with AD pathology (Vidoni et al., 2016). The increased expression of cathepsin D reported in this study may represent a compensatory mechanism to restore lysosomal function (Perez et al., 2015). This is supported by another study which showed that knockout of cathepsin D enhances abnormal tau phosphorylation, and oxidative stress in the brain (Khurana et al., 2010). Cathepsin D also has an important role of maintaining neuronal homeostasis, and optimal function of cathepsin D is therefore necessary for the clearance of unfolded/oxidized protein that are transported to lysosomes to facilitate their removal (Benes et al., 2008). Similarly, vesicular ATPases are required to acidify lysosomes and maintain an optimal acidic environment for lysosomes (Williamson et al., 2010). One study showed that degradation or reduced expression of V-ATPase can enhance AD pathology by making neurons more susceptible to insult following exposure to pathological concentrations of Aβ and tau (Williamson et al., 2010). Therefore, the observed changes in cathepsin D and V-ATPase may provide an additional mechanism for the failed protein degradation, abnormal accumulation of pathological hallmarks of AD, including Aβ (which was increased in this study), and age-related neurodegeneration.

Mutations, altered expression or aberrant functionality of several metal transporters belonging to the ATP7, ATP13, TRMPL, and ZnT families have also been implicated in neurodegenerative disorders (Lovell et al., 2005; Lyubartseva et al., 2010; Quadri et al., 2012; Yonova-Doing et al., 2012). Increased ATP13a2 expression has previously been shown to attenuate lysosomal dysfunction in fibroblasts derived from PD patients (Dehay et al., 2012a,b). Therefore, it is likely that downregulation of ATP13a2 in aged *O. degus* may impair lysosomal function. Neurodegenerative processes involving aberrant cellular metal trafficking may be intricately linked at the molecular level.

While Cu/Fe dysregulation may represent a useful biomarker for AD, on its own it is not conclusive. We analyzed the expression of several proteins, including: IRP2, a central regulator of intracellular iron and copper metabolism, and a downstream target of IRP2, the IRE-containing gene DMT1, FOS, and ALAS2 in the frontal cortex of *O. degus*. Alterations in the expression of regulatory proteins associated with the metabolism and transport of Cu and Fe suggest that homeostasis of these redox active metals is dysregulated in *O. degus*, and contributes to the age-related changes in the microdistribution of Cu and Fe (Mueller et al., 2012).

In the brain, Zn is transported by several members of the ZIP and ZnT family of transporters. A significant reduction in members of the ZIP family of transporters was observed in our study. In particular, our results are the first to show an age-related decline in the expression of ZIP7,8 and 14 in the brain of aged *O. degus*. It is likely that the loss of ZIP expression may stimulate perturbations in the metabolism of other redox-active metals (Dang et al., 2014; Grubman et al., 2014a,b). Excess unbound Zn may displace other redox-active transition metals from metalloproteins, and further promote formation of highly volatile free-radicals via Fenton chemistry (Wilson et al., 2012). Increased free Zn may also inhibit protein tyrosine phosphatases in the endoplasmic reticulum, inhibiting the propagation of "Zn wave" signals throughout cells, thus impairing pleiotropic cellular signaling processes important for cellular survival (Taylor et al., 2012).

Previous studies have reported upregulation of ZnT1, ZnT3, ZnT4, ZnT5, ZnT6, and ZnT7 protein isoforms in the hippocampus and neocortex in APP/PS1 mice (Lovell et al., 2005; Smith et al., 2006; Zhang et al., 2010). Similarly, we have shown that ZnT1, ZnT3, ZnT4, ZnT6, and ZnT7 are increased with age. On the other hand, a previous study showing that ZnT3 mRNA and protein levels are reduced in the human AD brain (Beyer et al., 2009). Two earlier studies have shown that ZnT1 is decreased significantly in the hippocampus of subjects with MCI (a stage from which 30% progress to develop dementia) and pre-clinical AD (PCAD; where subjects have no overt clinical manifestations of AD but pathology is revealed post-mortem) (Lovell et al., 2005), as well as in AD. Moreover, while ZnT4 and ZnT6 were elevated in PCAD and in the hippocampus of AD patients, no significant difference in levels was reported for either transporter between MCI and age-matched controls (Lyubartseva et al., 2010). ZnT3 is the main Zn transport protein and a major contributor to Zn^{2+} release from the synaptic cleft to Zn-enriched terminals, and may also be responsible for the abnormal distribution of Zn in the brain (Palmiter et al., 1996). Under normal physiological conditions, the levels of unbound Zn are regulated by metallothionein, and the endogenous antioxidant enzyme, SOD. However, under pathological conditions, Zn may remain unbound over a significant length of time, and may therefore interact with Aβ and enhance plaque deposition (Bosomworth et al., 2012).

Little information is known about the functional role of Zn10 in AD. A recent study has shown that ZnT10 expression is upregulated in post-mortem AD brain tissue (Bosomworth et al., 2013). By contrast, our results show that ZnT10 expression is reduced in the frontal cortex of aged *O. degus*. Under physiological conditions, ZnT10 is localized to the Golgi apparatus, and maybe translocated to the plasma membrane following an increase in Zn levels leading to a down-regulation of ZnT10 expression (Bosomworth et al., 2013). Therefore, it is possible that the increase in Zn may reduce ZnT10 expression early in disease course as reported in this study. Reduced expression of ZnT10 may exacerbate AD progression effluxing Zn

into the extracellular space, and providing Zn ions to facilitate Aβ deposition and senile plaque formation.

Our current study has utilized LA-ICPMS and neuromolecular biology techniques to examine the microdistribution of redox-active transition metals, and biometal trafficking pathways in the brain of aging *O. degus*. Since age is the greatest risk factor for AD, our present findings provide a rationale for further utilization of the metallomic approach in research and drug development to extend lifespan and maintain healthspan.

AUTHOR CONTRIBUTIONS

NB, AP, TJ, CM, and PS wrote the draft, reviewed and interpreted the bioimages. HR, AR, and B-EJ processed the images. NI and PS provided the conceptual foundation of the research, writing of drafts, and interpretation of data. Animal tissue was obtained from NI's laboratory.

FUNDING

This work was supported by a National Health and Medical Research Council of Australia Capacity Building Grant and a UNSW Faculty of Medicine Research Grant. NB is the recipient of an Alzheimer's Australia Viertel Foundation Postdoctoral Research Fellowship at the University of New South Wales. TJ is a recipient of the University of New South Wales Postgraduate Award (UPA).

ACKNOWLEDGMENTS

The authors thank the Rebecca Cooper Medical Research Foundation for their ongoing financial support and staff of the Bioanalytical Mass Spectrometry Facility at the University of New South Wales for assistance with GC-MS experiments. H&E sections were prepared by Ms Fei Shang.

REFERENCES

Andrasi, E., Farkas, E., Scheibler, H., Reffy, A., and Bezur, L. (1995). Al, Zn, Cu, Mn and Fe levels in brain in Alzheimer's disease. *Arch. Gerontol. Geriatr.* 21, 89–97. doi: 10.1016/0167-4943(95)00643-Y

Ardiles, A. O., Tapia-Rojas, C. C., Mandal, M., Alexandre, F., Kirkwood, A., Inestrosa, N. C., et al. (2012). Postsynaptic dysfunction is associated with spatial and object recognition memory loss in a natural model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 109, 13835–13840. doi: 10.1073/pnas.1201209109

Armstrong, A., Mattsson, N., Appelqvist, H., Janefjord, C., Sandin, L., Agholme, L., et al. (2014). Lysosomal network proteins as potential novel CSF biomarkers for Alzheimer's disease. *Neuromolecular Med.* 16, 150–160. doi: 10.1007/s12017-013-8269-3

Attems, J., Preusser, M., Grosinger-Quass, M., Wagner, L., Lintner, F., and Jellinger, K. (2008). Calcium-binding protein secretagogin-expressing neurones in the human hippocampus are largely resistant to neurodegeneration in Alzheimer's disease. *Neuropathol. Appl. Neurobiol.* 34, 23–32.

Baddeley, A. D., Bressi, S., Della Sala, S., Logie, R., and Spinnler, H. (1991). The decline of working memory in Alzheimer's disease. A longitudinal study. *Brain* 114(Pt 6), 2521–2542. doi: 10.1093/brain/114.6.2521

Barger, S. W. (2004). An unconventional hypothesis of oxidation in Alzheimer's disease: intersections with excitotoxicity. *Front. Biosci.* 9:3286–3295. doi: 10.2741/1481

Becker, J. S., Kumtabtim, U., Wu, B., Steinacker, P., Otto, M., and Matusch, A. (2012). Mass spectrometry imaging (MSI) of metals in mouse spinal cord by laser ablation ICP-MS. *Metallomics* 4, 284–288. doi: 10.1039/c2mt00166g

Becker, J. S., Matusch, A., Palm, C., Salber, D., Morton, K. A., and Becker, J. S. (2010). Bioimaging of metals in brain tissue by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) and metallomics. *Metallomics* 2, 104–111. doi: 10.1039/b916722f

Benes, P., Vetvicka, V., and Fusek, M. (2008). Cathepsin D-Many functions of one aspartic protease. *Crit. Rev. Oncol. Hematol.* 68, 12–28. doi: 10.1016/j.critrevonc.2008.02.008

Beyer, N., Coulson, D. T., Heggarty, S., Ravid, R., Irvine, G. B., Hellemans, J., et al. (2009). ZnT3 mRNA levels are reduced in Alzheimer's disease post-mortem brain. *Mol. Neurodegener.* 4:53. doi: 10.1186/1750-1326-4-53

Bharathi, Vasudevaraju, P., Govindaraju, M., Palanisamy, A. P., Sambamurti, K., and Rao, K. S. (2008). Molecular toxicity of aluminium in relation to neurodegeneration. *Indian J. Med. Res.* 128, 545–556.

Bonda, D. J., Lee, H. G., Blair, J. A., Zhu, X., Perry, G., and Smith, M. A. (2011). Role of metal dyshomeostasis in Alzheimer's disease. *Metallomics* 3, 267–270. doi: 10.1039/c0mt00074d

Borchardt, T., Schmidt, C., Camarkis, J., Cappai, R., Masters, C. L., Beyreuther, K., et al. (2000). Differential effects of zinc on amyloid precursor protein (APP) processing in copper-resistant variants of cultured Chinese hamster ovary cells. *Cell Mol. Biol.* 46, 785–795.

Bosomworth, H. J., Adlard, P. A., Ford, D., and Valentine, R. A. (2013). Altered expression of ZnT10 in Alzheimer's disease brain. *PLoS ONE* 8:e65475. doi: 10.1371/journal.pone.0065475

Bosomworth, H. J., Thornton, J. K., Coneyworth, L. J., Ford, D., and Valentine, R. A. (2012). Efflux function, tissue-specific expression and intracellular trafficking of the Zn transporter ZnT10 indicate roles in adult Zn homeostasis. *Metallomics* 4, 771–779. doi: 10.1039/c2mt20088k

Braidy, N., Munoz, P., Palacios, A. G., Castellano-Gonzalez, G., Inestrosa, N. C., Chung, R. S., et al. (2012). Recent rodent models for Alzheimer's disease: clinical implications and basic research. *J. Neural Transm.* 119, 173–195. doi: 10.1007/s00702-011-0731-5

Braidy, N., Poljak, A., Jayasena, T., Mansour, H., Inestrosa, N. C., and Sachdev, P. S. (2015). Accelerating Alzheimer's research through 'natural' animal models. *Curr. Opin. Psychiatry* 28, 155–164. doi: 10.1097/YCO.0000000000000137

Braidy, N., Poljak, A., Marjo, C., Rutlidge, H., Rich, A., Jayasena, T., et al. (2014). Metal and complementary molecular bioimaging in Alzheimer's disease. *Front. Aging Neurosci.* 6:138. doi: 10.3389/fnagi.2014.00138

Brun, A., and Englund, E. (2002). Regional pattern of degeneration in Alzheimer's disease: neuronal loss and histopathological grading. *Histopathology* 41, 40–55.

Burdo, J. R., Antonetti, D. A., Wolpert, E. B., and Connor, J. R. (2003). Mechanisms and regulation of transferrin and iron transport in a model blood-brain barrier system. *Neuroscience* 121, 883–890. doi: 10.1016/S0306-4522(03)00590-6

Burdo, J. R., and Connor, J. R. (2003). Brain iron uptake and homeostatic mechanisms: an overview. *Biometals* 16, 63–75. doi: 10.1023/A:1020718718550

Burdo, J. R., Martin, J., Menzies, S. L., Dolan, K. G., Romano, M. A., Fletcher, R. J., et al. (1999). Cellular distribution of iron in the brain of the Belgrade rat. *Neuroscience* 93, 1189–1196. doi: 10.1016/S0306-4522(99)00207-9

Bush, A. I. (2003). The metallobiology of Alzheimer's disease. *Trends Neurosci.* 26, 207–214. doi: 10.1016/S0166-2236(03)00067-5

Bush, A. I., Masters, C. L., and Tanzi, R. E. (2003). Copper, beta-amyloid, and Alzheimer's disease: tapping a sensitive connection. *Proc. Natl. Acad. Sci. U.S.A.* 100, 11193–11194. doi: 10.1073/pnas.2135061100

Cataldo, A. M., Hamilton, D. J., and Nixon, R. A. (1994). Lysosomal abnormalities in degenerating neurons link neuronal compromise to senile plaque development in Alzheimer-disease. *Brain Res.* 640, 68–80. doi: 10.1016/0006-8993(94)91858-9

Cataldo, A. M., and Nixon, R. A. (1990). Enzymatically active lysosomal proteases are associated with amyloid deposits in Alzheimer brain. *Proc. Natl. Acad. Sci. U.S.A.* 87, 3861–3865. doi: 10.1073/pnas.87.10.3861

Chen, M., and Nguyen, H. T. (2014). Our "energy-Ca(2(+) signaling deficits" hypothesis and its explanatory potential for key features of Alzheimer's disease. *Front. Aging Neurosci.* 6:329. doi: 10.3389/fnagi.2014.00329

Coffey, E. E., Beckel, J. M., Laties, A. M., and Mitchell, C. H. (2014). Lysosomal alkalization and dysfunction in human fibroblasts with the Alzheimer's disease-linked presenilin 1 A246E mutation can be reversed with cAMP. *Neuroscience* 263, 111–124. doi: 10.1016/j.neuroscience.2014.01.001

Colonnello, V., Iacobucci, P., Fuchs, T., Newberry, R. C., and Panksepp, J. (2011). Octodon degus. A useful animal model for social-affective neuroscience research: basic description of separation distress, social attachments and play. *Neurosci. Biobehav. Rev.* 35, 1854–1863. doi: 10.1016/j.neubiorev.2011.03.014

Copestake, P. (1993). Aluminium and Alzheimer's disease–an update. *Food Chem. Toxicol.* 31, 679–683. doi: 10.1016/0278-6915(93)90052-Z

da Silva, M. A., and Arruda, M. A. (2013). Laser ablation (imaging) for mapping and determining Se and S in sunflower leaves. *Metallomics* 5, 62–67. doi: 10.1039/c2mt20154b

Dang, T. N., Lim, N. K., Grubman, A., Li, Q. X., Volitakis, I., White, A. R., et al. (2014). Increased metal content in the TDP-43(A315T) transgenic mouse model of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Front. Aging Neurosci.* 6:15. doi: 10.3389/fnagi.2014.00015

De Sole, P., Rossi, C., Chiarpotto, M., Ciasca, G., Bocca, B., Alimonti, A., et al. (2013). Possible relationship between Al/ferritin complex and Alzheimer's disease. *Clin. Biochem.* 46, 89–93. doi: 10.1016/j.clinbiochem.2012.10.023

Deary, I. J., and Hendrickson, A. E. (1986). Calcium and Alzheimer's disease. *Lancet* 1, 1219. doi: 10.1016/s0140-6736(86)91205-5

Dehay, B., Martinez-Vicente, M., Ramirez, A., Perier, C., Klein, C., Vila, M., et al. (2012a). Lysosomal dysfunction in Parkinson disease: ATP13A2 gets into the groove. *Autophagy* 8, 1389–1391. doi: 10.4161/auto.21011

Dehay, B., Ramirez, A., Martinez-Vicente, M., Perier, C., Canron, M. H., Doudnikoff, E., et al. (2012b). Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration. *Proc. Natl. Acad. Sci. U.S.A.* 109, 9611–9616. doi: 10.1073/pnas.1112368109

Doll, R. (1993). Review: Alzheimer's disease and environmental aluminium. *Age Ageing* 22, 138–153. doi: 10.1093/ageing/22.2.138

Du, L. Y., Chang, L. Y., Ardiles, A. O., Tapia-Rojas, C., Araya, J., Inestrosa, N. C., et al. (2015). Alzheimer's disease-related protein expression in the retina of *Octodon degus*. *PLoS ONE* 10:e0135499. doi: 10.1371/journal.pone.0135499

Esposito, Z., Belli, L., Toniolo, S., Sancesario, G., Bianconi, C., and Martorana, A. (2013). Amyloid beta, glutamate, excitotoxicity in Alzheimer's disease: are we on the right track? *CNS Neurosci. Ther.* 19, 549–555. doi: 10.1111/cns.12095

Faux, N. G., Ritchie, C. W., Gunn, A., Rembach, A., Tsatsanis, A., Bedo, J., et al. (2010). PBT2 rapidly improves cognition in Alzheimer's disease: additional phase II analyses. *J. Alzheimers Dis.* 20, 509–516. doi: 10.3233/JAD-2010-1390

Feaga, H. A., Maduka, R. C., Foster, M. N., and Szalai, V. A. (2011). Affinity of Cu+ for the copper-binding domain of the amyloid-beta peptide of Alzheimer's disease. *Inorg. Chem.* 50, 1614–1618. doi: 10.1021/ic100967s

Finefrock, A. E., Bush, A. I., and Doraiswamy, P. M. (2003). Current status of metals as therapeutic targets in Alzheimer's disease. *J. Am. Geriatr. Soc.* 51, 1143–1148. doi: 10.1046/j.1532-5415.2003.51368.x

French, P., Gardner, M. J., and Gunn, A. M. (1989). Dietary aluminium and Alzheimer's disease. *Food Chem. Toxicol.* 27, 495–498. doi: 10.1016/0278-6915(89)90040-9

Garruto, R. M., and Brown, P. (1994). Tau protein, aluminium, and Alzheimer's disease. *Lancet* 343, 989. doi: 10.1016/S0140-6736(94)90119-8

Graham, S. F., Nasaruddin, M. B., Carey, M., Holscher, C., McGuinness, B., Kehoe, P. G., et al. (2014). Age-associated changes of brain copper, iron, and zinc in Alzheimer's disease and dementia with Lewy bodies. *J. Alzheimers Dis.* 42, 1407–1413. doi: 10.3233/JAD-140684

Greenamyre, J. T. (1991). Neuronal bioenergetic defects, excitotoxicity and Alzheimer's disease: use it and lose it. *Neurobiol. Aging* 12, 334–336; discussion 352–335. doi: 10.1016/0197-4580(91)90012-9

Grubman, A., Lidgerwood, G. E., Duncan, C., Bica, L., Tan, J. L., Parker, S. J., et al. (2014a). Deregulation of subcellular biometal homeostasis through loss of the metal transporter, Zip7, in a childhood neurodegenerative disorder. *Acta Neuropathol. Commun.* 2:25. doi: 10.1186/2051-5960-2-25

Grubman, A., White, A. R., and Liddell, J. R. (2014b). Mitochondrial metals as a potential therapeutic target in neurodegeneration. *Br. J. Pharmacol.* 171, 2159–2173. doi: 10.1111/bph.12513

Gupta, V. B., Anitha, S., Hegde, M. L., Zecca, L., Garruto, R. M., Ravid, R., et al. (2005). Aluminium in Alzheimer's disease: are we still at a crossroad? *Cell Mol. Life Sci.* 62, 143–158.

Guy, S. P., Jones, D., Mann, D. M., and Itzhaki, R. F. (1991). Human neuroblastoma cells treated with aluminium express an epitope associated with Alzheimer's disease neurofibrillary tangles. *Neurosci. Lett.* 121, 166–168. doi: 10.1016/0304-3940(91)90676-K

Halliwell, B. (1989). Oxidants and the central nervous system: some fundamental questions. Is oxidant damage relevant to Parkinson's disease, Alzheimer's disease, traumatic injury or stroke?. *Acta Neurol Scand. Suppl.* 126, 23–33. doi: 10.1111/j.1600-0404.1989.tb01779.x

Hancock, S. M., Finkelstein, D. I., and Adlard, P. A. (2014). Glia and zinc in ageing and Alzheimer's disease: a mechanism for cognitive decline? *Front. Aging Neurosci.* 6:137. doi: 10.3389/fnagi.2014.00137

Harkany, T., Penke, B., and Luiten, P. G. (2000). beta-Amyloid excitotoxicity in rat magnocellular nucleus basalis. Effect of cortical deafferentation on cerebral blood flow regulation and implications for Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 903, 374–386. doi: 10.1111/j.1749-6632.2000.tb06389.x

Harrington, C. R., Wischik, C. M., McArthur, F. K., Taylor, G. A., Edwardson, J. A., and Candy, J. M. (1994). Alzheimer's-disease-like changes in tau protein processing: association with aluminium accumulation in brains of renal dialysis patients. *Lancet* 343, 993–997. doi: 10.1016/S0140-6736(94)90124-4

Hynd, M. R., Scott, H. L., and Dodd, P. R. (2004). Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochem. Int.* 45, 583–595. doi: 10.1016/j.neuint.2004.03.007

Inestrosa, N. C., Reyes, A. E., Chacon, M. A., Cerpa, W., Villalon, A., Montiel, J., et al. (2005). Human-like rodent amyloid-beta-peptide determines Alzheimer pathology in aged wild-type *Octodon degu*. *Neurobiol. Aging* 26, 1023–1028. doi: 10.1016/j.neurobiolaging.2004.09.016

Inestrosa, N. C., Rios, J. A., Cisternas, P., Tapia-Rojas, C., Rivera, D. S., Braidy, N., et al. (2015). Age progression of neuropathological markers in the brain of the chilean rodent *Octodon degus*, a natural model of Alzheimer's disease. *Brain Pathol.* 25, 679–691. doi: 10.1111/bpa.12226

Joachim, C. L., Morris, J. H., Selkoe, D. J., and Kosik, K. S. (1987). Tau epitopes are incorporated into a range of lesions in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* 46, 611–622. doi: 10.1097/00005072-198711000-00001

Justin Thenmozhi, A., Raja, T. R., Janakiraman, U., and Manivasagam, T. (2015). Neuroprotective effect of hesperidin on aluminium chloride induced Alzheimer's disease in Wistar rats. *Neurochem. Res.* 40, 767–776. doi: 10.1007/s11064-015-1525-1

Kelliher, M., Fastbom, J., Cowburn, R. F., Bonkale, W., Ohm, T. G., Ravid, R., et al. (1999). Alterations in the ryanodine receptor calcium release channel correlate with Alzheimer's disease neurofibrillary and beta-amyloid pathologies. *Neuroscience* 92, 499–513. doi: 10.1016/S0306-4522(99)00042-1

Khachaturian, Z. S. (1985). Diagnosis of Alzheimer's disease. *Arch. Neurol.* 42, 1097–1105. doi: 10.1001/archneur.1985.04060100083029

Khurana, V., Elson-Schwab, I., Fulga, T. A., Sharp, K. A., Loewen, C. A., Mulkearns, E., et al. (2010). Lysosomal dysfunction promotes cleavage and neurotoxicity of Tau In Vivo. *PLoS Genet.* 6:e1001026. doi: 10.1371/journal.pgen.1001026

Koutsilieri, E., and Riederer, P. (2007). Excitotoxicity and new antiglutamatergic strategies in Parkinson's disease and Alzheimer's disease. *Parkinsonism Relat. Disord.* 13(Suppl. 3), S329–S331. doi: 10.1016/S1353-8020(08)70025-7

Kruck, T. P. (1993). Aluminium–Alzheimer's link? *Nature* 363, 119. doi: 10.1038/363119a0

Lemoine, L., Saint-Aubert, L., Marutle, A., Antoni, G., Eriksson, J. P., Ghetti, B., et al. (2015). Visualization of regional tau deposits using H-3-THK5117 in Alzheimer brain tissue. *Acta Neuropathol. Commun.* 3, 40. doi: 10.1186/s40478-015-0220-4

Leskovjan, A. C., Lanzirotti, A., and Miller, L. M. (2009). Amyloid plaques in PSAPP mice bind less metal than plaques in human Alzheimer's disease. *Neuroimage* 47, 1215–1220. doi: 10.1016/j.neuroimage.2009.05.063

Lisek, M., Boczek, T., Ferenc, B., and Zylinska, L. (2015). Regional brain dysregulation of Ca-handling systems in ketamine-induced rat model of experimental psychosis. *Cell Tissue Res.* 363, 609–620. doi: 10.1007/s00441-015-2332-3

Lovell, M. A., Robertson, J. D., Teesdale, W. J., Campbell, J. L., and Markesbery, W. R. (1998). Copper, iron and zinc in Alzheimer's disease senile plaques. *J. Neurol. Sci.* 158, 47–52. doi: 10.1016/S0022-510X(98)00092-6

Lovell, M. A., Smith, J. L., Xiong, S., and Markesbery, W. R. (2005). Alterations in zinc transporter protein-1 (ZnT-1) in the brain of subjects with mild cognitive impairment, early, and late-stage Alzheimer's disease. *Neurotox. Res.* 7, 265–271. doi: 10.1007/BF03033884

Lyubartseva, G., Smith, J. L., Markesbery, W. R., and Lovell, M. A. (2010). Alterations of zinc transporter proteins ZnT-1, ZnT-4 and ZnT-6 in preclinical Alzheimer's disease brain. *Brain Pathol.* 20, 343–350. doi: 10.1111/j.1750-3639.2009.00283.x

Makela, M., Paetau, A., Polvikoski, T., Myllykangas, L., and Tanskanen, M. (2016). Capillary amyloid-beta protein deposition in a population-based study (Vantaa 85+). *J. Alzheimers Dis.* 49, 149–157. doi: 10.3233/JAD-150241

Martyn, C. N. (1992). The epidemiology of Alzheimer's disease in relation to aluminium. *Ciba Found Symp.* 169, 69–79; discussion 79–86.

Martyn, C. N., Singh, S., and Wood, P. J. (1989). Calcium metabolism in Alzheimer's disease. A case-control study. *Gerontology* 35, 153–157.

Matusch, A., Depboylu, C., Palm, C., Wu, B., Hoglinger, G. U., Schafer, M. K., et al. (2010). Cerebral bioimaging of Cu, Fe, Zn, and Mn in the MPTP mouse model of Parkinson's disease using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). *J. Am. Soc. Mass Spectrom.* 21, 161–171. doi: 10.1016/j.jasms.2009.09.022

Maynard, C. J., Cappai, R., Volitakis, I., Cherny, R. A., White, A. R., Beyreuther, K., et al. (2002). Overexpression of Alzheimer's disease amyloid-beta opposes the age-dependent elevations of brain copper and iron. *J. Biol. Chem.* 277, 44670–44676. doi: 10.1074/jbc.M204379200

McCord, M. C., and Aizenman, E. (2014). The role of intracellular zinc release in aging, oxidative stress, and Alzheimer's disease. *Front. Aging Neurosci.* 6:77. doi: 10.3389/fnagi.2014.00077

McLachlan, D. R., Fraser, P. E., and Dalton, A. J. (1992). Aluminium and the pathogenesis of Alzheimer's disease: a summary of evidence. *Ciba Found Symp.* 169, 87–98; discussion 99–108.

Mera, S. L. (1991). Aluminium, amyloid, and Alzheimer's disease. *Med. Lab Sci.* 48, 283–295.

Mirra, S. S., Heyman, A., McKeel, D., Sumi, S. M., Crain, B. J., Brownlee, L. M., et al. (1991). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 41, 479–486. doi: 10.1212/WNL.41.4.479

Molaschi, M., Ponzetto, M., Bertacna, B., Berrino, E., and Ferrario, E. (1996). Determination of selected trace elements in patients affected by dementia. *Arch. Gerontol. Geriatr.* 22(Suppl. 1), 39–42. doi: 10.1016/0167-4943(96)86910-X

Mueller, C., Schrag, M., Crofton, A., Stolte, J., Muckenthaler, M. U., Magaki, S., et al. (2012). Altered serum iron and copper homeostasis predicts cognitive decline in mild cognitive impairment. *J. Alzheimers Dis.* 29, 341–350. doi: 10.3233/JAD-2011-111841

O'Day, D. H., and Myre, M. A. (2004). Calmodulin-binding domains in Alzheimer's disease proteins: extending the calcium hypothesis. *Biochem. Biophys. Res. Commun.* 320, 1051–1054. doi: 10.1016/j.bbrc.2004.06.070

Ong, W. Y., Tanaka, K., Dawe, G. S., Ittner, L. M., and Farooqui, A. A. (2013). Slow excitotoxicity in Alzheimer's disease. *J. Alzheimers Dis.* 35, 643–668.

Osterholt, T., Salber, D., Matusch, A., Becker, J. S., and Palm, C. (2011). IMAGENA: image generation and analysis – an interactive software tool handling LA-ICPMS data. *Int. J. Mass Spectrom.* 307, 232–239. doi: 10.1016/j.ijms.2011.03.010

Palmiter, R. D., Cole, T. B., Quaife, C. J., and Findley, S. D. (1996). ZnT-3, a putative transporter of zinc into synaptic vesicles. *Proc. Natl. Acad. Sci. U.S.A.* 93, 14934–14939. doi: 10.1073/pnas.93.25.14934

Parvathy, S., Turner, A. J., and Hooper, N. M. (2000). Inhibition of alpha-secretase by zinc metalloproteinase inhibitors. *Methods Mol. Med.* 32, 203–215. doi: 10.1385/1-59259-195-7:203

Perez, S. E., He, B., Nadeem, M., Wuu, J., Ginsberg, S. D., Ikonomovic, M. D., et al. (2015). Hippocampal endosomal, lysosomal, and autophagic dysregulation in mild cognitive impairment: correlation with a beta and tau pathology. *J. Neuropathol. Exp. Neurol.* 74, 345–358. doi: 10.1097/NEN.0000000000000179

Perl, D. P. (2006). Exposure to aluminium and the subsequent development of a disorder with features of Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 77:811. doi: 10.1136/jnnp.2006.090613

Peterson, C., Gibson, G. E., and Blass, J. P. (1985). Altered calcium uptake in cultured skin fibroblasts from patients with Alzheimer's disease. *N. Engl. J. Med.* 312, 1063–1065. doi: 10.1056/NEJM198504183121618

Peterson, C., Ratan, R., Shelanski, M., and Goldman, J. (1989). Changes in calcium homeostasis during aging and Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 568, 262–270. doi: 10.1111/j.1749-6632.1989.tb12515.x

Polizzi, S., Pira, E., Ferrara, M., Bugiani, M., Papaleo, A., Albera, R., et al. (2002). Neurotoxic effects of aluminium among foundry workers and Alzheimer's disease. *Neurotoxicology* 23, 761–774. doi: 10.1016/S0161-813X(02)00097-9

Quadri, M., Federico, A., Zhao, T., Breedveld, G. J., Battisti, C., Delnooz, C., et al. (2012). Mutations in SLC30A10 cause parkinsonism and dystonia with hypermanganesemia, polycythemia, and chronic liver disease. *Am. J. Hum. Genet.* 90, 467–477. doi: 10.1016/j.ajhg.2012.01.017

Rembach, A., Hare, D. J., Doecke, J. D., Burnham, S. C., Volitakis, I., Fowler, C. J., et al. (2014). Decreased serum zinc is an effect of ageing and not Alzheimer's disease. *Metallomics* 6, 1216–1219. doi: 10.1039/c4mt00060a

Rifat, S. L. (1993). Alzheimer's disease and environmental aluminium. *Age Ageing* 22, 476–477. doi: 10.1093/ageing/22.6.476

Sachdev, P. S., Zhuang, L., Braidy, N., and Wen, W. (2013). Is Alzheimer's a disease of the white matter? *Curr. Opin. Psychiatry* 26, 244–251. doi: 10.1097/YCO.0b013e32835ed6e8

Selkoe, D. J., Bell, D. S., Podlisny, M. B., Price, D. L., and Cork, L. C. (1987). Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. *Science* 235, 873–877. doi: 10.1126/science.3544219

Sensi, S. L., Paoletti, P., Bush, A. I., and Sekler, I. (2009). Zinc in the physiology and pathology of the CNS. *Nat. Rev. Neurosci.* 10, 780–791. doi: 10.1038/nrn2734

Singla, N., and Dhawan, D. K. (2014). Influence of zinc on calcium-dependent signal transduction pathways during aluminium-induced neurodegeneration. *Mol. Neurobiol.* 50, 613–625. doi: 10.1007/s12035-014-8643-7

Smith, D. G., Cappai, R., and Barnham, K. J. (2007). The redox chemistry of the Alzheimer's disease amyloid beta peptide. *Biochim. Biophys. Acta* 1768, 1976–1990. doi: 10.1016/j.bbamem.2007.02.002

Smith, J. L., Xiong, S., Markesbery, W. R., and Lovell, M. A. (2006). Altered expression of zinc transporters-4 and -6 in mild cognitive impairment, early and late Alzheimer's disease brain. *Neuroscience* 140, 879–888. doi: 10.1016/j.neuroscience.2006.02.049

Squitti, R., Lupoi, D., Pasqualetti, P., Dal Forno, G., Vernieri, F., Chiovenda, P., et al. (2002). Elevation of serum copper levels in Alzheimer's disease. *Neurology* 59, 1153–1161. doi: 10.1212/WNL.59.8.1153

Taddeo, M. A., Lee, S., and Shea, T. B. (2014). Synergistic inhibition of synaptic signaling in cortical cultures by subcytotoxic levels of oligomerized amyloid-beta and iron: alleviation by zinc. *J. Alzheimers Dis.* 41, 365–369. doi: 10.3233/JAD-132696

Taylor, G. A., Newens, A. J., Edwardson, J. A., Kay, D. W., and Forster, D. P. (1995). Alzheimer's disease and the relationship between silicon and aluminium in water supplies in northern England. *J. Epidemiol. Commun. Health* 49, 323–324. doi: 10.1136/jech.49.3.323

Taylor, K. M., Hiscox, S., Nicholson, R. I., Hogstrand, C., and Kille, P. (2012). Protein kinase CK2 triggers cytosolic zinc signaling pathways by phosphorylation of zinc channel ZIP7. *Sci. Signal.* 5, ra11. doi: 10.1126/scisignal.2002585

Teri, L., Borson, S., Kiyak, H. A., and Yamagishi, M. (1989). Behavioral disturbance, cognitive dysfunction, and functional skill. Prevalence and relationship in Alzheimer's disease. *J. Am. Geriatr. Soc.* 37, 109–116. doi: 10.1111/j.1532-5415.1989.tb05868.x

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., et al. (1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann. Neurol.* 30, 572–580. doi: 10.1002/ana.410300410

Thomas, M., and Jankovic, J. (2004). Neurodegenerative disease and iron storage in the brain. *Curr. Opin. Neurol.* 17, 437–442. doi: 10.1097/01.wco.0000137534.61244.d1

Tsai, V. W., Scott, H. L., Lewis, R. J., and Dodd, P. R. (2005). The role of group I metabotropic glutamate receptors in neuronal excitotoxicity in Alzheimer's disease. *Neurotox. Res.* 7, 125–141. doi: 10.1007/BF03033782

Uekita, T., and Okanoya, K. (2011). Hippocampus lesions induced deficits in social and spatial recognition in *Octodon degus*. *Behav. Brain Res.* 219, 302–309. doi: 10.1016/j.bbr.2011.01.042

van Groen, T., Kadish, I., Popovic, N., Popovic, M., Caballero-Bleda, M., Bano-Otalora, B., et al. (2011). Age-related brain pathology in Octodon degu: blood vessel, white matter and Alzheimer-like pathology. *Neurobiol. Aging* 32, 1651–1661. doi: 10.1016/j.neurobiolaging.2009.10.008

Vidoni, C., Follo, C., Savino, M., Melone, M. A. B., and Isidoro, C. (2016). The role of cathepsin D in the pathogenesis of human neurodegenerative disorders. *Med. Res. Rev.* 36, 845–870. doi: 10.1002/med.21394

Wang, G. T., Ladror, U. S., Holzman, T. F., Klein, W. L., and Krafft, G. A. (1994). Cleavage of fluorogenic substrates for APP-processing proteases by human brain extracts. Ca2(+)-substrate interaction is responsible for Ca2(+) stimulation of the neural protease activity. *Mol. Chem. Neuropathol.* 23, 191–199. doi: 10.1007/BF02815411

Wang, H. J., Wang, M., Wang, B., Li, M., Chen, H. Q., Yu, X. H., et al. (2012). The distribution profile and oxidation states of biometals in APP transgenic mouse brain: dyshomeostasis with age and as a function of the development of Alzheimer's disease. *Metallomics* 4, 289–296. doi: 10.1039/c2mt00104g

Wang, L. M., Becker, J. S., Wu, Q., Oliveira, M. F., Bozza, F. A., Schwager, A. L., et al. (2010). Bioimaging of copper alterations in the aging mouse brain by autoradiography, laser ablation inductively coupled plasma mass spectrometry and immunohistochemistry. *Metallomics* 2, 348–353. doi: 10.1039/c003875j

Watt, N. T., Griffiths, H. H., and Hooper, N. M. (2014). Lipid rafts: linking prion protein to zinc transport and amyloid-beta toxicity in Alzheimer's disease. *Front. Cell Dev. Biol.* 2:41. doi: 10.3389/fcell.2014.00041

Wilkinson, N., and Pantopoulos, K. (2014). The IRP/IRE system in vivo: insights from mouse models. *Front. Pharmacol.* 5:176. doi: 10.3389/fphar.2014.00176

Williamson, W. R., Wang, D., Haberman, A. S., and Hiesinger, P. R. (2010). A dual function of V0-ATPase a1 provides an endolysosomal degradation mechanism in *Drosophila melanogaster* photoreceptors. *J. Cell Biol.* 189, 885–899. doi: 10.1083/jcb.201003062

Wilson, M., Hogstrand, C., and Maret, W. (2012). Picomolar concentrations of free zinc(II) ions regulate receptor protein-tyrosine phosphatase beta activity. *J. Biol. Chem.* 287, 9322–9326. doi: 10.1074/jbc.C111.320796

Xue, X., Wang, L. R., Sato, Y., Jiang, Y., Berg, M., Yang, D. S., et al. (2014). Single-walled carbon nanotubes alleviate autophagic/lysosomal defects in primary glia from a mouse model of Alzheimer's disease. *Nano Lett.* 14, 5110–5117. doi: 10.1021/nl501839q

Ye, H., Jalini, S., Mylvaganam, S., and Carlen, P. (2010). Activation of large-conductance Ca(2+)-activated K(+) channels depresses basal synaptic transmission in the hippocampal CA1 area in APP (swe/ind) TgCRND8 mice. *Neurobiol. Aging* 31, 591–604. doi: 10.1016/j.neurobiolaging.2008.05.012

Yonova-Doing, E., Atadzhanov, M., Quadri, M., Kelly, P., Shawa, N., Musonda, S. T., et al. (2012). Analysis of LRRK2, SNCA, Parkin, PINK1, and DJ-1 in Zambian patients with Parkinson's disease. *Parkinsonism Relat. Disord.* 18, 567–571. doi: 10.1016/j.parkreldis.2012.02.018

Yuan, Y., Niu, F., Liu, Y., and Lu, N. (2014). Zinc and its effects on oxidative stress in Alzheimer's disease. *Neurol. Sci.* 35, 923–928. doi: 10.1007/s10072-014-1668-x

Zhang, L. H., Wang, X., Zheng, Z. H., Ren, H., Stoltenberg, M., Danscher, G., et al. (2010). Altered expression and distribution of zinc transporters in APP/PS1 transgenic mouse brain. *Neurobiol. Aging* 31, 74–87. doi: 10.1016/j.neurobiolaging.2008.02.018

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

CORRECTION
published: 15 May 2017
doi: 10.3389/fnagi.2017.00134

Corrigendum: Identification of Cerebral Metal Ion Imbalance in the Brain of Ageing *Octodon degus*

*Nady Braidy[1], Anne Poljak[1,2,3], Chris Marjo[2], Helen Rutlidge[2], Anne Rich[2], Bat-Erdene Jugder[4], Tharusha Jayasena[1], Nibaldo C. Inestrosa[1,5] and Perminder S. Sachdev[1,6]**

[1] Centre for Healthy Brain Ageing, School of Psychiatry, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia, [2] Mark Wainwright Analytical Centre, University of New South Wales, Sydney, NSW, Australia, [3] School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia, [4] School of Biotechnology and Biomolecular Sciences, Faculty of Science, University of New South Wales, Sydney, NSW, Australia, [5] Centre for Ageing and Regeneration, Faculty of Biological Sciences, Pontifical Catholic University of Chile, Santiago, Chile, [6] Neuropsychiatric Institute, Euroa Centre, Prince of Wales Hospital, Sydney, NSW, Australia

Keywords: LA-ICPMS, metals, Alzheimer's disease, bioimaging, *Octodon degus*

A corrigendum on

Identification of Cerebral Metal Ion Imbalance in the Brain of Aging *Octodon degus*
by Braidy, N., Poljak, A., Marjo, C., Rutlidge, H., Rich, A., Jugder, B.-E. (2017). Front. Aging Neurosci. 9:66. doi: 10.3389/fnagi.2017.00066

OPEN ACCESS

Edited and reviewed by:
Agustin Ibanez, Institute of Cognitive and Translational Neuroscience, Argentina

****Correspondence:***
Perminder S. Sachdev
p.sachdev@unsw.edu.au

Received: *19 April 2017*
Accepted: *21 April 2017*
Published: *15 May 2017*

Citation:
Braidy N, Poljak A, Marjo C, Rutlidge H, Rich A, Jugder B-E, Jayasena T, Inestrosa NC and Sachdev PS (2017) Corrigendum: Identification of Cerebral Metal Ion Imbalance in the Brain of Ageing Octodon degus. Front. Aging Neurosci. 9:134. doi: 10.3389/fnagi.2017.00134

Due to a misunderstanding, the *O. degus* brain specimen used in the study were obtained from the animal facility of the Pontificia Universidad Catholic University of Chile, not the animal facility of the University of Valparaiso as previously stated in the article.

Therefore, in the animal methods section, the correct paragraph should read:

Octodon degus were obtained from a breeding colony at the animal facility of the Pontifical Catholic University of Chile and maintained in a controlled temperature room (23 ± 1°C), under a 12:12 h light/dark cycle, with water and food provided *ad libitum*. At the time of this study, 16 male *O. degus* were grouped by age, from 12 to 36 months of age ($n = 8$ per group). Ages were selected to represent the development of AD-like pathology (36 months). All efforts were made to minimize animal discomfort and stress while also limiting the number of animals used. Aged animals were anesthetized with Equitesin (2.5 ml/kg, i.p.) and injected with heparin (4 USP/kg, i.p.). Afterward, brains were surgically removed from their skulls and frozen in isopentane at −78.5°C. All procedures were conducted according to animal protocols approved by the Institutional Animal Care and Use Committee at the Pontifical Catholic University of Chile.

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: 20 December 2016
doi: 10.3389/fnagi.2016.00310

Early Cognitive/Social Deficits and Late Motor Phenotype in Conditional Wild-Type TDP-43 Transgenic Mice

*Julio A. Alfieri, Pablo R. Silva and Lionel M. Igaz **

IFIBIO Houssay, Grupo de Neurociencia de Sistemas, Facultad de Medicina, Universidad de Buenos Aires - CONICET, Buenos Aires, Argentina

Frontotemporal Dementia (FTD) and amyotrophic lateral sclerosis (ALS) are two neurodegenerative diseases associated to mislocalization and aggregation of TAR DNA-binding protein 43 (TDP-43). To investigate in depth the behavioral phenotype associated with this proteinopathy, we used as a model transgenic (Tg) mice conditionally overexpressing human wild-type TDP 43 protein (hTDP-43-WT) in forebrain neurons. We previously characterized these mice at the neuropathological level and found progressive neurodegeneration and other features that evoke human TDP-43 proteinopathies of the FTD/ALS spectrum. In the present study we analyzed the behavior of mice at multiple domains, including motor, social and cognitive performance. Our results indicate that young hTDP-43-WT Tg mice (1 month after post-weaning transgene induction) present a normal motor phenotype compared to control littermates, as assessed by accelerated rotarod performance, spontaneous locomotor activity in the open field test and a mild degree of spasticity shown by a clasping phenotype. Analysis of social and cognitive behavior showed a rapid installment of deficits in social interaction, working memory (Y-maze test) and recognition memory (novel object recognition test) in the absence of overt motor abnormalities. To investigate if the motor phenotype worsen with age, we analyzed the behavior of mice after long-term (up to 12 months) transgene induction. Our results reveal a decreased performance on the rotarod test and in the hanging wire test, indicating a motor phenotype that was absent in younger mice. In addition, long-term hTDP-43-WT expression led to hyperlocomotion in the open field test. In sum, these results demonstrate a time-dependent emergence of a motor phenotype in older hTDP-43-WT Tg mice, recapitulating aspects of clinical FTD presentations with motor involvement in human patients, and providing a complementary animal model for studying TDP-43 proteinopathies.

Keywords: TDP-43, frontotemporal dementia, amyotrophic lateral sclerosis, transgenic mice, behavior, animal model, proteinopathy

OPEN ACCESS

Edited by:
Agustin Ibanez, Institute of Cognitive and Translational Neuroscience, Argentina

Reviewed by:
Emanuele Buratti, International Centre for Genetic Engineering, Italy
Christine Vande Velde, Université de Montréal, Canada

****Correspondence:***
Lionel M. Igaz
lmuller@fmed.uba.ar

Received: *22 September 2016*
Accepted: *06 December 2016*
Published: *20 December 2016*

Citation:
Alfieri JA, Silva PR and Igaz LM (2016) Early Cognitive/Social Deficits and Late Motor Phenotype in Conditional Wild-Type TDP-43 Transgenic Mice. Front. Aging Neurosci. 8:310. doi: 10.3389/fnagi.2016.00310

INTRODUCTION

Many neurodegenerative diseases are associated with characteristic changes in the behavioral profile of affected individuals. For example, frontotemporal dementia (FTD) comprises a group of clinical syndromes unified by underlying frontotemporal lobar degeneration (FTLD) pathology, which leads to disorders of behavior, language and executive function (Woollacott and Rohrer, 2016).

FTD is the second most common form of dementia after Alzheimer's disease in those under 65 years of age, and is characterized by progressive degeneration of frontal and anterior temporal lobes. In amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder that progressively affects upper and lower motor neurons, the primary symptoms are associated with motor function deficits (Morris, 2015). Recently, there has been a growing body of literature demonstrating a clinical and neuropathological overlap between FTD and ALS, disorders which can be viewed as representations of the extremes of a disease spectrum (Ferrari et al., 2011).

Several neurodegenerative diseases display TAR DNA-binding protein 43 (TDP-43) pathology and this protein was identified as the main component of the distinctive cytoplasmic aggregates seen in the vast majority of ALS cases and about half of the cases of FTD (FTLD-TDP; Neumann et al., 2006; Baralle et al., 2013). These and other neurodegenerative disorders with the presence of aggregated TDP-43 are now collectively referred to as "TDP-43 proteinopathies" (Kwong et al., 2008). Although it is clearly not possible to faithfully model every clinicopathological feature of the FTD/ALS spectrum in rodents, transgenic (Tg) mice have been shown to recapitulate major aspects of human FTD and ALS. These include animal models based on the genetic manipulation of Tau, TDP-43, SOD1 and C9ORF72, among others, each one displaying specific but partial features of these diseases (Roberson, 2012; Philips and Rothstein, 2015).

Behavioral phenotyping has been particularly challenging since these models often display multiple abnormalities, which can be heavily influenced by the gene, mutation and/or promoter used for the genetic manipulation (Vernay et al., 2016a). In particular, TDP-43 mouse models have been rapidly developed over the last few years, usually showing clear signs of neurodegeneration and other histopathological changes typical of the human disease (Liu et al., 2013; Picher-Martel et al., 2016). In terms of behavioral abnormalities, most TDP-43 rodent models display early motor deficits, frequently associated with the use of pan-neuronal, constitutive promoters affecting developmental milestones or proper motor function, conceivably masking other phenotypic domains. A subset of models have used promoters that allow restricted expression in terms of timing and/or cellular subgrouping. Among these, we have previously developed and characterized TDP-43 Tg mice with inducible, forebrain enriched neuronal expression using a CamKIIα promoter coupled to a tTA system (Igaz et al., 2011). These mice express either nuclear (TDP-43-WT) or cytoplasmic (TDP-43-ΔNLS) forms of the protein, and they recapitulated several aspects of TDP-43 proteinopathies, including time-dependent neuronal loss, gliosis, corticospinal tract degeneration and global changes in gene expression (Igaz et al., 2011). Subsequent in-depth behavioral analysis of inducible TDP-43-ΔNLS mice demonstrated an early establishment of motor, cognitive and social abnormalities (Alfieri et al., 2014), of relevance since these three behavioral domains are affected in patients with different presentations within the clinicopathological spectrum of FTD/ALS (Giordana et al., 2011; Seltman and Matthews, 2012; Gordon, 2013).

In this work, we sought to thoroughly study the behavioral changes in our inducible TDP-43-WT mouse model. Post-weaning 1 month induction of the Tg caused early deficits in social behavior, a characteristic early symptom of FTD patients (Shinagawa et al., 2006; Harciarek and Cosentino, 2013). A battery of cognitive tasks revealed early alterations in object recognition and working memory tests, while aversive memory was spared. Remarkably, motor tests demonstrated preserved function in the open field, rotarod and hanging wire tests at 1 month of induction, with only a mild clasping phenotype. Finally, we studied the time course of motor behavior up to 12 months of Tg induction and showed a gradual, time-dependent installment of motor phenotypes as evidenced by impaired performance in the rotarod and hanging wire test, and emergence of hyperlocomotion in the open field test. These results reveal different sensitivities to TDP-43-WT expression in the brain circuits underlying social/cognitive and motor behavior.

MATERIALS AND METHODS

Animals

The experimental protocol for this study was approved by the National Animal Care and Use Committee of the University of Buenos Aires (CICUAL). hTDP-43 Tg lines were generated by injection of linearized moPrP-tetP vector containing human wild-type TDP 43 protein (hTDP-43-WT) cDNA into pronucleus of fertilized eggs from C57BL/6J × C3HeJ F1 matings. Monogenic tetO-TDP-WT12 mice were bred to Camk2a-tTA mice (Mayford et al., 1996; Jackson Laboratory) generating non-Tg (nTg), tTA monogenic, single tetO-TDP-43 Tg mice (non-TDP-43 expressing control mice) and bigenic mice expressing hTDP-43-WT12 (hereinafter referred to as tTA/WT12).

Breeding mice and pups were treated with 0.2 mg/ml Dox (Doxycycline Hyclate, sc-204734A, Santa Cruz Biotechnology) in drinking water, in order to circumvent potential prenatal and postnatal developmental effects of Tg expression. Induction of hTDP-43 was achieved by switching mice to regular drinking water (without Dox) at weaning (postnatal day 28) and mice were analyzed at different time points (**Figure 1A**).

Genomic DNA isolated from ear biopsies were screened for the presence of the Tg by means of PCR amplification with the following primers: TDP-forward (TTG GTAATAGCAGAGGGGGTGGAG), MoPrP-reverse (TCCCCC AGCCTAGACCACGAGAAT), Camk2a-tTA-forward (CGCT GTGGGGCATTTTACTTTAG) and Camk2a-tTA-reverse (CA TGTCCAGATCGAAATCGTC) as previously described (Igaz et al., 2011; Alfieri et al., 2014). The TDP-43-WT12 Tg line used in these experiments was established by crossbreeding with C57BL/6J mice for 7–8 generations to homogenize genetic background and minimize variability. Both Tg and control animals of either sex were included in the experiments performed in the different age groups studied.

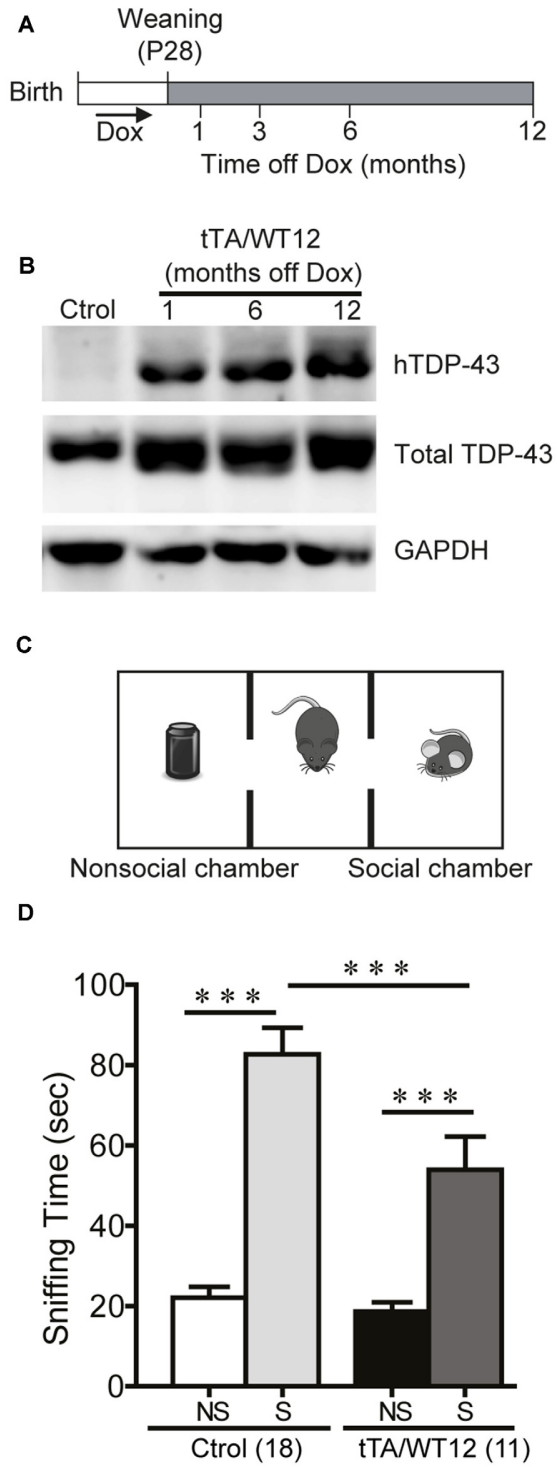


FIGURE 1 | Altered social behavior in TDP-43-WT transgenic (Tg) mice. **(A)** Experimental design: transgene expression was activated at weaning (postnatal day 28) by removing Dox from water. The behavioral responses of these Tg mice were analyzed at the indicated time points after weaning. **(B)** Expression of human TAR DNA-binding protein 43 (TDP-43) in Tg mice. Immunoblot of hTDP-43 or total TDP-43 (h+mTDP-43) in cortical RIPA extracts of control (non-Tg) and tTA/WT12 (1, 6 or 12 months off Dox) (*Continued*)

FIGURE 1 | Continued
mice. GAPDH is a loading control. **(C)** Schematic view of the three-chamber social interaction apparatus, consisting of a black Plexiglas rectangular box with three interconnected chambers. **(D)** Time spent sniffing the social (S; P21-P28 mouse) or the non–social (NS; black plastic object) stimulus during a 10 min session (test phase) was recorded. 1 month off Dox bigenic mice (tTA/WT12) presented a reduced social interaction time during the session (***$p < 0.001$, one-way ANOVA/Newman-Keuls *post hoc* test). Number of animals is indicated in parentheses. The data represent mean ± SEM.

Behavioral Studies

Mice were kept on a 12 h light/dark cycle under controlled conditions of temperature (23 ± 2°C) and humidity (40%–60%), with *ad libitum* access to food and water. All behavioral tasks were performed during the light phase (lights on 7 am; lights off 7 pm) with the exception of the Y-maze spontaneous alternation, which was conducted during the initial dark phase (7:00 p.m. to 9:00 p.m.) to maximize exploratory behavior and consistently obtain a high number of entries. All sessions were video recorded through a camera mounted above the arena (unless noticed) and mouse position was determined by automatic video tracking (ANY-maze, Stoelting Co). The animals were allowed to habituate in the experimental room (with attenuated light and sound) for at least 1 h prior to the tests. The objects, floor and walls of the mazes used in behavioral analysis were cleaned with ethanol 10% between sessions.

Similarly to what we previously demonstrated in experiments with TDP-43-NLS Tg mice (Alfieri et al., 2014), all non-bigenic offspring (nTg and both single Tg mice) exhibited similar behavioral responses. Thus, for all subsequent behavioral tests and other experimental analysis we grouped these genotypes under the Control group to compare against the Bigenic mice.

Social Interaction Test

This task was performed on a three-chamber box as previously described (Brodkin et al., 2004; Alfieri et al., 2014). Briefly, the test apparatus consisted of a black Plexiglas rectangular box (40.6 cm × 15 cm × 23 cm) with three interconnected chambers, placed under dim light (25 lux). Prior to the start of each test, one of the end chambers was randomly designated the "nonsocial side" and the other the "social side". During the habituation phase, two clear Plexiglas cylinders with multiple holes were placed in the apparatus, one in each end chamber. Animals (test mice) were placed in the central chamber and allowed to explore the whole apparatus during 5 min. After habituation a stimulus mouse (21–28 days old C57BL/6J male mouse) was placed into the cylinder on the side that had been designated the "social side" and a black plastic object was placed into the cylinder on the "nonsocial side" (nonsocial stimulus). Mice have a natural tendency to explore a novel conspecific over a novel object. The test mouse was able to freely explore the apparatus for 10 min (test phase). Time spent sniffing the social and nonsocial stimuli and time spent in each chamber was measured. Clean bedding was placed on the apparatus floor prior to the next test.

Novel Object Recognition

To analyze the impact of TDP-43-WT12 expression on recognition memory, we used the novel object recognition test. During habituation sessions, animals were introduced into an empty Plexiglas arena (40 cm × 23 cm × 15 cm) for 10 min during the first session (day 1) and two 5 min sessions during the second day. On the third day (training) the mice were exposed to two identical objects placed at opposite ends of the arena for 10 min. On test day (24 h later), the mice were allowed to explore one copy of the previously presented object (familiar) and a new object (novel) for 5 min. The time spent exploring the two objects was scored by an experimenter using the video recorded sessions. Exploration was defined as pointing the head toward an object at a distance of <2 cm from the object, with its neck extended and vibrissae moving. Turning around and sitting on the objects were not considered exploratory behaviors. The exploration time represents the percentage of time that mice spend exploring the object (familiar or novel) respect to the total exploration time (familiar + novel), as previously described (Alfieri et al., 2014).

Y-maze Spontaneous Alternation Test

A Y maze with three identical arms made of transparent Plexiglas (43 cm × 4 cm × 12.5 cm) placed at 120° angles to each other was used (Belforte et al., 2010; Alfieri et al., 2014) and placed in a room with clues to allow for visual orientation. Illumination was kept at 30 lux. Each mouse was placed at the end of one arm facing the center and allowed to explore the maze freely for 8 min without training, reward or punishment, while the experimenter remained out of sight. Entries into each arm were scored and alternation behavior was defined as a complete cycle of consecutive entrances into each of the three arms without repetition. The percentage of spontaneous alternation was defined as the number of actual alternations divided by the possible alternations [(# alternations)/(total arm entries − 2) × 100]. Total entries were scored as an index of ambulatory activity in the Y maze and mice with scores below 12 were excluded.

Step-Through Inhibitory (Passive) Avoidance

Inhibitory avoidance behavior was studied in a one-trial learning, step-through type situation which utilizes the natural preference of mice for a dark environment. The apparatus consists of two Plexiglas boxes (20 cm × 16 cm × 21 cm), one made of white Plexiglas and the other one of black Plexiglas, establishing two contiguous compartments connected through a sliding door. A stainless-steel grid floor spanned both compartments. The task was performed as previously described (Alfieri et al., 2014), with some modifications. Briefly, each mouse was placed during training in the light chamber for 60 s, then the door between the chambers was opened. The mouse received a footshock (0.2 mA, 50 Hz, 1 s) as it stepped into the dark compartment as described previously. Training latency was measured after door opening. Retention test was performed 24 h later. Each mouse was placed on the white compartment again (with the door open), and the step-through latency was recorded with a 300 s cutoff per session. In the retention test session the footshock was omitted.

Open Field

Assessment of general exploratory locomotion in a novel environment (open field test) was performed as previously described (Alfieri et al., 2014). Mice were placed in a clear Plexiglas (40 cm × 40 cm × 40 cm) arena with white floor divided into two zones: periphery and center (comprising 50% of the total area centered). Horizontal locomotor activity was assessed during 20 min. The open field arena was placed in the center of the room (1.8 m × 2 m) and illumination was kept at 50 lux. Total, peripheral and center distance traveled by the mice was quantified. Time bin analysis (every 5 min) was used.

Accelerated Rotarod

A rotarod apparatus (Ugo Basile, model 7600) was used to measure motor coordination and balance (Alfieri et al., 2014). For the accelerating rotarod test (4–40 rpm over 300 s) four trials per test were carried out during the test day, with a 2 min interval between trials. The latency to fall off the rotarod was recorded. Mice that rotated passively were scored as fallen.

Clasping Phenotype

hTDP-43-WT12 Tg mice as well as age-matched control mice were suspended by the tail 30 cm above an open cage for 30 s. Mice were slowly lowered toward the bottom of the cage, and a positive response was recorded for mice that clasped their limbs within 5 s of suspension while maintaining the clasping posture until lowered to the cage (Igaz et al., 2011; Alfieri et al., 2014).

Hanging Wire Grip Test

Grip strength was assessed using the hanging wire test, which was performed as described (Alfieri et al., 2014). Briefly, the mouse was placed on the top of a wire cage lid, then the lid was shaken lightly three times to cause the mouse to grip the wires and then the lid was turned upside down and held at a height approximately 20 cm above a cage containing fresh bedding. The latency to fall off the wire lid was quantified. A 60 s cutoff time was used.

Elevated Plus-Maze

Anxiety-like behavior was assessed as described (Braz et al., 2015) using an elevated plus maze consisting of two open arms (30 cm × 6 cm × 0.3 cm) and two closed arms (30 cm × 6 cm × 15 cm) with opaque walls. The apparatus was elevated 40 cm above the floor, and the duration of the test was 5 min. The maze was placed in the center of a homogenously illuminated room (2 m × 1.8 m; 100 lux across arms). At the beginning of the test, mice were placed in the central square facing the open arm opposite to the investigator. Number of open arm entries, percentage of time in open arms, total arm entries and total distance traveled was measured.

Visual Perception

Visual perception was evaluated in the visual cliff test as previously described (Alfieri et al., 2014). Briefly, a box with a simulated ledge was used and illumination was kept at 100 lux. The surface of the box (30 cm × 30 cm) and ledge (60 cm high) were covered with a black and white checkerboard pattern (2.5 cm × 2.5 cm) to emphasize the ledge dropoff. A piece of

clear Plexiglas spans the ledge, resulting in the visual appearance of a cliff. The behavior was scored as "positive" when the mouse stopped at the virtual edge before attempting to cross. Visually impaired animals walk across the Plexiglas without stopping, and percentage of animals stopping at the edge was quantified. Vibrissae were removed to eliminate tactile placing responses.

Brain Tissue Collection

After deep anesthesia with 5% chloral hydrate (1 ml/30 g), the mice were perfused transcardially with ice-cold PBS (0.1 M), pH 7.4 supplemented with 10 U/ml Heparin. The brains were immediately extracted and dissected into different areas including cerebral cortex, and then immediately frozen on dry ice and kept at −80°C for biochemical analysis.

SDS-PAGE and Immunoblot Analysis

Tissues were extracted with 10 volumes (ml/g tissue) of RIPA buffer (0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 5 mM EDTA, 150 mM NaCl, 50 mM Tris-HCl, pH 8.0) containing protease and phosphatase inhibitor cocktail (Roche), sonicated and centrifuged at 13,000 rpm for 20 min at 4°C. Protein concentrations were determined using the BCA assay kit (Pierce). Equal amounts (15 μg) of samples were subsequently resolved on 10% SDS-PAGE gels and transferred onto PVDF membranes (Immobilon-P, Millipore). Primary antibodies (described in Alfieri et al., 2014) were used as follows: rabbit anti-TDP-43 polyclonal antibody raised to amino acids 394–414 (1:20,000), human specific mouse anti-TDP-43 monoclonal antibody (60019-2, Proteintech, 1:2000) and anti-GAPDH mouse monoclonal antibody (6C5, Advanced ImmunoChemical Inc. 1:3000). Membranes were probed with corresponding secondary antibodies coupled to Alkaline Phosphatase and visualized with enhanced chemifluorescence (ECF, GE Healthcare Life Sciences). GAPDH was used as loading control. The blots were scanned in a Storm 845 PhosphorImager (GE Healthcare Life Sciences).

Statistical Analysis

Data are expressed as mean values ± SEM and statistical analysis of behavioral tests was performed using PRISM 6 (Graph Pad software). Differences were considered to be significant when the probability value was <0.05. The following tests were used as required: Student's *t* test (when comparing only two groups on one behavioral measure); one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison *post hoc* test (when comparing three or more groups); repeated measures ANOVA followed by Bonferroni's multiple comparison *post hoc* test (for accelerated rotarod and open field time segment analysis); Fisher exact test (for clasping analysis). When nonparametric tests were required, Mann-Whitney test (for visual cliff test) and Kruskal-Wallis one-way ANOVA followed by individual Mann-Whitney *U* test (inhibitory avoidance task) were used.

RESULTS

In order to analyze the impact of forebrain specific, neuronal wild-type hTDP-43 expression in multiple behavioral domains, we used previously generated TDP-43 Tg mice with a tet-off system and the CaMKIIα promoter that model aspects of human TDP-43 proteinopathies (Igaz et al., 2011). Tg expression was induced at weaning and animals were analyzed at different times after Dox removal (**Figure 1A**). To assess proper induction of the Tg, we performed immunoblot analysis of human and total (human + mouse) TDP-43 in cortical RIPA fractions (**Figure 1B**), demonstrating robust and sustained expression of hTDP-43 as previously reported for the WT12 line (Igaz et al., 2011).

There were no signs of illness or alterations in the physical appearance of Tg mice (i.e., abnormal growth, posture and gait) that could potentially interfere with behavioral testing. Tg mice displayed normal righting reflex, and a previous analysis of forelimb and hindlimb striated muscles by H&E staining showed no evidence of muscle atrophy (Igaz et al., 2011). The body weight curve of bigenic mice up to 12 months off Dox showed no significant differences compared to control littermates (not shown). All behavioral tests were conducted around 1 month after induction (7–9 weeks old) unless otherwise stated.

Conditional Overexpression of Human TDP-43 in Forebrain Neurons Leads to Impaired Social Behavior in Transgenic Mice

Social disinterest is observed as a recurrent feature of FTD patients (Shinagawa et al., 2006), and this behavior can be modeled in mice. We performed a variation of the three-chamber social interaction test (**Figure 1C**; Alfieri et al., 2014), which has been widely used in autism and FTD models (Yin et al., 2010; Gascon et al., 2014; Vernay et al., 2016a). This test measures the sociability of a mouse when given the choice between interacting with another mouse vs. an inanimate object. TDP-43-WT12 Tg mice showed a significant decrease in the exploration time of the social stimulus (another mouse) compared to control mice, while time exploring the nonsocial stimulus (object) was not different between groups (One-way ANOVA, $F_{(3,54)} = 33.06$, $p < 0.0001$; **Figure 1D**). Importantly, total exploration time in the social side showed non-significant differences, indicating that the decrease in social interaction time is not due to perception of the stimulus as aversive, increased anxiety-like behavior or decreased exploratory drive (254.4 ± 21.3 s vs. 201.3 ± 18.7 s for control and TDP-43-WT12 mice, respectively; $t_{(27)} = 1.717$, $p = 0.0974$). These results show that TDP-43-WT12 mice display sociability deficits.

TDP-43-WT Mice Develop Cognitive Deficits

Although some information is available regarding cognitive performance in FTD/ALS animal models, including those based in TDP-43 manipulation, the information has been highly heterogeneous due to the use of different promoters and variants/mutations of the pathological protein (Philips and Rothstein, 2015; Vernay et al., 2016a). Since (1) Tg expression

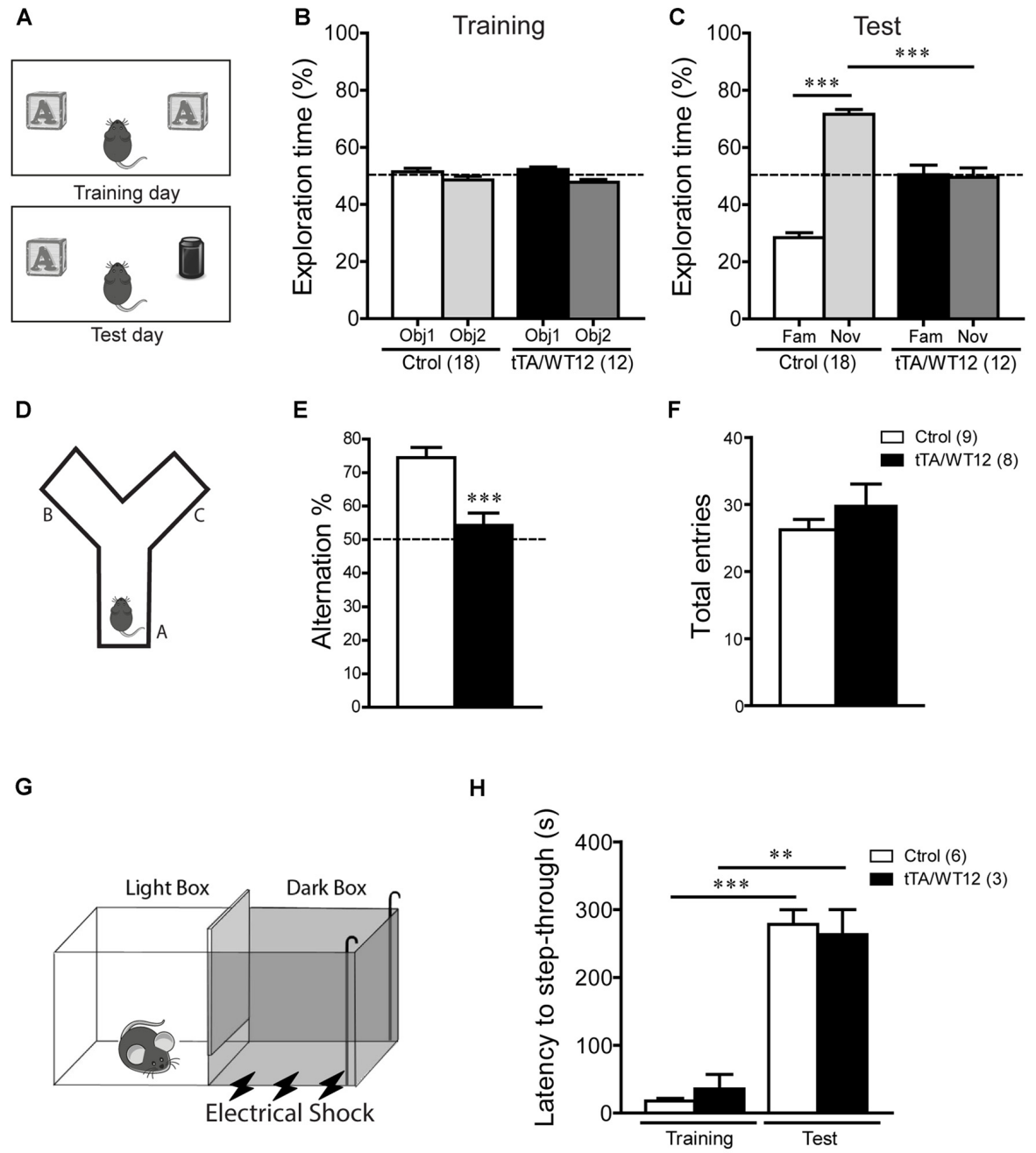


FIGURE 2 | Cognitive impairment in TDP-43-WT Tg mice. (A–C) Novel object recognition test. **(A)** Scheme of training and test phases for the novel object recognition test. **(B)** Training day. Both control and TDP-43-WT Tg mice were exposed to two identical objects for 10 min and the time spent exploring each object was recorded. No significant differences in the exploration time (%) of the two objects were found in training phase. **(C)** Test day. 24 h after training, the recognition memory was measured while the animals were allowed to explore the familiar (Fam) and novel (Nov) objects for 5 min. The exploration time (%) represents the percentage of time that mice spend exploring the object (familiar or novel) respect to the total exploration time (familiar + novel). tTA/WT12 animals displayed a deficit in object recognition memory (***$p < 0.001$, one-way ANOVA/Newman-Keuls *post hoc* test). **(D–F)** Y-maze spontaneous alternation task. **(D)** Scheme of the Y-maze. **(E)** Mice were placed at the end of one arm facing the center and allowed to explore the maze freely for 8 min without training, reward or punishment. Entries into each arm were scored and alternation behavior was defined as a complete cycle of consecutive entrances into each of the three arms without repetition. Bigenic tTA/WT12 mice alternated between the arms at the chance (≈50%) level (***$p < 0.001$ significantly different from control group, Student's *t* test; **F**) Total entries were scored as an index of locomotion activity in the Y maze. **(G,H)** Step-through inhibitory avoidance test. **(G)** Scheme of inhibitory avoidance apparatus. **(H)** During training, each mouse received a footshock (0.2 mA, 50 Hz, 1 s) as it stepped into the dark compartment. Retention test was performed 24 h later. The step-through latency was recorded; footshock was omitted during test session. No significant differences in latency values were found between controls and bigenic animals in either training or test phases, showing intact long term memory for this task (**$p < 0.01$, ***$p < 0.001$ Kruskal–Wallis one-way ANOVA followed by individual Mann-Whitney *U* test). Cognitive behavior was analyzed at 1 month off Dox. Number of animals is indicated in parentheses and data represent mean ± SEM.

is enriched in forebrain neurons; and (2) high expression was observed in the hippocampus and cortex (Igaz et al., 2011), a battery of behavioral tests was used to determine whether the expression of human wild-type TDP-43 influences cognitive performance. Therefore, we set out to evaluate different aspects of cognitive function using novel object recognition, Y-maze and inhibitory avoidance tests.

We first used the novel object recognition test to assess recognition memory (Alfieri et al., 2014), a task that requires intact function of perirhinal and prefrontal cortices (Warburton and Brown, 2010; **Figure 2A**). In this test, where preference for a novel object is evaluated, both control and bigenic mice did not show a preference for any of two identical objects during the training day (**Figure 2B**). However, during test day (24 h later), novel object preference was clearly demonstrated in control mice but completely absent in TDP-43-WT Tg mice (One-way ANOVA, $F_{(3,56)} = 66.0$, $p < 0.0001$; **Figure 2C**). We next used the Y-maze spontaneous alternation test, a prefrontal cortex and hippocampal-dependent task (Lalonde, 2002), to assess spatial working memory (**Figure 2D**). While control animals display the expected alternation values avoiding the previously visited arms, bigenic mice alternated at chance (≈50%) level, indicating impaired working memory ($t_{(15)} = 4.249$ $p = 0.0007$; **Figure 2E**). Locomotion, estimated by the number of arm entries, was similar between groups (**Figure 2F**). Lastly, we performed the inhibitory avoidance task to study aversive memory (**Figure 2G**; Boccia et al., 2004; Alfieri et al., 2014). No difference in step-through latency was observed between groups during the acquisition (training) session, where mice were given a footshock upon entrance to the dark chamber. In a test session performed 24 h later, both bigenic and control animals displayed high latency values (close to the cut-off time), indicating preserved long-term memory for this task (**Figure 2H**). In summary, post-weaning neuronal TDP-43 overexpression impairs cognitive function in some but not all the paradigms tested.

Preserved Motor Function in Young TDP-43-WT Mice

In the FTD/ALS spectrum of clinical presentations, motor deficits are a prominent feature of ALS but only a small fraction of FTD cases show motor abnormalities (Hodges et al., 2004; Seltman and Matthews, 2012). To evaluate this behavioral domain, we performed several tests, including open field, rotarod, hanging wire and clasping analysis.

General motor function and exploratory activity can be evaluated using the open field test. Distance traveled during this task was similar between control and TDP-43-WT groups, suggesting no motor or exploratory abnormalities in our mouse model (**Figure 3A**). In addition, relative center distance was also unaffected (**Figure 3B**). To unmask any potential effect on locomotion not revealed by measuring total values, we divided the traveled distance in time bins and confirmed that this parameter was similar between genotypes across the whole duration of the test (**Figure 3C**).

The accelerated rotarod was used to assess motor coordination and balance. Both groups of mice behaved similarly, although there was a non-significant trend in bigenic mice towards impaired performance (repeated-measures ANOVA, $F_{(1,23)} = 2.707$, $p = 0.1135$ for group; $F_{(3,69)} = 11.04$, $p < 0.0001$ for trial; $F_{(3,69)} = 2.498$, $p = 0.0668$ for interaction; **Figure 4A**). TDP-43-WT mice displayed no change in latency to fall in a hang wire test, suggesting intact grip strength (**Figure 4B**). Conditional expression of TDP-43-WT mainly in the forebrain of bigenic mice resulted in mild (22%) incidence of clasping abnormalities, without reaching significance (two-tailed Fisher exact test, $p = 0.1081$; **Figure 4C**).

It has been previously reported that cognitive and social performance can be negatively affected due to increased anxiety or sensory deficits (Kazdoba et al., 2016). Importantly, we found no abnormalities in visual function as assessed by the visual cliff test (**Figure 5A**). In order to assess both anxiety levels and exploratory activity in TDP-43 WT mice, we evaluated

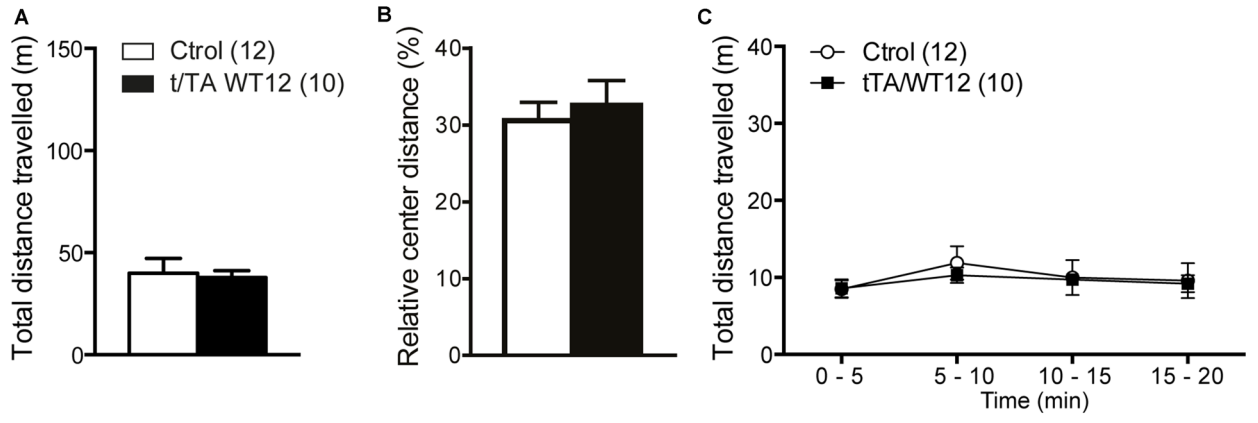


FIGURE 3 | Locomotor and exploratory behavior is preserved in young TDP-43-WT mice. To measure general motor function and exploratory activity we used an open field test. Animals were placed in a novel environment during a 20 min session. **(A)** Total distance traveled, **(B)** relative center distance and **(C)** detailed measurement of total distance traveled in time segments of 5 min. No significant differences were found between controls and bigenic animals in locomotion or exploration ($p > 0.05$, Student's *t* test for **A,B** or repeated-measures ANOVA in **C**). Number of animals is indicated in parentheses. Data represent mean ± SEM.

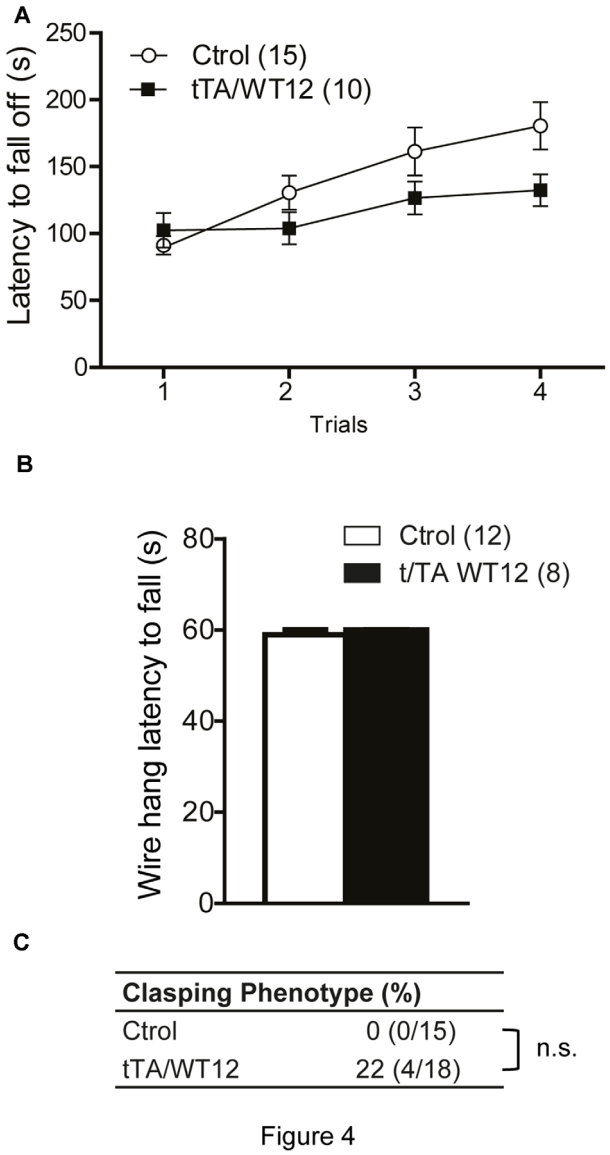


FIGURE 4 | Young TDP-43-WT Tg mice display normal motor coordination, balance and strength. (A) Accelerated rotarod performance (4–40 rpm/5 min). Four trials per test were performed during the test day with a 2 min interval between trials. Latency to fall off the rotarod was recorded. **(B)** Hanging wire grip test. Grip strength was assessed using a standard wire cage turned upside down. The latency to fall off the wire lid was quantified. A 60 s cutoff time was used. No significant differences were found between control and bigenic animals ($p > 0.05$, repeated-measures ANOVA in **A**, Student's *t* test in **B**). **(C)** Percentage of mice with clasping phenotype reveal mild incidence of spasticity, without reaching significance (Fischer Exact test, $p > 0.05$); n.s., non-significant differences respect to control group. Number of animals is indicated in parentheses. Data represent mean ± SEM.

performance in the elevated plus maze test. This task presents a conflict between the natural tendency of mice to explore a novel environment and the aversive properties of a brightly lit, open area (Lister, 1987). TDP-43-WT mice displayed a significant increase in the percentage of open arm entries (Student's *t* test, $t_{(28)} = 2.280$ $p = 0.0304$; **Figures 5B,C**) and a non-significant trend towards increased percentage of time in the open arms (Student's *t* test, $t_{(28)} = 1.459$ $p = 0.1557$, **Figure 5D**), suggesting decreased anxiety-like behavior compared to the control group. Consistent with the lack of altered locomotion in the open field test (**Figures 3A,C**), the total number of arm entries and the total distance traveled did not differ between groups (**Figures 5E,F**). These data argues against an unspecific effect on non-motor behaviors due to increased anxiety or impaired visual function. Additionally, they suggest that TDP-43-WT mice may show disinhibition, as reported in other mouse models for FTD (Yin et al., 2010; Ke et al., 2015; Przybyla et al., 2016).

Time-Dependent Motor Phenotypes Emerge After Prolonged hTDP-43 Overexpression

The early onset of social and cognitive symptoms with essentially intact motor function displayed by these Tg mice prompted us to analyze if there was a time-dependent decline in motor behavior, reflecting different sensitivity for TDP-43-WT overexpression in brain circuits underlying diverse behavioral domains. We analyzed TDP-43-WT and control littermates at 3, 6 and 12 months post induction (off Dox) in the open field test, and the total distance traveled showed a progressive increase starting at 6 months (Student's *t* test, $t_{(14)} = 1.890$ $p = 0.0797$), reaching significance 12 months post Tg induction (**Figure 6A**). Time bin analysis of this parameter also showed a departure from the average levels of control animals after 6 months of Tg expression that did not reach significance, becoming significant at 12 months off Dox (two way repeated-measures ANOVA/Bonferroni's *post hoc* test, $F_{(1,14)} = 7.296$, $p = 0.0172$ for group; $F_{(3,42)} = 2.208$, $p = 0.1012$ for time; $F_{(3,42)} = 2.550$, $p = 0.0685$ for interaction; **Figure 6B**). However, the relative center distance remained unchanged at all the post-induction time points evaluated (**Figure 6C**). In the accelerated rotarod, TDP-43-WT Tg mice demonstrated a poorer performance than controls after 3, 6 and 12 months of Tg induction, displaying a trend toward worse latencies over Tg expression time (two way repeated-measures ANOVA/Bonferroni's *post hoc* test, for 3 months: $F_{(1,22)} = 14.83$, $p = 0.0009$ for group; $F_{(3,66)} = 15.11$, $p < 0.0001$ for time; $F_{(3,66)} = 1.134$, $p = 0.3418$ for interaction; for 6 months: $F_{(1,14)} = 7.720$, $p = 0.0148$ for group; $F_{(3,42)} = 7.153$, $p = 0.0005$ for time; $F_{(3,42)} = 1.164$, $p = 0.3349$ for interaction; for 12 months: $F_{(1,15)} = 14.14$, $p = 0.0018$ for group; $F_{(3,45)} = 11.00$, $p < 0.0001$ for time; $F_{(3,45)} = 1.617$, $p = 0.1987$ for interaction; **Figure 6D**). Lastly, the hanging wire test at 12 months off Dox showed profound deficits in grip strength (Student's *t* test, $t_{(15)} = 6.551$ $p < 0.0001$; **Figure 6E**), which was normal after 1 month of overexpression (**Figure 4B**). As a whole, these results demonstrate a progressive motor phenotype in tTA/WT12 mice.

DISCUSSION

In the present study, we took advantage of a mouse model expressing wild-type hTDP-43 under a system that allows for temporal and regional control of Tg expression. In this

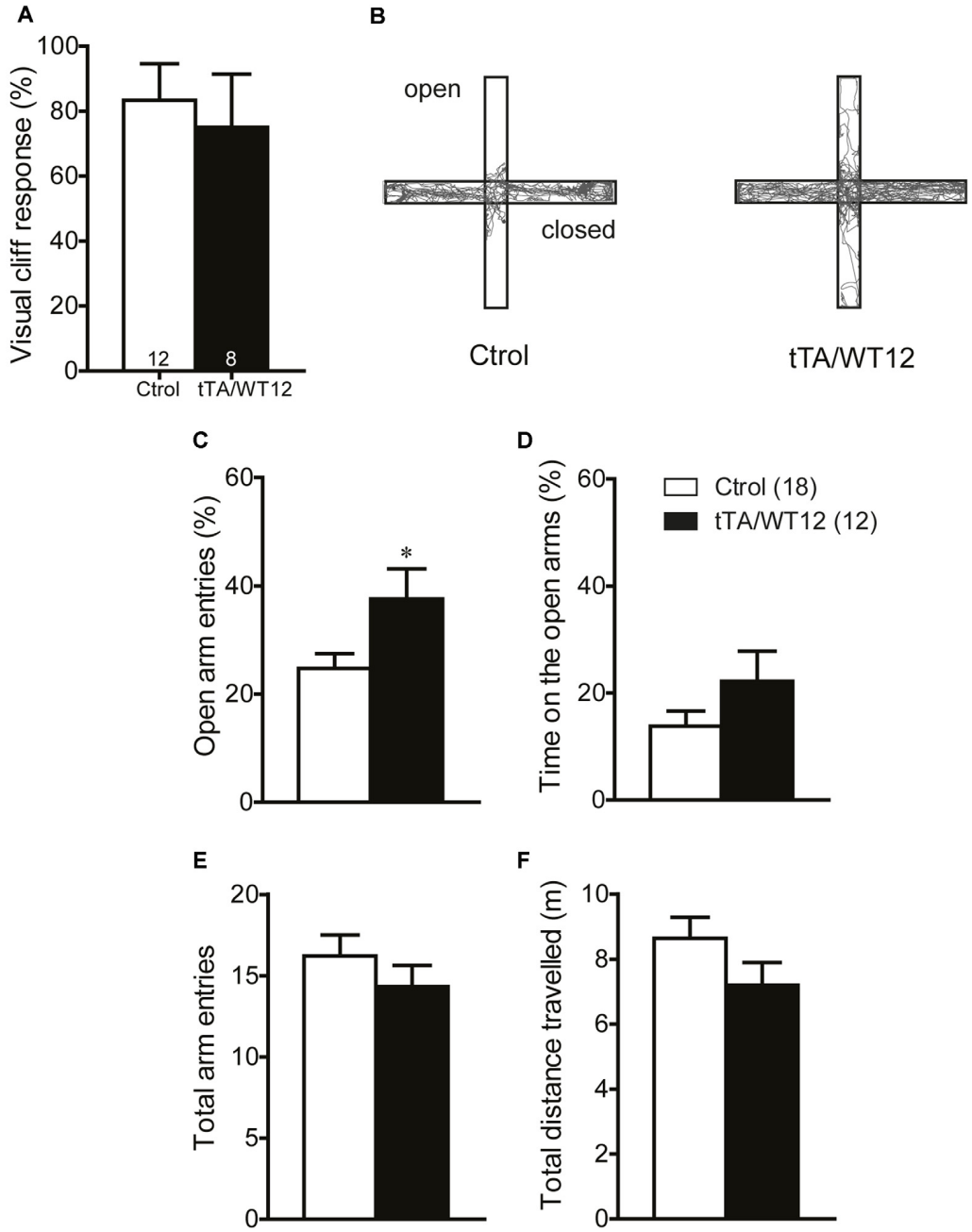


FIGURE 5 | TDP-43-WT bigenic mice display normal visual perception and signs of decreased anxiety. (A) Visual perception. Percentage of animals stopping at the edge in the visual cliff test. No significant differences in the response to the edge were found between control and bigenic animals ($p > 0.05$, Mann-Whitney *U* test). **(B–F)** Elevated plus maze test. Mice were placed at the center and allowed to explore the maze freely for 5 min. A mild decrease in anxiety-related behavior was found in bigenic mice. **(B)** Representative track plot. **(C)** Relative open arms entries (*$p < 0.05$ significantly different from control group, Student's *t* test). No difference between groups was found in **(D)** percentage of time on open arms, **(E)** total arms entries and **(F)** total distance traveled. ($p > 0.05$ in **D–F**, Student's *t* test). Number of animals is indicated in parentheses or inside plot bars. Data represent mean ± SEM.

way, we were able to analyze multiple domains of the animal behavioral repertoire which could be obscured by the use of constitutive and/or pan-neuronal promoters utilized in other Tg animals modeling the FTD/ALS spectrum of disease.

The main findings from our study are as follows. First, post-weaning overexpression of wild-type hTDP-43 leads to a rapid installment of impairments in social behavior, a characteristic feature of behavioral variant FTD (bvFTD) patients, which is the most common clinical subtype of

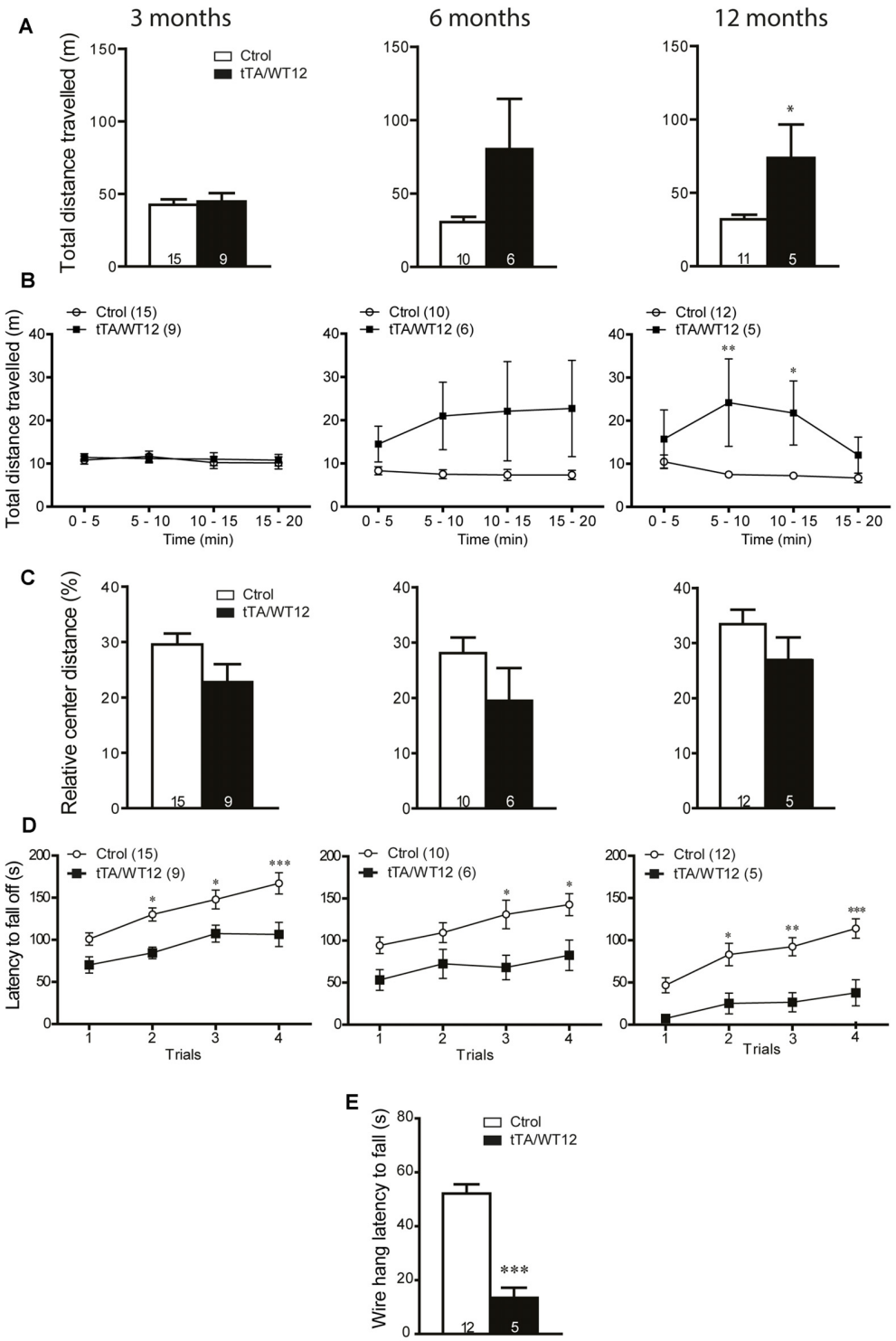


FIGURE 6 | Time-dependent appearance of motor deficits in TDP-43-WT mice. (A–D) Motor behavior was analyzed at 3, 6 and 12 months off Dox. **(A–C)** In order to assess general exploratory locomotion in a novel environment, open field test were performed. Mice were placed in a clear (40 cm × 40 cm × 40 cm) arena, and a 20 min session was used. **(A)** Total distance traveled in the open field chamber. An increased trend at 6 months became significant at 12 months of Tg expression (*$p < 0.05$ significantly different from control group, Student's *t* test). **(B)** Open field time bin (segments of 5 min) analysis of total distance traveled show a significant difference at 12 months after Tg expression (*$p < 0.05$; **$p < 0.01$ significantly different from control group, repeated-measures two-way ANOVA/Bonferroni *post hoc* test). **(C)** Relative center distance during the open field session show no significant differences at any time post Tg induction.

(*Continued*)

FIGURE 6 | Continued
(D) Accelerated rotarod test. TDP-43-WT mice display impaired coordination and balance at 3, 6 and 12 months after Tg induction (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ significantly different from control group, repeated-measures two-way ANOVA/Bonferroni *post hoc* test). **(E)** Grip strength was evaluated at 12 months after Tg expression using the hanging wire grip test. The latency to fall off the wire lid was quantified using a 60 s cutoff time. TDP-43-WT mice show significant deficits in grip strength (***$p < 0.001$ significantly different from control group, Student's *t* test). Number of animals is indicated in parentheses or inside plot bars. Data represent mean ± SEM.

FTD (Seltman and Matthews, 2012; Laforce, 2013). Second, tTA/WT12 mice display early deficits in cognitive function, most remarkably impaired working memory, a frontal cortex-dependent function. They also show signs of a mild decrease in anxiety in the elevated plus maze test, which some authors interpret as disinhibition and might indicate alterations in amygdala or prefrontal cortex function (Roberson, 2012; Koss et al., 2016). These phenotypes are consistent with those observed in other FTD mouse models (Takeuchi et al., 2011; Przybyla et al., 2016; Vernay et al., 2016a), although in some FTD models an increase in anxiety was also reported (Koss et al., 2016; Liu et al., 2016). Further analysis are required to clearly define if some form of disinhibition is present in tTA/WT12 mice. Third, there is a surprising preservation of motor function at the early time point post-induction (1 month off Dox) when cognitive and social deficits are manifest. Most TDP-43 mouse models present with robust, rapid motoric phenotypes, regardless of appearance of cognitive deficits, and most likely this is at least in part related to the use of pan-neuronal promoters (Tsao et al., 2012; Liu et al., 2013; Philips and Rothstein, 2015). Fourth, we studied in these mice the time course of motor function and identified a progressive appearance of abnormalities including hyperlocomotion, loss of coordination/balance and decreased grip strength.

In the past few years, there has been enormous activity and effort applied to animal model development for FTD/ALS, making good use of the information from recent genetic and neuropathological findings (Roberson, 2012; Liu et al., 2013; Philips and Rothstein, 2015). Specifically, Tg mice based in modulating the expression of C9ORF72, progranulin (PRGN), VCP, CHMP2B and other genes are being generated at a swift pace, and are available for comparison with the variety of TDP-43 based rodent models. In this context, it is becoming more and more difficult to parse out commonalities and differences in the associated neuropathological and behavioral changes. The unique combination of early social/cognitive deficits without motor involvement, which emerges later on with extended periods of TDP-43 expression, suggest that tTA/WT12 mice provide an interesting platform to perform behavioral studies combined with pharmacological approaches difficult to design in other Tg models of ALS/FTD.

In light of the behavioral results reported in this work, we consider that it is relevant to compare them with those observed in a closely related mouse model, termed for short tTA/△NLS. This Tg mouse model, developed in parallel with the tTA/WT12 mice (Igaz et al., 2011) and recently behaviorally analyzed (Alfieri et al., 2014), use the same combination of promoter (CamKIIα) and Dox-regulated expression system as the one used in the current study, but with a mutant form of TDP-43 that is cytoplasmically localized due to mutation of the nuclear localization sequence (NLS) in the protein (hTDP-43-△NLS). tTA/△NLS mice displayed a similar -although more aggressive- degenerative, behavioral and transcriptional phenotype (Igaz et al., 2011; Alfieri et al., 2014). We demonstrate here that tTA/WT12 mice show early (1 month post-weaning Tg induction) social and cognitive deficits, while tTA/△NLS mice also rapidly developed a penetrant and florid motor phenotype, which nonetheless allowed the mice to perform most behavioral tasks appropriately, as indicated by control experiments (Alfieri et al., 2014). The reasons for this interesting difference are less than clear at this point, but since both animal models share the same promoter system there should be additional sources for this divergence. One relevant underlying cause might be the different impact of elevated hTDP-43 species on gene expression, as evidenced by genome-wide microarray analysis of cortical samples at early time points post Tg induction (Igaz et al., 2011). Transcriptional changes in tTA/WT12 mice were relatively modest, although they did separate from nTg group after principal component analysis, establishing an "intermediate" gene expression signature. On the other hand, tTA/△NLS mice showed >4700 differentially expressed genes respect to nTg mice. This robust alteration in the transcriptional profile of tTA/△NLS mice was recently corroborated by RNA-seq analysis, although that study did not analyze tTA/WT12 mice (Amlie-Wolf et al., 2015). Alternative explanations for the different early motor phenotype include the difference in percentage of cells expressing either Tg form (lower in tTA/WT12 mice) and total expression levels of the Tg protein (Igaz et al., 2011), higher in tTA/△NLS probably due to override of endogenous autoregulatory mechanisms that govern nuclear TDP-43 levels (Ayala et al., 2011; Budini and Buratti, 2011).

Although many behavioral studies focus predominantly in motor dysfunction, other animal models have shown cognitive and social deficits consistent mostly with the FTD end of the FTD/ALS disease spectrum, i.e., those based in FTD-associated genes including tau, PRGN, CHMP2B and others. The first description of social dysfunction in a TDP-43 based rodent model was our study of behavioral phenotypes in tTA/△NLS mice (Alfieri et al., 2014). PRGN deficiency has been used to model PGRN haploinsufficiency-related FTD, and these mice display early social and cognitive phenotypes (Ghoshal et al., 2012; Filiano et al., 2013). A recently described Tg mouse line expressing the human CHMP2B^{intron5} mutant in a neuron specific manner progressively developed FTD-relevant behavioral modifications such as disinhibition, stereotypies, decrease in social interactions and compulsivity (Vernay et al., 2016b). In this context, our behavioral data from tTA/WT12 mice constitutes further evidence for a link between TDP-43 dysregulation and sociability. A forebrain specific, human mutated Tau (hTauP301L + R406W) knock-in mouse showed FTD-relevant phenotypes related to semantic memory,

anxiety, anhedonia, sleep and activity (Koss et al., 2016), and mice expressing human P301S mutant tau protein recapitulated neurological deficits of human tauopathies, including early abnormalities in the open field test, the elevated plus-maze test, Y-maze test, Barnes maze test and the Morris water maze test (Takeuchi et al., 2011). Importantly, most of these studies were performed when motor deficits were not preeminent, although in some cases detailed characterization was not reported, in contrast to the current analysis of tTA/WT12 mice. In our TDP-43 WT model, it is not clear yet if certain cognitive and social phenotypes are progressively deteriorated over time, although some cognitive tests performed at 1 month post induction indicate they rapidly reach a performance that cannot worsen. Further studies will be required to better understand the dynamics of these behavioral changes, including also those related to anxiety and disinhibition.

The existence of other animal models with early and profound motor dysfunction (which at this point are the majority of rodent models of FTD/ALS) exemplify the usefulness of having available Tg mice with dissociation of social/cognitive phenotype from motor decline as the one described in this study. Of note, Walker et al. (2015) have recently developed a TDP-43-ΔNLS mouse model (termed rNLS8) with regulatable pan-neuronal expression by means of the NEFH promoter coupled with the same tet-Off system used in this study. Although rNLS8 mice rapidly develop neuropathology and motor deficits consistent with changes observed in ALS, early and robust motor decline leading to death within a few weeks after Tg expression precluded the study of cognitive and social phenotypes more commonly associated with FTD (Walker et al., 2015; Spiller et al., 2016). Moreover, as motor abnormalities gradually emerge in tTA/WT12 mice over time, they provide a unique opportunity to study different presentations or stages shown in the clinicopathological spectrum of human FTD/ALS associated with TDP-43 dysfunction.

Another point that should be noted is that, although relatively scarce, there is evidence that modulating TDP-43 levels changes specific aspects of the biochemical, morphological and electrophysiological properties of neurons. Some authors propose that TDP-43 is an activity-related factor, suggesting roles in mRNA transport and translation in dendrites (Wang et al., 2008) and axons (Alami et al., 2014). There is also recent evidence that hyperexcitability of somatostatin interneurons in TDP-43(A315T) mice contribute to excitotoxicity (Zhang et al., 2016). TDP-43 might alter neuronal morphology and connectivity via regulation of both spinogenesis and neurite outgrowth through small GTPases Rac1 and Rho, respectively (Iguchi et al., 2009; Majumder et al., 2012). Constitutive CamKIIα-TDP-43 mice display attenuated long-term potentiation and decreased levels of plasticity-associated proteins or phosphorylation events, which are concomitant to motor and cognitive phenotypes (Tsai et al., 2010). Besides the functional implications of TDP-43 overexpression, there might be differences in sensitivity to cell death in the neurons composing the brain circuits underlying the different types of behaviors. Further studies investigating these possibilities are warranted.

In summary, we have behaviorally characterized a new conditional mouse model with neuronal expression of hTDP-43 and defined time windows to study early social and cognitive deficits in the absence of overt motor dysfunction, which could better model aspects of "pure" FTD. With progression of Tg expression over time, motor abnormalities become established, giving rise to phenotypes more related to FTD with motor neuron disease. These unique pattern of phenotypic evolution suggest that these mice might be useful to study mechanisms underlying pathological changes associated with TDP-43 proteinopathies.

AUTHOR CONTRIBUTIONS

JAA carried out the motor, social, cognitive behavior experiments in young mice, analyzed the data, discussed the results and helped to write the manuscript. PRS performed motor behavior tests in older mice and analyzed data. LMI conceived the project, wrote the manuscript and supervised all aspects of the project.

FUNDING

This work was supported by research grants to LMI from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (PICT-PRH 2009-0073 and PICT 2011-1727), the International Brain Research Organization (IBRO Return Home Fellowship 2009), Fundación Florencio Fiorini, Fundación Alberto Roemmers and the University of Buenos Aires (UBACyT; grant no. 20020110200160). LMI is a member of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). JAA and PRS were supported by doctoral fellowships from CONICET.

ACKNOWLEDGMENTS

We would like to thank Dr. Virginia M.-Y. Lee and Dr. John Q. Trojanowski (University of Pennsylvania) for the kind gift of TDP-43-WT mice (these mice were developed through support by NIH grants AG032953 and AG-17586). The authors also thank P. Bekinschtein, J. Belforte, F. Gallo, C. Katche, F. Morici, J. Medina, G. Nieva, J. Piriz and N. Weisstaub for helpful discussion of the manuscript, Graciela Ortega for her technical help and Denise Martin for husbandry support.

REFERENCES

Alami, N. H., Smith, R. B., Carrasco, M. A., Williams, L. A., Winborn, C. S., Han, S. S., et al. (2014). Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. *Neuron* 81, 536–543. doi: 10.1016/j.neuron.2013.12.018

Alfieri, J. A., Pino, N. S., and Igaz, L. M. (2014). Reversible behavioral phenotypes in a conditional mouse model of TDP-43 proteinopathies. *J. Neurosci.* 34, 15244–15259. doi: 10.1523/JNEUROSCI.1918-14.2014

Amlie-Wolf, A., Ryvkin, P., Tong, R., Dragomir, I., Suh, E., Xu, Y., et al. (2015). Transcriptomic changes due to cytoplasmic TDP-43 expression

reveal dysregulation of histone transcripts and nuclear chromatin. *PLoS One* 10:e0141836. doi: 10.1371/journal.pone.0141836

Ayala, Y. M., De Conti, L., Avendaño-Vázquez, S. E., Dhir, A., Romano, M., D'Ambrogio, A., et al. (2011). TDP-43 regulates its mRNA levels through a negative feedback loop. *EMBO J.* 30, 277–288. doi: 10.1038/emboj.2010.310

Baralle, M., Buratti, E., and Baralle, F. E. (2013). The role of TDP-43 in the pathogenesis of ALS and FTLD. *Biochem. Soc. Trans.* 41, 1536–1540. doi: 10.1042/BST20130186

Belforte, J. E., Zsiros, V., Sklar, E. R., Jiang, Z., Yu, G., Li, Y., et al. (2010). Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat. Neurosci.* 13, 76–83. doi: 10.1038/nn.2447

Boccia, M. M., Acosta, G. B., Blake, M. G., and Baratti, C. M. (2004). Memory consolidation and reconsolidation of an inhibitory avoidance response in mice: effects of i.c.v. injections of hemicholinium-3. *Neuroscience* 124, 735–741. doi: 10.1016/j.neuroscience.2004.01.001

Braz, B. Y., Galiñanes, G. L., Taravini, I. R., Belforte, J. E., and Murer, M. G. (2015). Altered corticostriatal connectivity and exploration/exploitation imbalance emerge as intermediate phenotypes for a neonatal dopamine dysfunction. *Neuropsychopharmacology* 40, 2576–2587. doi: 10.1038/npp.2015.104

Brodkin, E. S., Hagemann, A., Nemetski, S. M., and Silver, L. M. (2004). Social approach-avoidance behavior of inbred mouse strains towards DBA/2 mice. *Brain Res.* 1002, 151–157. doi: 10.1016/j.brainres.2003.12.013

Budini, M., and Buratti, E. (2011). TDP-43 autoregulation: implications for disease. *J. Mol. Neurosci.* 45, 473–479. doi: 10.1007/s12031-011-9573-8

Ferrari, R., Kapogiannis, D., Huey, E. D., and Momeni, P. (2011). FTD and ALS: a tale of two diseases. *Curr. Alzheimer Res.* 8, 273–294. doi: 10.2174/156720511795563700

Filiano, A. J., Martens, L. H., Young, A. H., Warmus, B. A., Zhou, P., Diaz-Ramirez, G., et al. (2013). Dissociation of frontotemporal dementia-related deficits and neuroinflammation in progranulin haploinsufficient mice. *J. Neurosci.* 33, 5352–5361. doi: 10.1523/JNEUROSCI.6103-11.2013

Gascon, E., Lynch, K., Ruan, H., Almeida, S., Verheyden, J. M., Seeley, W. W., et al. (2014). Alterations in microRNA-124 and AMPA receptors contribute to social behavioral deficits in frontotemporal dementia. *Nat. Med.* 20, 1444–1451. doi: 10.1038/nm.3717

Ghoshal, N., Dearborn, J. T., Wozniak, D. F., and Cairns, N. J. (2012). Core features of frontotemporal dementia recapitulated in progranulin knockout mice. *Neurobiol. Dis.* 45, 395–408. doi: 10.1016/j.nbd.2011.08.029

Giordana, M. T., Ferrero, P., Grifoni, S., Pellerino, A., Naldi, A., and Montuschi, A. (2011). Dementia and cognitive impairment in amyotrophic lateral sclerosis: a review. *Neurol. Sci.* 32, 9–16. doi: 10.1007/s10072-010-0439-6

Gordon, P. H. (2013). Amyotrophic lateral sclerosis: an update for 2013 clinical features, pathophysiology, management and therapeutic trials. *Aging Dis.* 4, 295–310. doi: 10.14336/AD.2013.0400295

Harciarek, M., and Cosentino, S. (2013). Language, executive function and social cognition in the diagnosis of frontotemporal dementia syndromes. *Int. Rev. Psychiatry* 25, 178–196. doi: 10.3109/09540261.2013.763340

Hodges, J. R., Davies, R. R., Xuereb, J. H., Casey, B., Broe, M., Bak, T. H., et al. (2004). Clinicopathological correlates in frontotemporal dementia. *Ann. Neurol.* 56, 399–406. doi: 10.1002/ana.20203

Igaz, L. M., Kwong, L. K., Lee, E. B., Chen-Plotkin, A., Swanson, E., Unger, T., et al. (2011). Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. *J. Clin. Invest.* 121, 726–738. doi: 10.1172/JCI44867

Iguchi, Y., Katsuno, M., Niwa, J.-I., Yamada, S.-I., Sone, J., Waza, M., et al. (2009). TDP-43 depletion induces neuronal cell damage through dysregulation of Rho family GTPases. *J. Biol. Chem.* 284, 22059–22066. doi: 10.1074/jbc.M109.012195

Kazdoba, T. M., Leach, P. T., and Crawley, J. N. (2016). Behavioral phenotypes of genetic mouse models of autism. *Genes Brain Behav.* 15, 7–26. doi: 10.1111/gbb.12256

Ke, Y. D., van Hummel, A., Stevens, C. H., Gladbach, A., Ippati, S., Bi, M., et al. (2015). Short-term suppression of A315T mutant human TDP-43 expression improves functional deficits in a novel inducible transgenic mouse model of FTLD-TDP and ALS. *Acta Neuropathol.* 130, 661–678. doi: 10.1007/s00401-015-1486-0

Koss, D. J., Robinson, L., Drever, B. D., Plucinska, K., Stoppelkamp, S., Veselcic, P., et al. (2016). Mutant Tau knock-in mice display frontotemporal dementia relevant behaviour and histopathology. *Neurobiol. Dis.* 91, 105–123. doi: 10.1016/j.nbd.2016.03.002

Kwong, L. K., Uryu, K., Trojanowski, J. Q., and Lee, V. M. (2008). TDP-43 proteinopathies: neurodegenerative protein misfolding diseases without amyloidosis. *Neurosignals* 16, 41–51. doi: 10.1159/000109758

Laforce, R., Jr. (2013). Behavioral and language variants of frontotemporal dementia: a review of key symptoms. *Clin. Neurol. Neurosurg.* 115, 2405–2410. doi: 10.1016/j.clineuro.2013.09.031

Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. *Neurosci. Biobehav. Rev.* 26, 91–104. doi: 10.1016/s0149-7634(01)00041-0

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 92, 180–185. doi: 10.1007/bf00177912

Liu, Y. C., Chiang, P. M., and Tsai, K. J. (2013). Disease animal models of TDP-43 proteinopathy and their pre-clinical applications. *Int. J. Mol. Sci.* 14, 20079–20111. doi: 10.3390/ijms141020079

Liu, Y., Pattamatta, A., Zu, T., Reid, T., Bardhi, O., Borchelt, D. R., et al. (2016). C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD. *Neuron* 90, 521–534. doi: 10.1016/j.neuron.2016.04.005

Majumder, P., Chen, Y. T., Bose, J. K., Wu, C. C., Cheng, W. C., Cheng, S. J., et al. (2012). TDP-43 regulates the mammalian spinogenesis through translational repression of Rac1. *Acta Neuropathol.* 124, 231–245. doi: 10.1007/s00401-012-1006-4

Mayford, M., Bach, M. E., Huang, Y. Y., Wang, L., Hawkins, R. D., and Kandel, E. R. (1996). Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274, 1678–1683. doi: 10.1126/science.274.5293.1678

Morris, J. (2015). Amyotrophic Lateral Sclerosis (ALS) and related motor neuron diseases: an overview. *Neurodiagn. J.* 55, 180–194. doi: 10.1080/21646821.2015.1075181

Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Chou, T. T., et al. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314, 130–133. doi: 10.1126/science.1134108

Philips, T., and Rothstein, J. D. (2015). Rodent models of amyotrophic lateral sclerosis. *Curr. Protoc. Pharmacol.* 69, 5.67.1–5.67.21. doi: 10.1002/0471141755.ph0567s69

Picher-Martel, V., Valdmanis, P. N., Gould, P. V., Julien, J. P., and Dupre, N. (2016). From animal models to human disease: a genetic approach for personalized medicine in ALS. *Acta Neuropathol. Commun.* 4:70. doi: 10.1186/s40478-016-0340-5

Przybyla, M., Stevens, C. H., van der Hoven, J., Harasta, A., Bi, M., Ittner, A., et al. (2016). Disinhibition-like behavior in a P301S mutant tau transgenic mouse model of frontotemporal dementia. *Neurosci. Lett.* 631, 24–29. doi: 10.1016/j.neulet.2016.08.007

Roberson, E. D. (2012). Mouse models of frontotemporal dementia. *Ann. Neurol.* 72, 837–849. doi: 10.1002/ana.23722

Seltman, R. E., and Matthews, B. R. (2012). Frontotemporal lobar degeneration: epidemiology, pathology, diagnosis and management. *CNS Drugs* 26, 841–870. doi: 10.2165/11640070-000000000-00000

Shinagawa, S., Ikeda, M., Fukuhara, R., and Tanabe, H. (2006). Initial symptoms in frontotemporal dementia and semantic dementia compared with Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 21, 74–80. doi: 10.1159/000090139

Spiller, K. J., Restrepo, C. R., Khan, T., Stieber, A. M., Kwong, L. K., Trojanowski, J. Q., et al. (2016). Progression of motor neuron disease is accelerated and the ability to recover is compromised with advanced age in rNLS8 mice. *Acta Neuropathol. Commun.* 4:105. doi: 10.1186/s40478-016-0377-5

Takeuchi, H., Iba, M., Inoue, H., Higuchi, M., Takao, K., Tsukita, K., et al. (2011). P301S mutant human tau transgenic mice manifest early symptoms of human tauopathies with dementia and altered sensorimotor gating. *PLoS One* 6:e21050. doi: 10.1371/journal.pone.0021050

Tsai, K. J., Yang, C. H., Fang, Y. H., Cho, K. H., Chien, W. L., Wang, W. T., et al. (2010). Elevated expression of TDP-43 in the forebrain of mice is sufficient to cause neurological and pathological phenotypes mimicking FTLD-U. *J. Exp. Med.* 207, 1661–1673. doi: 10.1084/jem.20092164

Tsao, W., Jeong, Y. H., Lin, S., Ling, J., Price, D. L., Chiang, P. M., et al. (2012). Rodent models of TDP-43: recent advances. *Brain Res.* 1462, 26–39. doi: 10.1016/j.brainres.2012.04.031

Vernay, A., Sellal, F., and René, F. (2016a). Evaluating behavior in mouse models of the behavioral variant of frontotemporal dementia: which test for which symptom? *Neurodegener. Dis.* 16, 127–139. doi: 10.1159/000439253

Vernay, A., Therreau, L., Blot, B., Risson, V., Dirrig-Grosch, S., Waegaert, R., et al. (2016b). A transgenic mouse expressing CHMP2B^{intron5} mutant in neurons develops histological and behavioural features of amyotrophic lateral sclerosis and frontotemporal dementia. *Hum. Mol. Genet.* doi: 10.1093/hmg/ddw182 [Epub ahead of print].

Walker, A. K., Spiller, K. J., Ge, G., Zheng, A., Xu, Y., Zhou, M., et al. (2015). Functional recovery in new mouse models of ALS/FTLD after clearance of pathological cytoplasmic TDP-43. *Acta Neuropathol.* 130, 643–660. doi: 10.1007/s00401-015-1460-x

Wang, I. F., Wu, L. S., Chang, H. Y., and Shen, C. K. (2008). TDP-43, the signature protein of FTLD-U, is a neuronal activity-responsive factor. *J. Neurochem.* 105, 797–806. doi: 10.1111/j.1471-4159.2007.05190.x

Warburton, E. C., and Brown, M. W. (2010). Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory. *Neuropsychologia* 48, 2262–2272. doi: 10.1016/j.neuropsychologia.2009.12.022

Woollacott, I. O., and Rohrer, J. D. (2016). The clinical spectrum of sporadic and familial forms of frontotemporal dementia. *J. Neurochem.* 138, 6–31. doi: 10.1111/jnc.13654

Yin, F., Dumont, M., Banerjee, R., Ma, Y., Li, H., Lin, M. T., et al. (2010). Behavioral deficits and progressive neuropathology in progranulin-deficient mice: a mouse model of frontotemporal dementia. *FASEB J.* 24, 4639–4647. doi: 10.1096/fj.10-161471

Zhang, W., Zhang, L., Liang, B., Schroeder, D., Zhang, Z. W., Cox, G. A., et al. (2016). Hyperactive somatostatin interneurons contribute to excitotoxicity in neurodegenerative disorders. *Nat. Neurosci.* 19, 557–559. doi: 10.1038/nn.4257

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: 27 March 2017
doi: 10.3389/fnagi.2017.00078

Spontaneously Hypertensive Rats (SHR) Are Resistant to a Reserpine-Induced Progressive Model of Parkinson's Disease: Differences in Motor Behavior, Tyrosine Hydroxylase and α-Synuclein Expression

*Anderson H. F. F. Leão[1,2,3], Ywlliane S. R. Meurer[1], Anatildes F. da Silva[2], André M. Medeiros[3], Clarissa L. C. Campêlo[1], Vanessa C. Abílio[4], Rovena C. G. K. Engelberth[5], Jeferson S. Cavalcante[5], Geison S. Izídio[6], Alessandra M. Ribeiro[7] and Regina H. Silva[1,3]**

[1]Memory Studies Laboratory, Department of Physiology, Federal University of Rio Grande do Norte, Natal, Brazil, [2]Brain Institute, Federal University of Rio Grande do Norte, Natal, Brazil, [3]Behavioral Neuroscience Laboratory, Department of Pharmacology, Federal University of São Paulo, São Paulo, Brazil, [4]Department of Pharmacology, Federal University of São Paulo, São Paulo, Brazil, [5]Neurochemical Studies Laboratory, Department of Physiology, Federal University of Rio Grande do Norte, Natal, Brazil, [6]Laboratory of Behavioral Genetics, Department of Cellular Biology, Embryology and Genetics, Federal University of Santa Catarina, Florianopolis, Brazil, [7]Department of Biosciences, Federal University of São Paulo, Santos, Brazil

OPEN ACCESS

Edited by:
Patricia Cogram, Fraunhofer Research, Chile

Reviewed by:
Neha Sehgal, Children's Hospital of Philadelphia, USA
Michael James Hurley, Imperial College London, UK

***Correspondence:**
Regina H. Silva
reginahsilva@gmail.com

Received: *28 July 2016*
Accepted: *13 March 2017*
Published: *27 March 2017*

Citation:
Leão AHFF, Meurer YSR, da Silva AF, Medeiros AM, Campêlo CLC, Abílio VC, Engelberth RCGK, Cavalcante JS, Izídio GS, Ribeiro AM and Silva RH (2017) Spontaneously Hypertensive Rats (SHR) Are Resistant to a Reserpine-Induced Progressive Model of Parkinson's Disease: Differences in Motor Behavior, Tyrosine Hydroxylase and α-Synuclein Expression. Front. Aging Neurosci. 9:78. doi: 10.3389/fnagi.2017.00078

Reserpine is an irreversible inhibitor of vesicular monoamine transporter-2 (VMAT2) used to study Parkinson's disease (PD) and screening for antiparkinsonian treatments in rodents. Recently, the repeated treatment with a low-dose of reserpine was proposed as a progressive model of PD. Rats under this treatment show progressive catalepsy behavior, oral movements and spontaneous motor activity decrement. In parallel, compared to Wistar rats, spontaneously hypertensive rats (SHR) are resistant to acute reserpine-induced oral dyskinesia. We aimed to assess whether SHR would present differential susceptibility to repeated reserpine-induced deficits in the progressive model of PD. Male Wistar and SHR rats were administered 15 subcutaneously (s.c.) injections of reserpine (0.1 mg/kg) or vehicle, every other day and motor activity was assessed by the catalepsy, oral movements and open field tests. Only reserpine-treated Wistar rats presented increased latency to step down in the catalepsy test and impaired spontaneous activity in the open field. On the other hand, there was an increase in oral movements in both reserpine-treated strains, although with reduced magnitude and latency to instauration in SHR. After a 15-day withdrawn period, both strains recovered from motor impairment, but SHR animals expressed reduced latencies to reach control levels. Finally, we performed immunohistochemistry for tyrosine hydroxylase (TH) and α-synuclein (α-syn) 48 h after the last injection or 15 days after withdrawn. Reserpine-treated animals presented a reduction in TH and an increase in α-syn immunoreactivity in the substantia nigra and dorsal striatum (dSTR), which were both recovered after 15 days of withdraw. Furthermore, SHR rats were resistant to reserpine-induced TH decrement in the substantia nigra, and presented reduced immunoreactivity to α-syn in

the dSTR relative to Wistar rats, irrespective of treatment. This effect was accompanied by increase of malondaldhyde (MDA) in the striatum of reserpine-treated Wistar rats, while SHR presented reduced MDA in both control and reserpine conditions relative to Wistar strain. In conclusion, the current results show that SHR are resilient to motor and neurochemical impairments induced by the repeated low-dose reserpine protocol. These findings indicate that the neurochemical, molecular and genetic differences in the SHR strain are potential relevant targets to the study of susceptibility to PD.

Keywords: reserpine, Parkinson's disease, SHR, α-synuclein, tyrosine hydroxylase

INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, estimated to affect 1%–2% of those older than 60 years, and 7–10 million people worldwide (Mayeux, 2003; Van Den Eeden et al., 2003). Most importantly, it is a disorder with progressive onset and escalating deterioration of life quality (Braak et al., 2003). Therefore, the resulting social and economic burdens to countries with increasing life expectancy justify a growing scientific effort to understand its physiopathology.

The disease is characterized by motor impairments including muscular weakness, rigidity, postural instability, tremors and bradykinesia (Klockgether, 2004; Duty and Jenner, 2011). However, non-motor alterations such as anxiety, depression, anhedonia, deficit in executive function, dementia and sleep disturbances are also present (Comella, 2003; Schneider et al., 2003; Bowers et al., 2006; Ishihara and Brayne, 2006; Barone, 2011; Gómez-Esteban et al., 2011; Voon and Dalley, 2011). The motor symptoms are the result of the progressive and irreversible loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Such loss of dopaminergic inputs concurrently progresses with the accumulation of intracellular α-synuclein (α-syn)-rich protein inclusions, known as Lewy bodies (McNaught et al., 2001; Lotharius and Brundin, 2002).

The scientific literature highlights a number of genes associated to the inheritance of PD, such as *α-syn*, *parkin*, *PINK1*, *DJ-1* and *LRRK*. Nevertheless, familial forms of PD explains only 10%–15% of the cases (Gao and Hong, 2011). The majority of cases are reported as idiopathic or sporadic, and are associated to environmental factors such as exposition to pesticides, rural life, infection, head trauma, and emotional disturbances like anxiety and depression (Godeiro et al., 2010; Noyce et al., 2012). Nonetheless, these epidemiological findings suggest that there are cumulative interactions between genetic and environmental factors that lead to the disease (Kim et al., 2005).

Despite many studies investigating the relevance of punctual genetic mutations in neurotoxic PD animal models (von Bohlen und Halbach et al., 2004; von Bohlen und Halbach, 2006), few of them investigated the differences between strains upon the expression of the parkinsonian phenotype. The use of distinct rodent strains could help to enlighten mechanisms related to sensitivity to neurotoxic insults. Specifically, physiological variations related to susceptibility to a dopaminergic insult in different rat strains would be relevant in the context of the multifactorial etiology of PD. For example, a study associated the resistance to the dopaminergic insult induced by the parkinsonian agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to autosomic alleles of the C57BL/J6 mice strain (Hamre et al., 1999). Of notice, spontaneously hypertensive rats (SHR) are resistant to the high-dose reserpine-induced oral dyskinesia. Further, this resistance is attributed to the increased activity of the antioxidant enzyme catalase in the striatum exhibited by the SHR animals (Queiroz et al., 1998; Abílio et al., 2004). Interestingly, studies have shown that SHR present other neurochemical differences compared to normotensive strains that could be related to the pathophysiology of PD, such as variances in dopaminergic systems (Viggiano et al., 2004) and decreased α-syn expression in the hippocampus (Chiavegatto et al., 2009). However the effects of parkinsonian-inducing agents on these parameters have not been investigated in this strain yet.

Reserpine is a vesicular monoamine transporter-2 (VMAT2) inhibitor extracted from *Rauwolfia serpentina*. This drug depletes monoamines in the central nervous system and produces lethargy, depression and dyskinesia (Leão et al., 2015). Due to these properties, reserpine was employed to model the motor and non-motor deficits related to PD in rodents and screen for candidate pharmacological treatments to PD (Jurna et al., 1973; Goldstein et al., 1975; Ossowska et al., 1994; Fernandes et al., 2012; Santos et al., 2013; Leão et al., 2015). Briefly, the reserpine model recapitulates substantial features of symptomatology, neurochemistry and pharmacology of the disease (for a review see Leão et al., 2015). Recently, we have proposed a repeated low-dose reserpine treatment that produces progressive motor and non-motor symptoms of PD, oxidative damage to membrane lipids, monoamine depletion and reduction in tyrosine hydroxylase (TH) in the nigrostriatal pathway (Fernandes et al., 2012; Santos et al., 2013; Leão et al., 2015). In view of Abílio et al.'s (2004) findings, which were focused on high-dose acute reserpine-induced dyskinesia, we predict that SHR animals would also be more resistant to repeated low-dose reserpine-induced progressive motor impairment and cellular alterations. We suggest that the comparison between SHR and Wistar rats under this treatment could provide relevant information to the understanding of susceptibility to factors involved in PD etiology. Thus, we aimed to compare the development of motor impairments and cellular hallmarks of PD (expression

of TH and α-syn, and membrane lipid peroxidation) between SHR and Wistar rats using the repeated low-dose reserpine protocol.

MATERIALS AND METHODS

Animals

We used 7-month-old male Wistar and SHR rats. All animals were housed in groups of 4–5 per cage (30 cm× 37 cm× 16 cm) under conditions of acoustic isolation and controlled airflow and temperature (23 ± 1°C), with a 12 h light/12 h dark cycle (lights on 6:30 a.m.). Food and water were available *ad libitum*. Animals used in this study were handled in accordance with the guidelines of the Brazilian law for the use of animals in research (Law Number 11.794). All procedures were approved by the local ethics committee (Comissao de Etica no uso de animais da Universidade Federal do Rio Grande do Norte, CEUA-UFRN #005/2014, and Comissao de Etica no uso de animais da Universidade Federal de Sao Paul, CEUA-UNIFESP #9239070815) and all efforts were made to minimize animal pain, suffering or discomfort.

Drugs and General Procedures

Reserpine (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in two drops of glacial acetic acid and diluted in distilled water (0.1 mg/ml). Vehicle consisted of the same amount of acetic acid and water as in the reserpine solution. These solutions were injected subcutaneously (s.c.). We briefly handled animals for 2 min during 3 days before the beginning of the experimental procedures.

Experimental Design

The rats from each strain (Wistar and SHR) were randomly assigned to one of three groups: control (WIS-CTR: $n = 8$; SHR-CTR: $n = 10$), reserpine-treated (WIS-RESt: $n = 8$; SHR-RESt: $n = 10$) and reserpine-withdrawn (WIS-RESw/d: $n = 8$; SHR-RESw/d: $n = 10$) groups. The animals received 15 s.c injections of vehicle (CTR) or 0.1 mg/kg of reserpine (RESt and RESw/d) at a volume of 1 ml/kg body weight, every other day. The rats of the RESt group were euthanized 48 h after the 15th injection, while CTR and RESw/d animals were euthanized 15 days after the 15th injection, for immunohistochemmical procedures. Motor behavior data from RESt and RESw/d were considered together for analysis until RESt group was euthanized (RES group). Rats went through the following behavioral procedures (from 8:00 h to 16:00 h): (1) catalepsy test before the 1st injection and every other day (8:00–9:00 h) throughout the treatment and for 15 days after the last injection; (2) evaluation of spontaneous activity in the open field after the 2nd, 6th and 15th injections, and 15 days after withdraw (9:00–12:00 h); and (3) oral movements quantification after the 2nd, 6th, 10th and 15th injection, and 10 and 15 days after withdraw (13:00–16:00 h). The behavioral tests listed were performed ∼48 h after the referred injection, before the animals received the following injection (16:00–17:00 h). Experimental design is shown in **Figure 1A**.

Motor Behavior

Catalepsy Test

The catalepsy behavior was assessed by placing the animal's forepaws on a horizontal bar positioned at 9 cm above the bench surface. Catalepsy was defined as an immobile posture, keeping both forepaws on the bar. The duration of catalepsy was measured up to a maximum of 180 s. Three trials were carried out for each animal in each observation day and the mean value of the three trials was considered for analysis.

Spontaneous Activity in the Open Field

The apparatus was a circular open field arena (84 cm in diameter) with 32 cm high walls, made of wood and covered in black laminated plastic. Animals were placed in the center of the apparatus for free exploration during 5 min. A digital camera above the apparatus recorded the sessions and an animal video-tracking software (ANY-maze, Stoelting, Wood Dale, IL, USA) registered the behavioral parameters. We quantified the distance traveled (in meters) and the time moving above the speed of 0.05 m/s in the apparatus (in seconds).

Oral Movements

Rats were individually placed in a wired cage (40 cm × 40.5 cm × 20 cm). Mirrors were positioned under the floor and behind the back wall of the cage to allow behavioral quantification when the animal faced away from the observer. The number of tongue protrusions (projection of the tongue out of the oral cavity) and the frequency of vacuous chewing movements (mouth openings in the vertical plane not directed toward physical material) were measured continuously for 10 min.

TH and α-syn Immunohistochemistry

Upon completion of the behavioral procedures, all animals were anesthetized with an intraperitoneal injection of sodium thiopental (40 mg/kg) and perfused transcardially with 200 ml phosphate-buffered saline (PBS), pH 7.4, containing 500 IU heparin (Liquemin, Roche, Brazil), followed by 300 ml 4.0% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed from the skull, postfixed in the same fixative solution for 2–4 h, and transferred to a solution containing sucrose 30% in 0.1 M PBS, pH 7.4. Each brain was serially cut in the coronal plane into 50-μm thick sections with a cryostat microtome (Leica, Germany) at a temperature of −20°C. The sections were placed sequentially in five compartments (one section per compartment, 250-μm apart) and stored in antifreeze solution. Free-floating sections were incubated for 18–24 h with a polyclonal anti-TH (cat # AB152 Chemicon, USA, 1:10,000, as described by Santos et al. (2013) or anti-α-syn (cat # sc-7011-R, Santa Cruz Inc., Santa Cruz, CA, USA, 1:500) primary antibody raised in rabbit, containing 2% goat normal serum diluted in 0.3% Triton X-100 and 0.1 M phosphate buffer, pH 7.4. Sections were incubated with the biotinylated secondary antibody anti-rabbit (1:1000; cat # S-1000 Vector Labs, Burlingame, CA, USA) obtained in goat for 2 h at room temperature, washed, and incubated with avidin–biotin-peroxidase solution (ABC Elite

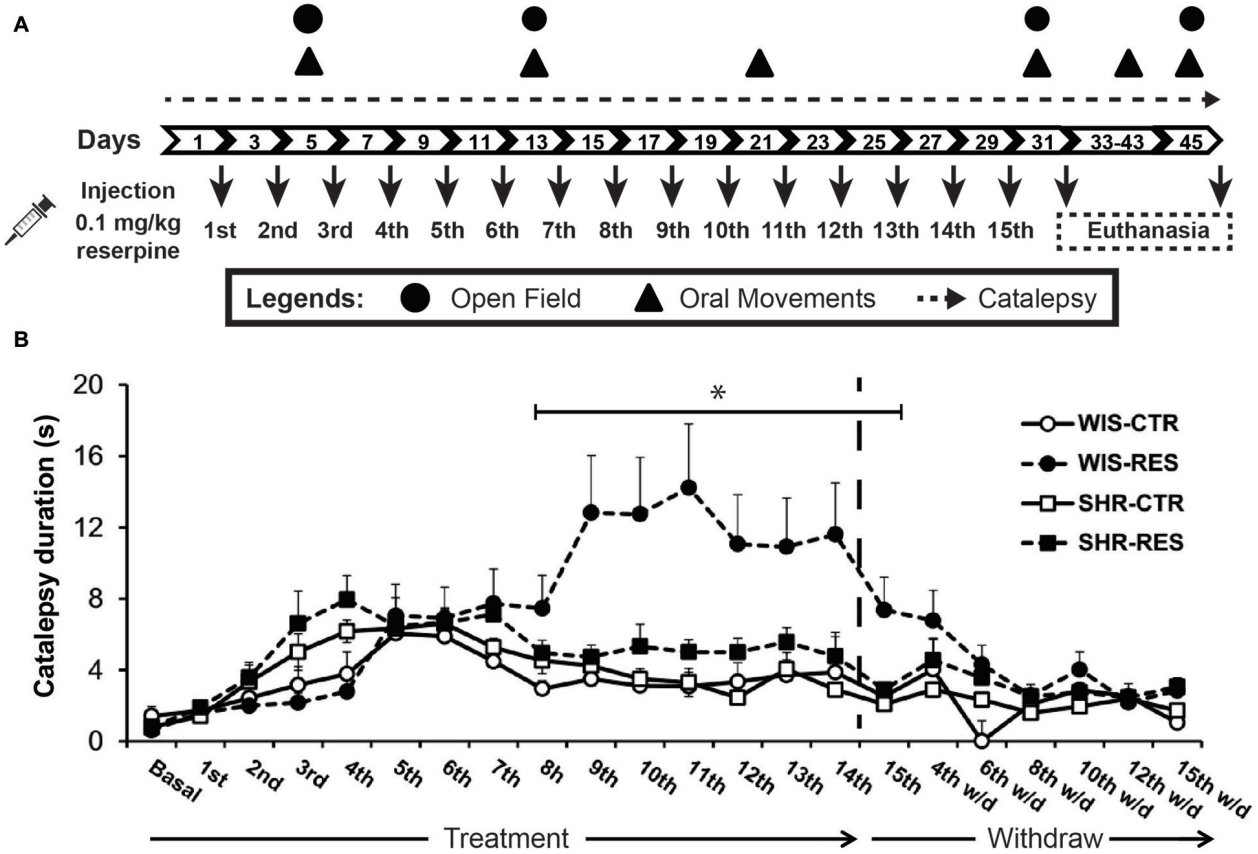


FIGURE 1 | (A) Schematic representation of the experimental design. **(B)** Effects of repeated administration of 0.1 mg/kg reserpine on catalepsy behavior in Wistar and spontaneously hypertensive rats (SHR) rats. Data are expressed as mean ± SEM relative to WIS-CTR group. *$p < 0.05$ comparing WIS-RES to WIS-CTR (Two-way analysis of variance (ANOVA) with repeated measures followed by Sidak's *post hoc* test).

kit, Vector Labs, Burlingame, CA, USA) for 90 min. The reaction was developed by the addition of diaminobenzidine tetrahydrochloride (Sigma, NY, USA) and 0.01% H_2O_2 in 0.1 M phosphate buffer, pH 7.4. The sections were washed (4×, 5 min) with 0.1 M phosphate buffer, pH 7.4, between each step and at the end of the procedure. Then, the sections were dried, dehydrated in a graded alcohol series, cleared in xylene, and cover slipped with Entellan (Merck). The immunostainings were performed in four sessions with balanced number of animals per group in each session, minimizing possible differences in background between the groups due to small variations in immunostaining. Sections were examined under brightfield illumination (Olympus Microscope, BX-41), images were captured using a CCD camera (Nikon, DXM-1200) and the locations of areas were determined using the atlas of Paxinos and Watson (2009).

Immunohistochemistry Quantification

In order to estimate the number of dopaminergic cells in the SNpc four sections of each animal were selected: one at the rostral level, two at the medium level and one at the caudal level, representative of the rostrocaudal extension of SNpc. The exact location of the region was determined on the basis of the Paxinos and Watson rat brain atlas (2009; AP: −5.04; −5.40; −5.52; −6.00 relative to the bregma). Briefly, using an automated plate microscope (Olympus Microscope, BX-41) and the software Stereo Investigator (MBF Bioscience, Williston, VT, USA) we delineated the SNpc bilaterally in the coronal sections previously described and sampled the number of TH+ neuron bodies from 75 × 75 (μm) frames into a 200 × 200 (μm) grid under a 100× oil immersion objective. The TH+ cell count was performed for the whole extension within each section. Number of neuron count was then expressed relative to WIS-CTR group (Santos et al., 2013).

Additionally, TH and α-syn levels were assessed by analysis of relative optical densitometry (ROD) in the dorsal striatum (dSTR; AP: 1.08; 0.36; 0.00; −0.72 relative to bregma) and substantia nigra pars reticulada (SNpr, in the same coronal sections as for the SNpc) using the software ImageJ (Version 1.46i, NIH). α-syn expresses in synaptic terminals, and a previous study has shown immunoreaction for this protein in the SNpr, co-localized with inhibitory terminals from the dSTR. Of notice, that study did not show any protein inclusion,

fibrils or neuron body staining in the SNpc (Taguchi et al., 2016). The SNpr receives abundant afferences from striatal medium spiny neurons and is one of the two output nuclei from the basal ganglia to the motor thalamus, working as a fast-spiking pacemaker in the absence of synaptic input (DeLong and Wichmann, 2007; Da Cunha et al., 2009). Thus, the quantification of α-syn expression in the SNpr may provide some understanding on the status of the indirect pathway in the basal ganglia.

Four representative sections of the rostrocaudal extension of each region were chosen. In each section, five fields evenly distributed throughout the areas of interest and control region were analyzed. The medium pixels in the target area were subtracted from the medium values of a control region (areas that should not have specific TH staining) of the same tissue (cortex or corpus calosum). Finally, all values were normalized considering the Wistar control group, in order to evaluate proportional alterations (Santos et al., 2013).

Lipid Peroxidation Assay

We performed an experiment to assess membrane lipid peroxidation after reserpine treatment. We administered the repeated-reserpine treatment protocol (15 s.c. injections of 0.1 mg/kg, every other day) to Wistar and SHR (WIS-CTR = 7; WIS-RES = 7; SHR-CTR = 8; SHR-RES = 8). Forty-eight hours after the last injection animals were euthanized by decapitation, the brains were removed and the striatum were dissected billateraly. The tissue was immediately weighed and homogenized in phosphate buffer 0.1 M (1:5). Samples were centrifuged for 15 min at 3500 rpm and 4–5°C and duplicates of the supernatant of each sample were used in the reaction. Lipid peroxidation was estimated by the quantification of malondialdehyde (MDA)—a fluorescent product formed from the reaction of this aldehyde with thiobarbituric acid (TBA), as described by Tanizawa et al. (1981). The reaction was initiated by the addition of 250 μL of SDS 3%, 1.5 ml of acetic acid buffer 2M, and 1.5 ml of TBA 0.8% to 50 μL of the tissue homogenate. The volume was completed with 4 ml of ultrapure water, incubated in a water bath at 95°C for 60 min and cooled in an ice-cold water bath. Finally, 2.5 ml from a n-butanol:pyridine (15:1) mixture was added and samples were vigorously agitated for 30 s. The results were expressed in nanomoles (nm) of MDA per gram (g) of wet tissue calculated by plotting the obtained fluorescence (excitation at 315 nm, emission at 553 nm) against a standard MDA concentration curve.

Data Analysis

Kolmogorov-Smirnov's and Levene's tests were used to analyze normality of data and homogeneity of variance, respectively. Parametric tests were used accordingly to data distribution and homogeneity of variance. Two-way analysis of variance (ANOVA) with repeated measures—with treatment and strain as between-subject factors and sessions as within-subject factor—were applied to catalepsy, open field and oral movement motor parameters to assess effects throughout treatment and withdraw phases. A two-way ANOVA was applied to immunohistochemistry parameters with treatment and strain as between-subject factors. Both tests were followed by Sidak's *post hoc* test to highlight differences between strain and treatment groups. Results were expressed as mean + SEM and $p < 0.05$ was considered to reflect significant differences. Exact *p*-values were expressed for each factor and factor interactions for the two-way ANOVA, while differences highlighted by the Sidak's *post hoc* test are assumed $p < 0.05$.

RESULTS

Motor Behavior

Catalepsy Test

Two-way ANOVA with repeated measures revealed effects of session ($F_{(15,750)} = 3.187$; $p < 0.001$), session*strain ($F_{(15,750)} = 2.766$; $p < 0.001$), session*treatment interaction ($F_{(15,750)} = 3.162$; $p < 0.001$), time*treatment*strain interaction ($F_{(15,750)} = 1.950$; $p = 0.016$) and treatment ($F_{(1,50)} = 4.151$); $p = 0.047$) during the treatment phase. The same analysis to the withdraw phase did not reveal significant effects, and pointed out only a marginal effect of time ($F_{(6,192)} = 2.120$); $p = 0.053$). Sidak's *post hoc* test yielded differences only to WIS-RES group, with animals spending more time in the bar than any other group from 48 h after the 7th injection (day 17) to 48 h after the 15th injection (day 31) of reserpine (**Figure 1B**). That is, the repeated administration of reserpine resulted in the progressive increase of latency to step down the bar only in the Wistar strain, and this motor impairment was restored after treatment withdrawn (**Figure 1B**).

Spontaneous Locomotion in the Open Field

Total distance traveled

Two-way ANOVA with repeated measures in the treatment phase revealed effects of treatment ($F_{(1,50)} = 6.18$; $p = 0.016$), session ($F_{(2,100)} = 69.17$; $p < 0.001$), session*treatment interaction ($F_{(2,100)} = 3.37$; $p = 0.038$) and session*strain interaction ($F_{(2,100)} = 3.21$; $p = 0.044$). Two-way ANOVA did not found significant effects in the withdraw phase. Differences between groups in each time point were yielded by Sidak's *post hoc* test and shown in **Figure 2A**. Only WIS-RES group traveled a shorter distance relative to WIS-CTR group 48 h after the 15th injection.

Speed above 0.05 m/s

Two-way ANOVA with repeated measures in the treatment phase revealed effects of session ($F_{(2,100)} = 29.26$; $p < 0.001$) and session*strain interaction ($F_{(2,100)} = 3.08$; $p = 0.050$). Two-way ANOVA did not found significant effects in the withdraw phase. Differences between groups in each time point were yielded by Sidak's *post hoc* test and shown in **Figure 2B**. Briefly, only WIS-RES group spent less time moving above 0.05 m/s relative to WIS-CTR and SHR-RES groups 48 h after the 15th injection.

Taken together, open field data indicate that the repeated administration of reserpine resulted in the progressive

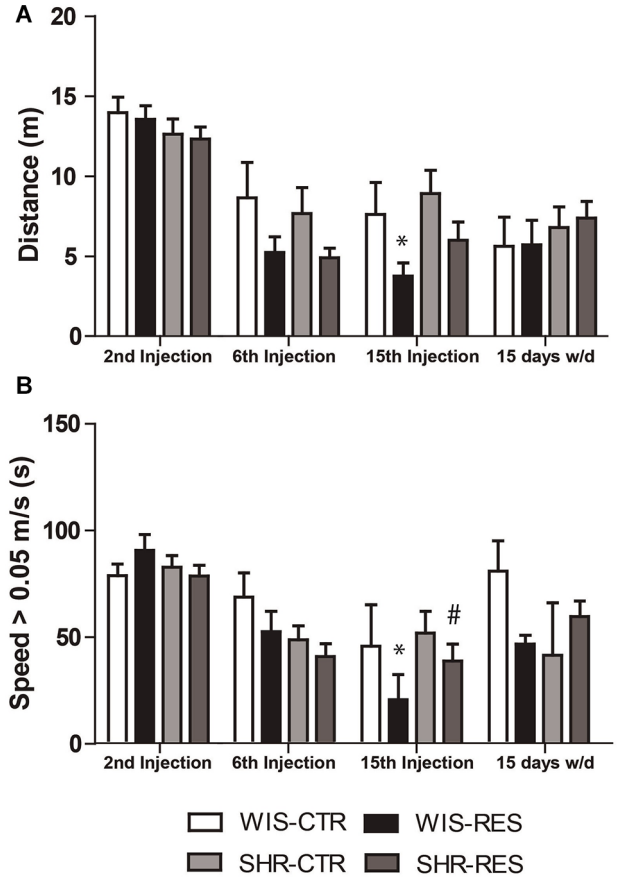


FIGURE 2 | Effects of repeated administration of 0.1 mg/kg reserpine on (A) total distance traveled and **(B)** time in speed above 0.05 m/s. Data are expressed as mean ± SEM relative to WIS-CTR group. $^{*}p < 0.05$ compared to respective CTR group; $^{\#}p < 0.05$ compared to respective treatment in WIS strain (Two-way ANOVA with repeated measures followed by Sidak's *post hoc* test).

impairment of spontaneous motor activity in both strains, but with higher magnitude for the Wistar strain. Also, motor impairment was restored after treatment withdrawn.

Oral Movements

Vacuous chewing

Two-way ANOVA with repeated measures in the treatment phase revealed effects of session ($F_{(3,150)} = 13.81$; $p < 0.001$), treatment ($F_{(1,50)} = 23.33$; $p < 0.001$), session*strain interaction ($F_{(3,150)} = 3.16$; $p = 0.026$) and session*treatment interaction ($F_{(3,150)} = 11.33$; $p < 0.001$). The same analysis revealed effect of session ($F_{(1,32)} = 12.56$; $p < 0.001$), treatment ($F_{(1,32)} = 12.56$; $p = 0.001$), session*treatment*strain interaction ($F_{(1,32)} = 4.83$; $p = 0.035$) and strain*treatment interaction ($F_{(1,32)} = 4.42$; $p = 0.043$) in the withdraw phase. Differences between groups in each time point were yielded by Sidak's *post hoc* test and shown in **Figure 3A**. WIS-RES presented increase in the vacuous chewing movements relative to respective strain control from the 6th injection to 10 days after withdraw, while SHR-RES presented increase in vacuous chewing only after the 10th injection. As well, differences in the magnitude of increase in vacuous chewing between reserpine-treated strains occurred from the 10th injection to 15 days after withdraw, with WIS-RES presenting higher values than SHR-RES group.

Tongue protrusion

Two-way ANOVA with repeated measures in the treatment phase revealed effects of session ($F_{(3,150)} = 5.15$; $p < 0.05$), treatment ($F_{(1,50)} = 18.59$; $p < 0.001$) and session*treatment interaction ($F_{(3,150)} = 5.94$; $p < 0.001$). The same analysis revealed effect of time ($F_{(1,32)} = 8.17$; $p < 0.05$) and treatment ($F_{(1,32)} = 8.48$; $p < 0.05$) in the withdraw phase. Differences between groups in each time point were yielded by Sidak's *post hoc* test and shown in **Figure 3B**. WIS-RES groups presented increase in the tongue protrusion relative to WIS-CTR from the 6th injection to 10 days after withdraw, while SHR-RES (relative to SHR-CTR) presented increased values only after the 10th and 15th injections. No differences in the magnitude of increase in tongue protrusion between reserpine-treated strains was detected.

Overall, repeated reserpine treatment resulted in the progressive increase in oral movements in both strains, but with higher magnitude in the Wistar strain. Nevertheless, this effect upon oral movements was restored after treatment withdrawn.

Neurochemistry

Tyrosine Hydroxylase

Two-way ANOVA for the number of TH positive neurons in the SNpc revealed effects of treatment ($F_{(2,54)} = 3.39$; $p = 0.041$) and strain ($F_{(1,54)} = 7.44$; $p = 0.009$; **Figure 4A**). Sidak's *post hoc* test revealed that WIS-RESw/d group presented reduction in the number of TH+ neurons in the SNpc relative to WIS-CTR. Moreover, SHR-RESt and SHR-RESw/d groups did not present any differences in the TH+ neuron count compared to SHR-CTR. Further, SHR-RESw/d displayed increased TH+ neuron count in the SNpc compared to WIS-RESw/d group. That is, only WIS rats presented reductions in TH expression in the SNpc after reserpine treatment.

Two-way ANOVA for TH expression in the dSTR revealed only effects of treatment ($F_{(2,54)} = 7.61$; $p = 0.001$), but a marginal effect for strain ($F_{(1,54)} = 2.48$; $p = 0.068$) was pointed out (**Figure 4B**). Both WIS-RESt and SHR-RESt groups presented a reduction in TH expression in the dSTR compared to respective CTR groups. Despite this tendency, RESw/d groups did not differ from CTR groups in any strain. Nevertheless, they did not differ from RESt groups either. Differences between groups revealed by Sidak's *post hoc* test are shown in **Figures 4A,B**.

Overall, reserpine treatment resulted in a reduced immunoreactivity to TH in the nigrostriatal pathway, but SHR animals appeared to be more resistant to such decrease.

α-Synuclein

α-syn expression in the SNpc did not reveal any protein inclusion, fibrils or neuron body staining. Two-way ANOVA for α-syn expression in the SNpr revealed effects of treatment ($F_{(2,54)} = 5.48$; $p = 0.007$) and strain ($F_{(1,54)} = 4.27$; $p = 0.045$).

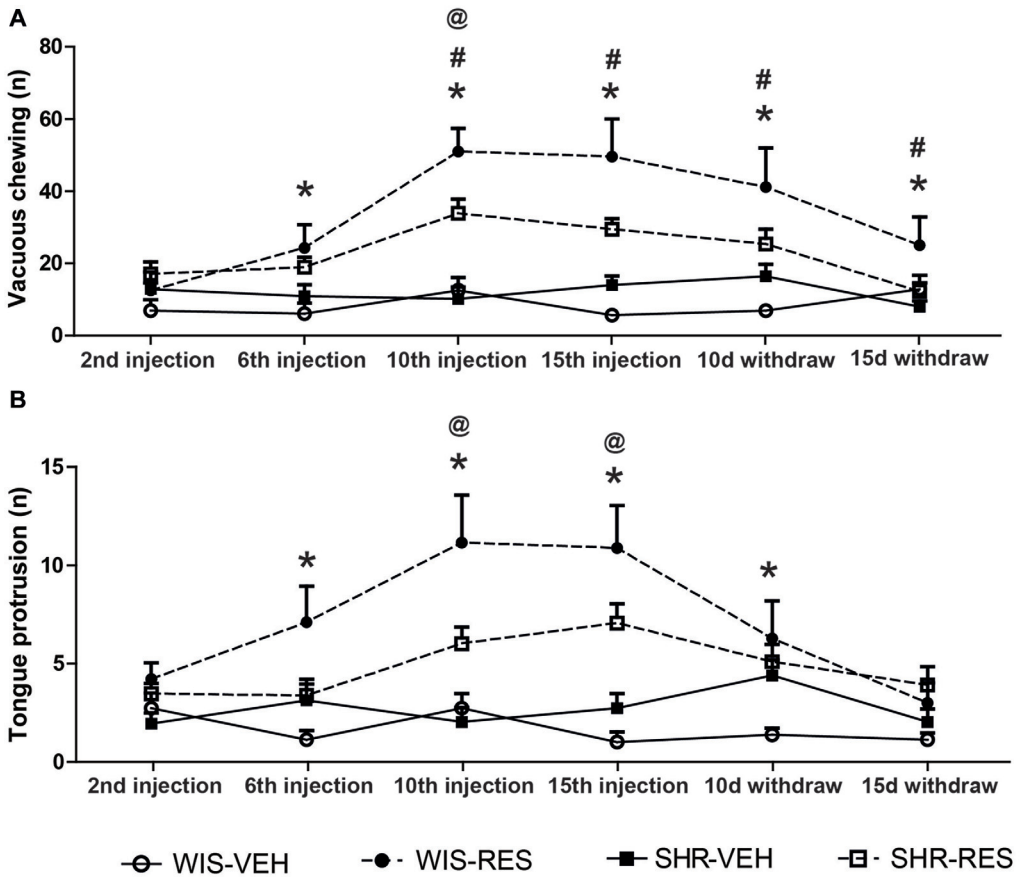


FIGURE 3 | Effects of repeated administration of 0.1 mg/kg reserpine on (A) vacuous chewing movements, and **(B)** tongue protrusions in oral movement test in Wistar and SHR rats. Data are expressed as mean ± SEM. $^{*}p < 0.05$ WIS-RES compared to WIS-CTR; $^{@}p < 0.05$ SHR-RES compared to SHR-CTR; $^{\#}p < 0.05$ WIS-RES compared to SHR-RES (Two-way ANOVA with repeated measures followed by Sidak's *post hoc* test).

Sidak's *post hoc* test revealed that the SHR-RESt group presented increased immunoreactivity to α-syn in the SNpr compared to the SHR-RESw/d group (**Figure 4C**).

Two-way ANOVA for α-syn expression in the dSTR revealed effects of treatment ($F_{(2,54)} = 5.39$; $p = 0.008$) and strain ($F_{(1,54)} = 9.59$; $p = 0.003$). Both RESt groups presented an increase in α-syn immunostaining compared to respective CTR and RESw groups. Importantly, SHR-CTR and SHR-RESt group presented a reduction of expression compared to respective treatment groups in the WIS strain. Differences between groups revealed by Sidak's *post hoc* test and shown in **Figure 4D**.

Overall, reserpine treatment increased α-syn immunostaining, and this effect was restored after withdraw. Of notice, SHR strain displayed reduced α-syn expression regardless of treatment.

Representative sections of each region analyzed for TH and α-syn immunohistochemistry are presented in **Figure 5**.

Lipid Peroxidation Assay

Two-way ANOVA for MDA quantification revealed effects of treatment ($F_{(1,28)} = 7.817$; $p = 0.01$) and strain ($F_{(1,28)} = 28.536$; $p < 0.001$; **Figure 6**). Sidak's *post hoc* test revealed that WIS-RES group presented increased lipid peroxidation in the striatum compared to WIS-CTR. Moreover, SHR-CTR and SHR-RES groups presented decreased MDA concentration in the striatum compared to the respective treatment group in Wistar strain. Nonetheless, SHR-CTR and SHR-RES were not different from each other (**Figure 6**). That is, SHR strain presented reduced basal levels of MDA compared to Wistar, and reserpine-treatment induced an increase in lipid peroxidation only in the Wistar strain.

DISCUSSION

We found that SHR are resistant to catalepsy, spontaneous locomotion and oral movement impairments in a reserpine-induced progressive animal model of PD. As expected, reserpine-treated Wistar rats presented a progressive increase in the latency to step down the catalepsy bar, which was significant from the 8th injection onwards (**Figure 1B**). Nevertheless, the SHR reserpine-treated rats presented no increase in the latency to stepdown whatsoever. Thus, the SHR strain was resistant to the difficulty to

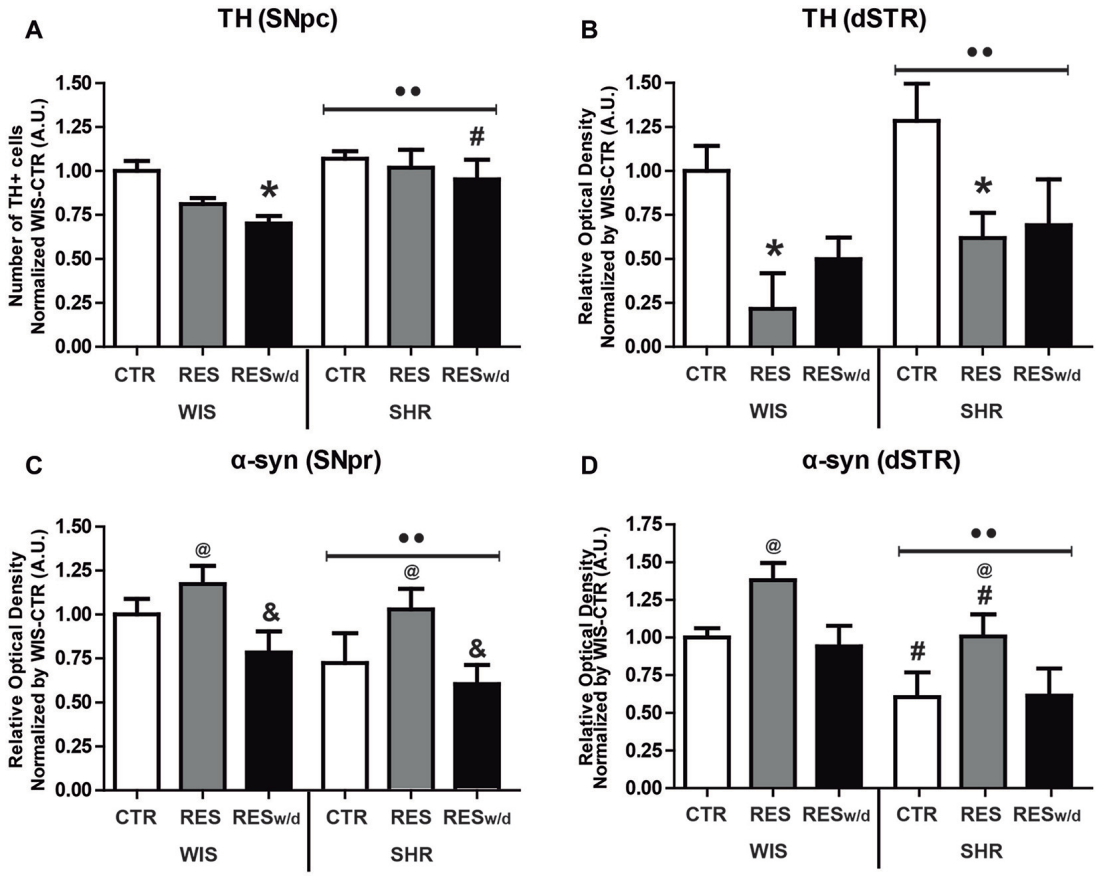


FIGURE 4 | Effects of repeated administration of 0.1 mg/kg reserpine on immunostaining for tyrosine hydroxylase (TH) in the (A) substantia nigra pars compacta (SNpc) and **(B)** dorsal striatum (dSTR), and α-synuclein (α-syn) in the **(C)** SNpc and **(D)** dSTR in Wistar and SHR rats. Data are expressed as mean ± SEM relative to WIS-CTR group. $^{*}p < 0.05$ compared to respective CTR group; $^{\#}p < 0.05$ compared to respective treatment in WIS strain; $^{\&}p < 0.05$ compared to respective RESt group; $^{\bullet\bullet}p < 0.05$ effect of strain; $^{@}p < 0.05$ effect of treatment (Two-way ANOVA followed by Sidak's *post hoc* test).

start a movement induced by the low-dose reserpine treatment. As follows, the same profile of motor impairment was evident in the spontaneous locomotion in the open field. Despite both strains significantly reduced locomotion and speed throughout sessions due to habituation, only reserpine-treated Wistar rats presented impaired spontaneous locomotion 48 h after the 15th injection—to both total distance traveled and time in speed above 0.05 m/s (**Figures 2A,B**).

Despite the lower susceptibility to motor impairment in the catalepsy and spontaneous locomotion test, reserpine-treated SHR rats presented some extent of oral dyskinesia. However, SHR-RES group presented an increase in oral movements only after the 10th injection, which remained until the 10th day of withdraw. Conversely, WIS-RES group displayed the motor impairment after the 6th injection until the 15th day of withdraw (**Figures 3A,B**). Moreover, the magnitude of effects on oral movements was higher in Wistar rats than in SHR rats. In summary, the motor profile of reserpine-treated Wistar rats corroborates previous studies (Fernandes et al., 2012; Santos et al., 2013). Importantly, the present results show that the resistance to the acute high-dose reserpine-induced oral dyskinesia that was previously reported (Queiroz et al., 1998; Abílio et al., 2004) was extended to the low-dose repeated regimen and to other motor parameters (catalepsy and spontaneous activity) in the SHR strain.

Regarding the neurochemical analysis, we found a progressive reduction in TH staining in the SNpc that was significant only to WIS-RESw/d group, despite a tendency towards reduction in WIS-RESt ($p = 0.069$; **Figure 4A**). Oppositely, the reserpine treatment did not result in any reduction of TH staining in the SNpc of SHR rats. On the other hand, both strains presented a reduction in the TH staining after treatment with reserpine in the dSTR, as well as a partial recovery relative to CTR and RESt group in RESw/d groups (**Figure 4B**). The same profile of recovery was reported for Wistar rats (10 injections of 0.1 mg/kg every other day) by Santos et al. (2013) and Swiss mice (four injections of 1 mg/kg every other day) by de Freitas et al. (2016), which found partial recovery of immunoreactivity for TH in the dSTR after 30 and 60 days of reserpine withdrawn, respectively. These findings suggest a path of progression of the reserpine-induced TH decrement from the fibers (dSTR) to the nucleus (SNpc). Studies with other drug-induced models or

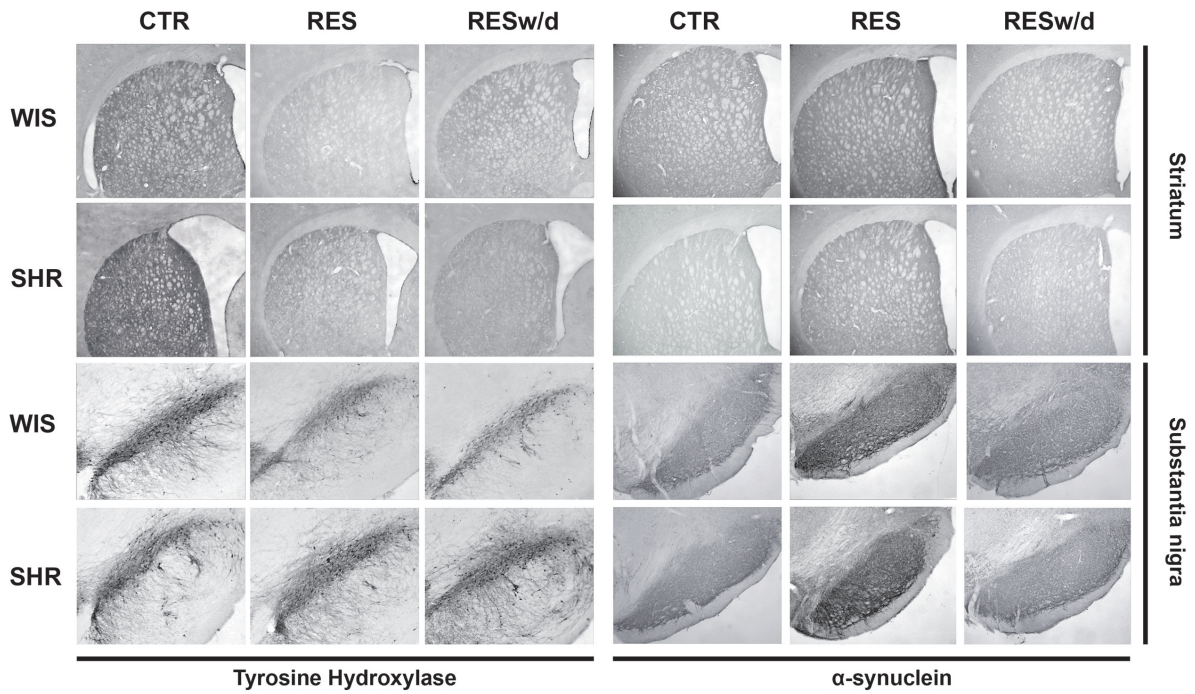


FIGURE 5 | Representative photomicrographs of brain coronal sections of dSTR and substantia nigra of rats repeatedly treated with vehicle (CTR) or 0.1 mg/kg reserpine euthanized 48 h after last injection (RESt) or 15 days (RESw/d) after the last injection. Scale bar in dSTR: 1000 μm; and substantia nigra: 200 μm.

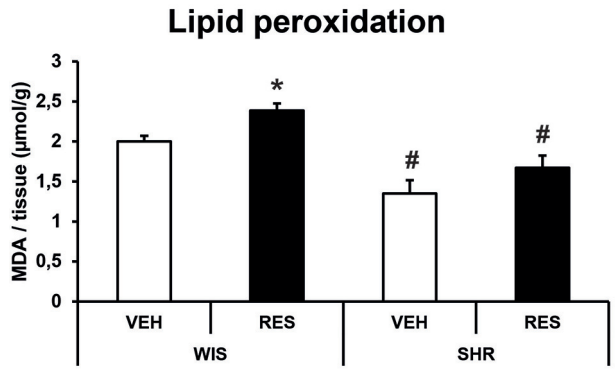


FIGURE 6 | Effects of repeated administration of 0.1 mg/kg reserpine on lipid peroxidation (malondyaldehyde—MDA formation) in the striatum of Wistar and SHR rats. Data are expressed as mean ± SEM. *$p < 0.05$ compared to respective CTR group; #$p < 0.05$ compared to respective treatment in WIS strain (Two-way ANOVA followed by Sidak's *post hoc* test).

parkinsonism also reported a retrograde profile of progression (Zhu et al., 2004; Korecka et al., 2013). Importantly, this trend of progression corroborates contemporary evidence that during early PD progression the dopaminergic loss begins in the projection sites, and subsequently advances to the nucleus (Cheng et al., 2010).

Regarding the present results, the fact that the TH decrement was found in the dSTR but not in the SNpc of SHR reinforces the notion that the dopaminergic loss did not progress in this strain in the same magnitude as it did in Wistar animals. Indeed, the reduction in TH staining in the dSTR of SHR strain was not enough to cause expressive motor impairments. Thus, it is likely that the impairing effects on motor activity of reserpine treatment requires the decrement of TH in the SNpc, which occurred only in the Wistar strain (**Figures 4A,B**). That is in accordance with the motor progression of PD, because the motor impairments in patients are observed only after the loss of at least a quarter of neurons in the SNpc (Arkadir et al., 2014). Nevertheless, WIS-RESw/d group, despite reduced TH+ neuron count in the SNpc, presented recovery of motor impairment (**Figure 4A**). Thus, other adaptive mechanisms could be related to this finding. As far as the SHR strain is concerned, other neurochemical alterations—than the TH expression—could have precluded the motor deficit induced by reserpine repeated treatment. Recently, we have found that the magnitude of motor deficit corresponded to the extent of dopamine depletion in Wistar compared to SHR animals (unpublished data).

In this respect, such neurochemical findings could be secondary to alterations in catecholamine levels and metabolism imbalances. Indeed, catecholamines regulate TH activity and expression in the cytoplasm (Dickson and Briggs, 2013). Thus, the resulting increase of DA in the cytoplasm by reserpine treatment may reduce the TH activity in the nigrostriatal pathway. Alternatively, NO and nitrite formation in the cytoplasm also regulates the expression of TH (Daubner et al.,

2011; Nakashima et al., 2011). In this respect, reserpine treatment results in oxidative stress and accumulation of reactive oxygen and nitrogen specimens, which ultimately may result in the decrement of TH expression (Arora et al., 2011; Arora and Chopra, 2013; Santos et al., 2013). Here we report reduced lipid peroxidation (evaluated by MDA formation) in SHR relative to Wistar rats. Further, reserpine treatment resulted in increased MDA formation only in the Wistar strain (**Figure 6**), corroborating findings by Abílio et al. (2004) and Fernandes et al. (2012). That is, SHR rats have a reduced baseline for the formation of oxidative stress products, which ultimately may confer resistance to reserpine-induced oxidative stress. In fact, Abílio et al. (2004) have associated such resistance to increased activity of catalase in the SHR strain. Further investigations of which phosphorylated site of TH is involved in the regulation of TH expression in response to the treatment with reserpine is required to clarify the mechanisms of TH downregulation. Likewise, epigenetic studies could also help to clarify how such cellular oxidative imbalance would modulate the expression of TH (Yang et al., 2011).

From another standpoint, the present results also showed strain differences in α-syn expression. Specifically, both RES-treated groups presented an increase in the expression of α-syn relative to CTR and RESw/d groups (particularly in the dSTR, **Figures 4C,D**). However, SHR rats presented a reduction of α-syn expression relative to WIS rats in both SNpc and dSTR (**Figures 4C,D**), irrespective of treatment. This findings corroborate the study of Chiavegatto et al. (2009) that report reduced expression of α-syn in the hippocampus of SHR rats compared to the Lewis strain.

Thus, the reported reduced expression of α-syn in this strain may reflect a significant relevance to the resistance to motor impairments showed by SHR animals. It is well known that the stable form of α-syn is localized in membranes and vesicles of presynaptic terminals in a stable membrane-bound state (Burré, 2015). The stable form is in dynamic equilibrium with the soluble cytosolic α-syn, a monomeric and natively unfolded protein that can be converted into β-sheet containing oligomers (protofibrils), which eventually form amyloid-like fibrils and Lewy bodies (Burré et al., 2013). Thus, an increase in the soluble cytosolic form of α-syn is detrimental to catecholaminergic neurons because there is an increased probability of formation of β-sheet and protofibrils. Nevertheless, despite the increase in α-syn immunostaining, we did not detect any protein inclusions or fibrils formation with the length of repeated reserpine treatment used here (**Figure 5**). Even so, the reduced α-syn expression in SHR compared to Wistar strain (**Figures 4C,D**) denotes a mechanism by which reserpine-treated SHR rats may cope with DA imbalances and TH downregulation.

Indeed, α-syn is involved in the homeostasis of DA by inhibiting the expression and activity of TH and VMAT2 (Baptista et al., 2003; Guo et al., 2008), and interrupting dopamine balance by causing increased cytosolic dopamine levels (Perez et al., 2002). Conversely, catecholamine metabolites, such as 5-S-Cysteinyldopamine, modulate α-syn expression through oxidative stress (Aureli et al., 2014). Thus, reserpine-induced increases in catecholamine metabolites and oxidative imbalances may be in the core of the neurochemical alterations reported here (Leão et al., 2015). Accordingly, SHR rats, which show decreased imunostainning for α-syn (**Figure 4D**), also presented reduced levels of lipid peroxidation (**Figure 6**). Therefore, SHR rats appear to be better suited to cope with oxidative imbalances resulting from reserpine treatment, and this feature could be related to the different profiles of TH and α-syn expressions between Wistar and SHR strains.

Of notice, irrespective of strains differences, this is the first study to show increased expression of α-syn in reserpine-treated mammals. To our knowledge, only one previous study investigated the effects of reserpine on α-syn expression in a *Droshophila melanogaster* model. That study reported inhibition of the autophagic flux, disruption in protein degradation, and accumulation of α-syn-rich protein aggregates (Lee et al., 2015). Accordingly, VMAT2-deficient mice presented age-dependent neurodegeneration in the SNpc, followed by α-syn accumulation and TH immunostaining reduction (Caudle et al., 2007; Taylor et al., 2009). Despite overall motor and neurochemical results pointing towards a reversible reserpine-induced insult to the nigrostriatal pathway (present results; Fernandes et al., 2012; Santos et al., 2013), we do not discard some extent of neurodegeneration and α-syn rich protein inclusions or fibrils in longer treatment regimens. As follows, long-term VMAT2 blockade or loss of function results in irreversible neurochemical and behavioral alterations (Neisewander et al., 1991), as well as neurodegeneration (Taylor et al., 2011). Thus, investigation of neurodegenerative markers and defective protein accumulation are still required to clarify this matter. For instance, reserpine treatment results in increased expression of caspase-3 (Arora and Chopra, 2013; Liu et al., 2014) and reduction of Bcl-2 (Liu et al., 2014; El-Ghazaly et al., 2015), which are important markers of apoptotic pathways commitment. Finally, the increase in the α-syn found in reserpine-treated animals is a putative secondary mechanism by which TH is downregulated. Indeed, α-syn reduces TH expression through inhibition of phosphorylation at Ser40 of TH (Peng et al., 2005; Wu et al., 2011).

In summary, even though we do not report any sign of α-syn positive protein inclusions, we suggest that the behavioral and neurochemical alterations here reported resembles a closer relationship to early PD molecular alterations. Nonetheless, other chemically induced PD models do not account for this histopathological hallmark (Meredith et al., 2008; Blesa et al., 2012). Importantly, the differences between the two strains could shed light on these initial cellular alterations. That is, the different profiles described here may result from compensatory and/or plastic mechanisms related to the reduced α-syn expression, resistance to the TH decrement in the SNpc, and reduced oxidative damage exhibited by the SHR strain.

In view of such findings, it is clear that the interaction between the genetic background of rat strains (genetic factors) and neurotoxic treatments (environmental factors) is substantial to

the expression of motor and cellular alterations in the progressive PD model induced by repeated reserpine treatment. Beyond the aforementioned evidence, SHR rats present other cellular features of the dopaminergic system that are relevant to the understanding of PD. For example, SHR rats express more D1 and DAT in the striatum (Watanabe et al., 1997), but present reduced function of the latter (Russell, 2003). They also seem to express less TH in the pre-frontal cortex (King et al., 2000) among other neurochemical alterations (for a review see Viggiano et al., 2004). Ideally, the investigation of the SHR strain neurochemical differences in the catecholaminergic system should be extended to other parameters and rat strains.

In conclusion, here we demonstrate that SHR rats are resistant to a progressive reserpine-induced PD model. In the Wistar strain, the motor impairments were followed by compatible neurochemical alterations that reflected PD progression, namely reduced TH and increased α-syn expressions in the substantia nigra and dSTR. On the other hand, motor and neurochemical data from the SHR strain revealed a dissociation of neurochemical alterations and motor behavior—for example, reduced TH expression in the dSTR, but no motor impairment. Here we highlight some of the neurochemical differences between these strains that may be adaptive to PD resistance, such as reduced expression of α-syn and oxidative stress products in the SHR strain. Nevertheless, the underlying mechanism by which such resistance is granted to SHR rats remains to be investigated. In view of these findings, we suggest the SHR resistant strain and the repeated low-dose reserpine protocol as valuable animal resources to investigate the relevance of genetic, cellular and behavioral features to the of progression of PD.

AUTHOR CONTRIBUTIONS

AHFFL, GSI, AMR and RHS designed the study. AHFFL, YSRM, AFS, AMM, CLCC and AMR performed the experiments. AHFFL analyzed data and wrote the manuscript. VCA, RCGKE, JSC and AMR contributed with theoretical discussions and technical insights. RHS contributed in analysis and writing, and revised the final version.

ACKNOWLEDGMENTS

The research was supported by fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Apoio à Pesquisa do Estado do Rio Grande do Norte (FAPERN), Pró-reitoria de Pesquisa da Universidade Federal do Rio Grande do Norte (PROPESQ/UFRN) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; grant #2015/12308-5 and #2015/03354-3).

REFERENCES

Abílio, V. C., Silva, R. H., Carvalho, R. C., Grassl, C., Calzavara, M. B., Registro, S., et al. (2004). Important role of striatal catalase in aging- and reserpine-induced oral dyskinesia. *Neuropharmacology* 47, 263–272. doi: 10.1016/j.neuropharm.2004.04.003

Arkadir, D., Bergman, H., and Fahn, S. (2014). Redundant dopaminergic activity may enable compensatory axonal sprouting in Parkinson disease. *Neurology* 82, 1093–1098. doi: 10.1212/WNL.0000000000000243

Arora, V., and Chopra, K. (2013). Possible involvement of oxido-nitrosative stress induced neuro-inflammatory cascade and monoaminergic pathway: underpinning the correlation between nociceptive and depressive behaviour in a rodent model. *J. Affect. Disord.* 151, 1041–1052. doi: 10.1016/j.jad.2013.08.032

Arora, V., Kuhad, A., Tiwari, V., and Chopra, K. (2011). Curcumin ameliorates reserpine-induced pain-depression dyad: behavioural, biochemical, neurochemical and molecular evidences. *Psychoneuroendocrinology* 36, 1570–1581. doi: 10.1016/j.psyneuen.2011.04.012

Aureli, C., Cassano, T., Masci, A., Francioso, A., Martire, S., Cocciolo, A., et al. (2014). 5-S-cysteinyldopamine neurotoxicity: influence on the expression of α-synuclein and ERp57 in cellular and animal models of Parkinson's disease. *J. Neurosci. Res.* 92, 347–358. doi: 10.1002/jnr.23318

Baptista, M. J., O'Farrell, C., Daya, S., Ahmad, R., Miller, D. W., Hardy, J., et al. (2003). Co-ordinate transcriptional regulation of dopamine synthesis genes by α-synuclein in human neuroblastoma cell lines. *J. Neurochem.* 85, 957–968. doi: 10.1046/j.1471-4159.2003.01742.x

Barone, P. (2011). Treatment of depressive symptoms in Parkinson's disease. *Eur. J. Neurol.* 18, 11–15. doi: 10.1111/j.1468-1331.2010.03325.x

Blesa, J., Phani, S., Jackson-Lewis, V., and Przedborski, S. (2012). Classic and new animal models of Parkinson's disease. *J. Biomed. Biotechnol.* 2012:845618. doi: 10.1155/2012/845618

Bowers, D., Miller, K., Mikos, A., Kirsch-Darrow, L., Springer, U., Fernandez, H., et al. (2006). Startling facts about emotion in Parkinson's disease: blunted reactivity to aversive stimuli. *Brain* 129, 3356–3365. doi: 10.1093/brain/awl301

Braak, H., Del Tredici, K., Rüb, U., de Vos, R. A. I., Jansen Steur, E. N. H., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24, 197–211. doi: 10.1016/s0197-4580(02)00065-9

Burré, J. (2015). The synaptic function of α-synuclein. *J. Parkinsons Dis.* 5, 699–713. doi: 10.3233/JPD-150642

Burré, J., Vivona, S., Diao, J., Sharma, M., Brunger, A. T., and Südhof, T. C. (2013). Properties of native brain α-synuclein. *Nature* 498, E4–E6. doi: 10.1038/nature12125

Caudle, W. M., Richardson, J. R., Wang, M. Z., Taylor, T. N., Guillot, T. S., McCormack, A. L., et al. (2007). Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *J. Neurosci.* 27, 8138–8148. doi: 10.1523/JNEUROSCI.0319-07.2007

Cheng, H.-C., Ulane, C. M., and Burke, R. E. (2010). Clinical progression in Parkinson disease and the neurobiology of axons. *Ann. Neurol.* 67, 715–725. doi: 10.1002/ana.21995

Chiavegatto, S., Izidio, G. S., Mendes-Lana, A., Aneas, I., Freitas, T. A., Torrão, A. S., et al. (2009). Expression of α-synuclein is increased in the hippocampus of rats with high levels of innate anxiety. *Mol. Psychiatry* 14, 894–905. doi: 10.1038/mp.2008.43

Comella, C. L. (2003). Sleep disturbances in Parkinson's disease. *Curr. Neurol. Neurosci. Rep.* 3, 173–180. doi: 10.1007/s11910-003-0073-2

Da Cunha, C., Wietzikoski, E. C., Dombrowski, P., Bortolanza, M., Santos, L. M., Boschen, S. L., et al. (2009). Learning processing in the basal ganglia: a mosaic of broken mirrors. *Behav. Brain Res.* 199, 157–170. doi: 10.1016/j.bbr.2008.10.001

Daubner, S. C., Le, T., and Wang, S. (2011). Tyrosine hydroxylase and regulation of dopamine synthesis. *Arch. Biochem. Biophys.* 508, 1–12. doi: 10.1016/j.abb.2010.12.017

de Freitas, C. M., Busanello, A., Schaffer, L. F., Peroza, L. R., Krum, B. N., Leal, C. Q., et al. (2016). Behavioral and neurochemical effects induced by reserpine in mice. *Psychopharmacology (Berl)* 233, 457–467. doi: 10.1007/s00213-015-4118-4

DeLong, M. R., and Wichmann, T. (2007). Circuits and circuit disorders of the basal ganglia. *Arch. Neurol.* 64, 20–24. doi: 10.1001/archneur.64.1.20

Dickson, P. W., and Briggs, G. D. (2013). Tyrosine hydroxylase: regulation by feedback inhibition and phosphorylation. *Adv. Pharmacol.* 68, 13–21. doi: 10.1016/B978-0-12-411512-5.00002-6

Duty, S., and Jenner, P. (2011). Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. *Br. J. Pharmacol.* 164, 1357–1391. doi: 10.1111/j.1476-5381.2011.01426.x

El-Ghazaly, M. A., Sadik, N. A., Rashed, E. R., and Abd-El-Fattah, A. A. (2015). Neuroprotective effect of EGb761® and low-dose whole-body γ-irradiation in a rat model of Parkinson's disease. *Toxicol. Ind. Health* 31, 1128–1143. doi: 10.1177/0748233713487251

Fernandes, V. S., Santos, J. R., Leão, A. H. F. F., Medeiros, A. M., Melo, T. G., Izídio, G. S., et al. (2012). Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's disease. *Behav. Brain Res.* 231, 154–163. doi: 10.1016/j.bbr.2012.03.008

Gao, H.-M., and Hong, J.-S. (2011). Gene-environment interactions: key to unraveling the mystery of Parkinson's disease. *Prog. Neurobiol.* 94, 1–19. doi: 10.1016/j.pneurobio.2011.03.005

Godeiro, C., Aguiar, P. M. D. C., Felício, A. C., Barsottini, O. G. P., Silva, S. M. A., Borges, V., et al. (2010). PINK1 polymorphism IVS1–7 A→G, exposure to environmental risk factors and anticipation of disease onset in Brazilian patients with early-onset Parkinson's disease. *Neurosci. Lett.* 469, 155–158. doi: 10.1016/j.neulet.2009.11.064

Goldstein, J., Barnett, A., and Malick, J. (1975). The evaluation of anti-parkinson drugs on reserpine-induced rigidity in rats. *Eur. J. Pharmacol.* 33, 183–188. doi: 10.1016/0014-2999(75)90154-5

Gómez-Esteban, J. C., Tijero, B., Somme, J., Ciordia, R., Berganzo, K., Rouco, I., et al. (2011). Impact of psychiatric symptoms and sleep disorders on the quality of life of patients with Parkinson's disease. *J. Neurol.* 258, 494–499. doi: 10.1007/s00415-010-5786-y

Guo, J. T., Chen, A. Q., Kong, Q., Zhu, H., Ma, C. M., and Qin, C. (2008). Inhibition of vesicular monoamine transporter-2 activity in α-synuclein stably transfected SH-SY5Y cells. *Cell. Mol. Neurobiol.* 28, 35–47. doi: 10.1007/s10571-007-9227-0

Hamre, K., Tharp, R., Poon, K., Xiong, X., and Smeyne, R. J. (1999). Differential strain susceptibility following 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) administration acts in an autosomal dominant fashion: quantitative analysis in seven strains of Mus musculus. *Brain Res.* 828, 91–103. doi: 10.1016/S0006-8993(99)01273-1

Ishihara, L., and Brayne, C. (2006). A systematic review of depression and mental illness preceding Parkinson's disease. *Acta Neurol. Scand.* 113, 211–220. doi: 10.1111/j.1600-0404.2006.00579.x

Jurna, I., Grossmann, W., and Nell, T. (1973). Depression by amantadine of tremor induced by reserpine and oxotremorine in the rat. *Naunyn Schmiedebergs Arch. Pharmacol.* 280, 141–152. doi: 10.1007/bf00499175

Kim, S. W., Ko, H. S., Dawson, V. L., and Dawson, T. M. (2005). Recent advances in our understanding of Parkinson's disease. *Drug Discov. Today Dis. Mech.* 2, 427–433. doi: 10.1016/j.ddmec.2005.11.015

King, J. A., Barkley, R. A., Delville, Y., and Ferris, C. F. (2000). Early androgen treatment decreases cognitive function and catecholamine innervation in an animal model of ADHD. *Behav. Brain Res.* 107, 35–43. doi: 10.1016/s0166-4328(99)00113-8

Klockgether, T. (2004). Parkinson's disease: clinical aspects. *Cell Tissue Res.* 318, 115–120. doi: 10.1007/s00441-004-0975-6

Korecka, J. A., Eggers, R., Swaab, D. F., Bossers, K., and Verhaagen, J. (2013). Modeling early Parkinson's disease pathology with chronic low dose MPTP treatment. *Restor. Neurol. Neurosci.* 31, 155–167. doi: 10.3233/RNN-110222

Leão, A. H. F. F., Sarmento-Silva, A. J., Santos, J. R., Ribeiro, A. M., and Silva, R. H. (2015). Molecular, neurochemical and behavioral hallmarks of reserpine as a model for Parkinson's disease: new perspectives to a long-standing model. *Brain Pathol.* 25, 377–390. doi: 10.1111/bpa.12253

Lee, K. II, Kim, M. J., Koh, H., Lee, J. I., Namkoong, S., Oh, W. K., et al. (2015). The anti-hypertensive drug reserpine induces neuronal cell death through inhibition of autophagic flux. *Biochem. Biophys. Res. Commun.* 462, 402–408. doi: 10.1016/j.bbrc.2015.04.145

Liu, S.-B., Zhao, R., Li, X.-S., Guo, H.-J., Tian, Z., Zhang, N., et al. (2014). Attenuation of reserpine-induced pain/depression dyad by gentiopicroside through downregulation of GluN2B receptors in the amygdala of mice. *Neuromolecular Med.* 16, 350–359. doi: 10.1007/s12017-013-8280-8

Lotharius, J., and Brundin, P. (2002). Pathogenesis of Parkinson's disease: dopamine, vesicles and α-synuclein. *Nat. Rev. Neurosci.* 3, 932–942. doi: 10.1038/nrn983

Mayeux, R. (2003). Epidemiology of neurodegeneration. *Annu. Rev. Neurosci.* 26, 81–104. doi: 10.1146/annurev.neuro.26.043002.094919

McNaught, K. S. P., Olanow, C. W., Halliwell, B., Isacson, O., and Jenner, P. (2001). Failure of the ubiquitin-proteasome system in Parkinson's disease. *Nat. Rev. Neurosci.* 2, 589–594. doi: 10.1038/35086067

Meredith, G. E., Sonsalla, P. K., and Chesselet, M.-F. (2008). Animal models of Parkinson's disease progression. *Acta Neuropathol.* 115, 385–398. doi: 10.1007/s00401-008-0350-x

Nakashima, A., Mori, K., Kaneko, Y. S., Hayashi, N., Nagatsu, T., and Ota, A. (2011). Phosphorylation of the N-terminal portion of tyrosine hydroxylase triggers proteasomal digestion of the enzyme. *Biochem. Biophys. Res. Commun.* 407, 343–347. doi: 10.1016/j.bbrc.2011.03.020

Neisewander, J. L., Lucki, I., and McGonigle, P. (1991). Neurochemical changes associated with the persistence of spontaneous oral dyskinesia in rats following chronic reserpine treatment. *Brain Res.* 558, 27–35. doi: 10.1016/0006-8993(91)90710-d

Noyce, A. J., Bestwick, J. P., Silveira-Moriyama, L., Hawkes, C. H., Giovannoni, G., Lees, A. J., et al. (2012). Meta-analysis of early nonmotor features and risk factors for Parkinson disease. *Ann. Neurol.* 72, 893–901. doi: 10.1002/ana.23687

Ossowska, K., Lorenc-Koci, E., and Wolfarth, S. (1994). Antiparkinsonian action of MK-801 on the reserpine-induced rigidity: a mechanomyographic analysis. *J. Neural Transm. Park. Dis. Dement. Sect.* 7, 143–152. doi: 10.1007/bf02260969

Paxinos, G., and Watson, C. (2009). *The Rat Brain in Stereotaxic Coordinates.* 6th Edn. Cambridge, MA: Academic Press.

Peng, X. M., Tehranian, R., Dietrich, P., Stefanis, L., and Perez, R. G. (2005). α-synuclein activation of protein phosphatase 2A reduces tyrosine hydroxylase phosphorylation in dopaminergic cells. *J. Cell Sci.* 118, 3523–3530. doi: 10.1242/jcs.02481

Perez, R. G., Waymire, J. C., Lin, E., Liu, J. J., Guo, F., and Zigmond, M. J. (2002). A role for alpha-synuclein in the regulation of dopamine biosynthesis. *J. Neurosci.* 22, 3090–3099.

Queiroz, C. M., Piovezan, R. D., and Frussa-Filho, R. (1998). Reserpine does not induce orofacial dyskinesia in spontaneously hypertensive rats. *Eur. J. Pharmacol.* 356, 105–108. doi: 10.1016/s0014-2999(98)00546-9

Russell, V. A. (2003). Dopamine hypofunction possibly results from a defect in glutamate-stimulated release of dopamine in the nucleus accumbens shell of a rat model for attention deficit hyperactivity disorder—the spontaneously hypertensive rat. *Neurosci. Biobehav. Rev.* 27, 671–682. doi: 10.1016/j.neubiorev.2003.08.010

Santos, J. R., Cunha, J. A. S., Dierschnabel, A. L., Campêlo, C. L. C., Leão, A. H. F. F., Silva, A. F., et al. (2013). Cognitive, motor and tyrosine hydroxylase temporal impairment in a model of parkinsonism induced by reserpine. *Behav. Brain Res.* 253, 68–77. doi: 10.1016/j.bbr.2013.06.031

Schneider, F., Habel, U., Volkmann, J., Regel, S., Kornischka, J., Sturm, V., et al. (2003). Deep brain stimulation of the subthalamic nucleus enhances emotional processing in Parkinson disease. *Arch. Gen. Psychiatry* 60, 296–302. doi: 10.1001/archpsyc.60.3.296

Taguchi, K., Watanabe, Y., Tsujimura, A., and Tanaka, M. (2016). Brain region-dependent differential expression of alpha-synuclein. *J. Comp. Neurol.* 524, 1236–1258. doi: 10.1002/cne.23901

Tanizawa, H., Sazuka, Y., and Takino, Y. (1981). Micro-determination of lipoperoxide in the mouse myocardium by thiobarbituric acid fluorophotometry. *Chem. Pharm. Bull. (Tokyo)* 29, 2910–2914. doi: 10.1248/cpb.29.2910

Taylor, T. N., Caudle, W. M., and Miller, G. W. (2011). VMAT2-deficient mice display nigral and extranigral pathology and motor and nonmotor symptoms of Parkinson's disease. *Parkinsons Dis.* 2011:124165. doi: 10.4061/2011/124165

Taylor, T. N., Caudle, W. M., Shepherd, K. R., Noorian, A., Jackson, C. R., Iuvone, P. M., et al. (2009). Nonmotor symptoms of Parkinson's disease revealed in an animal model with reduced monoamine storage capacity. *J. Neurosci.* 29, 8103–8113. doi: 10.1523/JNEUROSCI.1495-09.2009

Van Den Eeden, S. K., Tanner, C. M., Bernstein, A. L., Fross, R. D., Leimpeter, A., Bloch, D. A., et al. (2003). Incidence of Parkinson's disease:

variation by age, gender and race/ethnicity. *Am. J. Epidemiol.* 157, 1015–1022. doi: 10.1093/aje/kwg068

Viggiano, D., Vallone, D., and Sadile, A. (2004). Dysfunctions in dopamine systems and ADHD: evidence from animals and modeling. *Neural Plast.* 11, 97–114. doi: 10.1155/np.2004.97

von Bohlen und Halbach, O. (2006). Modeling neurodegenerative diseases *in vivo* review. *Neurodegener. Dis.* 2, 313–320. doi: 10.1159/000092318

von Bohlen und Halbach, O., Schober, A., and Krieglstein, K. (2004). Genes, proteins, and neurotoxins involved in Parkinson's disease. *Prog. Neurobiol.* 73, 151–177. doi: 10.1016/j.pneurobio.2004.05.002

Voon, V., and Dalley, J. W. (2011). Parkinson disease: impulsive choice-parkinson disease and dopaminergic therapy. *Nat. Rev. Neurol.* 7, 541–542. doi: 10.1038/nrneurol.2011.139

Watanabe, Y., Fujita, M., Ito, Y., Okada, T., Kusuoka, H., and Nishimura, T. (1997). Brain dopamine transporter in spontaneusly hypertensive rat. *J. Nucl. Med.* 38, 470–474.

Wu, B., Liu, Q., Duan, C., Li, Y., Yu, S., Chan, P., et al. (2011). Phosphorylation of α-synuclein upregulates tyrosine hydroxylase activity in MN9D cells. *Acta Histochem.* 113, 32–35. doi: 10.1016/j.acthis.2009.07.007

Yang, J. W., Choi, E. Y., Park, M. J., and Lee, M. A. (2011). Expression of tyrosine hydroxylase is epigenetically regulated in neural stem cells. *Biochem. Biophys. Res. Commun.* 414, 712–718. doi: 10.1016/j.bbrc.2011.09.141

Zhu, C., Vourc'h, P., Fernagut, P.-O., Fleming, S. M., Lacan, S., Dicarlo, C. D., et al. (2004). Variable effects of chronic subcutaneous administration of rotenone on striatal histology. *J. Comp. Neurol.* 478, 418–426. doi: 10.1002/cne.20305

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: 14 March 2017
doi: 10.3389/fnagi.2017.00061

A Novel Genetic Screen Identifies Modifiers of Age-Dependent Amyloid β Toxicity in the *Drosophila* Brain

*Lautaro F. Belfiori-Carrasco[1†], María S. Marcora[1†], Nadia I. Bocai[1], M. Fernanda Ceriani[2], Laura Morelli[1] and Eduardo M. Castaño[1]**

[1]*Laboratorio de Amiloidosis y Neurodegeneración, Fundación Instituto Leloir-Instituto de Investigaciones Bioquímicas de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina,* [2]*Laboratorio de Genética del Comportamiento, Fundación Instituto Leloir-Instituto de Investigaciones Bioquímicas de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*

The accumulation of amyloid β peptide (Aβ) in the brain of Alzheimer's disease (AD) patients begins many years before clinical onset. Such process has been proposed to be pathogenic through the toxicity of Aβ soluble oligomers leading to synaptic dysfunction, phospho-tau aggregation and neuronal loss. Yet, a massive accumulation of Aβ can be found in approximately 30% of aged individuals with preserved cognitive function. Therefore, within the frame of the "amyloid hypothesis", compensatory mechanisms and/or additional neurotoxic or protective factors need to be considered and investigated. Here we describe a modifier genetic screen in *Drosophila* designed to identify genes that modulate toxicity of Aβ42 in the CNS. The expression of Aβ42 led to its accumulation in the brain and a moderate impairment of negative geotaxis at 18 days post-eclosion (d.p.e) as compared with genetic or parental controls. These flies were mated with a collection of lines carrying chromosomal deletions and negative geotaxis was assessed at 5 and 18 d.p.e. Our screen is the first to take into account all of the following features, relevant to sporadic AD: (1) pan-neuronal expression of wild-type Aβ42; (2) a quantifiable complex behavior; (3) Aβ neurotoxicity associated with progressive accumulation of the peptide; and (4) improvement or worsening of climbing ability only evident in aged animals. One hundred and ninety-nine deficiency (Df) lines accounting for ∼6300 genes were analyzed. Six lines, including the deletion of 52 *Drosophila* genes with human orthologs, significantly modified Aβ42 neurotoxicity in 18-day-old flies. So far, we have validated *CG11796* and identified *CG17249* as a strong candidate (whose human orthologs are *HPD* and *PRCC*, respectively) by using RNAi or mutant hemizygous lines. *PRCC* encodes proline-rich protein PRCC (ppPRCC) of unknown function associated with papillary renal cell carcinoma. *HPD* encodes 4-hydroxyphenylpyruvate dioxygenase (HPPD), a key enzyme in tyrosine degradation whose Df causes autosomal recessive Tyrosinemia type 3, characterized by mental retardation. Interestingly, lines with a partial Df of *HPD* ortholog showed increased

OPEN ACCESS

Edited by:
Patricia Cogram,
Fraunhofer Chile Research, Chile

Reviewed by:
Isidre Ferrer,
University of Barcelona, Spain
Christian Gonzalez-Billault,
University of Chile, Chile

****Correspondence:***
Eduardo M. Castaño
ecastano@leloir.org.ar

†These authors have contributed equally to this work.

Received: *26 July 2016*
Accepted: *28 February 2017*
Published: *14 March 2017*

Citation:
Belfiori-Carrasco LF, Marcora MS, Bocai NI, Ceriani MF, Morelli L and Castaño EM (2017) A Novel Genetic Screen Identifies Modifiers of Age-Dependent Amyloid β Toxicity in the Drosophila Brain. Front. Aging Neurosci. 9:61. doi: 10.3389/fnagi.2017.00061

Abbreviations: AD, Alzheimer's disease; Aβ, amlyoid β peptide; Aβ42, amyloid β peptide 1-42; CK, creatine kinases; Df, deficiency; DIOPT, *Drosophila* RNAi Screen Center Integrative Ortholog Prediction Tool; dpe, days post-eclosion; G4, Gal4; H&E, hematoxylin-eosin; HPPD, 4-hydroxy-phenylpyruvate dioxygenase; PBS, phosphate buffered saline; Ppil2, peptidylprolyl isomerase-like 2; ppPRCC, proline-rich protein PRCC; qRT-PCR, quantitative real-time PCR; RING, Rapid Iterative Negative Geotaxis; RM, repeated measures; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ThS, thioflavine S; UAS, upstream activating sequence.

intraneuronal accumulation of Aβ42 that coincided with geotaxis impairment. These previously undetected modifiers of Aβ42 neurotoxicity in *Drosophila* warrant further study to validate their possible role and significance in the pathogenesis of sporadic AD.

Keywords: amyloid β, Alzheimer's disease, neurodegeneration, genetic screen, *Drosophila*, dementia

INTRODUCTION

Alzheimer's disease (AD) is the most prevalent form of dementia in the aged population worldwide and its impact is steadily growing due to the extension of life expectancy (Cacace et al., 2016; Scheltens et al., 2016). More than 95% of AD cases are sporadic, with age and the epsilon 4 allele of the apolipoprotein E gene as the major risk factors. Rare familial forms are associated with mutations in the amyloid precursor protein and presenilin 1–2 genes (Campion et al., 1995; Newman et al., 2007; Kandimalla et al., 2011, 2012; De Strooper and Karran, 2016).

AD brain is characterized by a pervasive synaptic loss and the accumulation of protein aggregates mostly composed of Aβ42 and microtubule-associated protein tau. Oligomeric species of Aβ42 have been proposed as early pathogenic molecules by inducing mitochondrial and endoplasmic reticulum stress, an increase in reactive oxygen species formation and action potential abnormalities (Karran et al., 2011; De Strooper and Karran, 2016). AD tau is excessively phosphorylated and aggregates intracellularly leading to microtubule instability and organelle failure (Khan and Bloom, 2016). However, the accumulation of Aβ and phospho-tau is not sufficient for the development of AD. Large autopsy series show that about 30%–40% of individuals can sustain a normal or nearly normal cognitive function at a very old age despite extensive Aβ and phospho-tau pathology (Bennett et al., 2006; Maarouf et al., 2011; Perez-Nievas et al., 2013). Several hypothesis have been put forward to explain such clinico-pathological dissociation, including differences in "cognitive/brain reserve" or the presence of compensatory mechanisms at a functional or molecular level (Maarouf et al., 2011; Steffener and Stern, 2012). In this context, the search for novel genetic and epigenetic factors that partake in neurotoxicity mechanisms related to Aβ is of key importance for understanding the disease process.

Drosophila is widely used for genetic screens applied to study the molecular bases of neurodegenerative disorders including AD (Crowther et al., 2005; Moloney et al., 2010; Lenz et al., 2013; Prüßing et al., 2013; Shulman et al., 2014; Fernandez-Funez et al., 2015; Liu et al., 2015). Major advantages of this animal model include a complex CNS, the fact that about 70% of human genetic diseases have a *Drosophila* genetic counterpart (Jackson, 2008; Bouleau and Tricoire, 2015; Lim et al., 2016) and the availability of large collections of mutant and transgenic lines.

Forward genetic screens in *Drosophila* have been used to identify modifiers of Aβ neurotoxicity. Cao et al. (2008) used a collection of transgenic lines carrying directionally inserted P elements and screened for enhancers or suppressors of a rough eye phenotype induced by Aβ42. In this way, they identified candidate genes involved in cellular processes such as transcription regulation, proteolysis in the secretory pathway and cholesterol metabolism (Finelli et al., 2004; Cao et al., 2008). By screening a collection of chromosomal deletions, the same group found that the toll-NFκB pathway enhanced both Aβ-induced rough eye and a negative effect upon life span (Tan et al., 2008). Rival et al. (2009) screened 3000 lines carrying P element inserts for modifiers of a shorter life span induced by the "Arctic" variant of Aβ42 (AβE22G) associated with familial AD. Notably, they found that genes associated with redox or antioxidant activities were strong modifiers of AβE22G neurotoxicity (Rival et al., 2009). By inducing misexpression of genes involved in specific developmental pathways, several modifiers of Aβ42 toxicity upon photoreceptors have been described (Moran et al., 2013). In addition to the eye phenotype and life span, the gravitaxis behavior (negative geotaxis) can be used for genetic screening. This test provides easily quantifiable data, explores a complex behavior of the *Drosophila* CNS and allows a rapid assessment of age-dependent Aβ toxicity. Recently, Liu et al. (2015) developed an automatic device for the Rapid Iterative Negative Geotaxis (RING) assay and screened a collection of chromosomal deletions to find modifiers of AβE22G neurotoxicity upon the giant fiber system neurons (Gargano et al., 2005; Liu et al., 2015).

The aim of the present study was to develop a modifier screen designed to study the effect of chromosomal deletions upon neuronal toxicity mediated by pan-neural expression of wild-type Aβ42 in the CNS (the major isoform that accumulates in the brain of sporadic AD patients). Fly lines with defined genomic deletions were found to exert a dominant effect under the presence of Aβ42. Deficiency (Df) lines that significantly enhanced age-dependent Aβ42 toxicity included *CG17249* and *CG11796* whose human orthologs are *PRCC* and *HPD*, respectively. *PRCC* encodes proline-rich protein PRCC (ppPRCC), a protein of unknown function associated with renal cell carcinomas. *HPD* encodes 4-hydroxyphenylpyruvate dioxygenase (HPPD), a key enzyme in tyrosine degradation.

MATERIALS AND METHODS

Fly Stocks

Flies were raised at 25°C in a standard corn meal with a light:dark cycle of 12 h:12 h. The line expressing Aβ1-42 fused with the rat pre-proenkephalin signal peptide was kindly provided by Dr. Mary Konsolaki (Rutgers University). The upstream activating sequence (UAS)-Aβ42 construct is inserted in the 2nd chromosome. Lines w^{1118} #5905 (+), $elav^{c155}$ [Gal4] #458 (G4), lines from the Df kit and the mutants for *CG11796* #51528 and

CG17249 #16098 were obtained from Bloomington *Drosophila* Stock Center (NIH P0OD018537). The CG11796 RNAi line #103482 was obtained from VDRC Stock Center. The *elav* [Gal4]; [UAS] Aβ42/Cyo line (G4 > Aβ42) was generated for the screen.

RING Assay

Groups of 30–40 male flies were raised at 25°C in 4-inch glass vials with food replacement every 2–3 days. The geotaxis behavior was tested using the RING assay as described (Gargano et al., 2005). The day before the test, 10 flies were shortly anesthetized with CO_2 and placed into a fresh vial. They were let to recover overnight at 25°C, transferred to clear glass vials and placed them in the negative geotaxis device. The device was tapped three times in rapid succession to initiate the response and climbing was recorded for 10 s. The climbed distance in cm was measured for each fly and the average height from five technical replicates per genotype was calculated using the Scion Image software.

SDS-PAGE and Western Blots

Forty heads from 5 to 18-day-old flies were homogenized in 60 μl of RIPA buffer, pH 7.4, containing 1% SDS, 5 mM EDTA, 5 mM EGTA, 1 mM PMSF, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, 1 mg/ml pepstatin and 50 mM NaF. Homogenates were centrifuged at 10,000× g for 1 h at 4°C. Twenty μl of the supernatant containing ∼150 μg of total proteins, were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 12.5% Tris-tricine gel. After transfer to polyvinylidene fluoride membranes, proteins were analyzed by Western blot. Aβ42 was detected with anti-Aβ monoclonal 6E10 (Biolegend Co.) used at 1:1000. Actin was detected with rabbit polyclonal anti-actin (Sigma) at 1:1000. After washing with PBS-T, membranes were incubated anti-rabbit or anti-mouse horseradish peroxidase-labeled IgGs (Dako, Denmark) at 1:10000. Immunoreactivity was visualized by chemiluminescence with ECL Prime (GE Bioscience, Piscataway, NJ, USA) and scanned with an Image Quant LAS 4000 apparatus (GE Bioscience, Piscataway, NJ, USA). For relative quantitation, optical densities from each lane were obtained and analyzed with the ImageJ software. Synthetic Aβ1-42 was obtained from American Peptide Co.

Inmunohistochemistry and Thioflavin S Staining

Adult heads were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 45 min at room temperature (RT). Fly brains were dissected in PBS containing 0.1% Triton X-100 (PT). Brains were blocked in 10% normal goat serum for 1 h in PT and incubated with antibody 6E10 at 4°C overnight. After incubation with Cy3-labeled anti-mouse antibody (Jackson InmunoResearch, West Grove, PA, USA) for 2 h at RT, brain tissue was stained with DAPI, washed with PBS and mounted in PBS containing 80% glycerol. For amyloid fibril staining, brains were incubated in 50% ethanol containing 1% thioflavine S (ThS; Sigma, St.Louis, MO, USA) overnight at 4°C. Samples were washed with PBS containing 50% ethanol and mounted in 80% glycerol. Brain samples from a transgenic mouse carrying the "Swedish" mutation of amyloid precursor protein (Tg2576) were used as positive controls. Images were captured with a Zeiss LSM 510 Meta Confocal microscope.

Histology and Vacuolization Assessment

Fly heads were fixed overnight in Carnoy solution (60% ethanol, 30% chloroform, 10% acetic acid) at 4°C and dehydrated in increasing concentrations of ethanol. Then, they were treated with butanol:ethanol (1:1), butanol:toluene (1:1) and toluene 30 min each, and finally soaked in toluene:paraffin (1:1) for 30 min at 65°C. After a 2-h incubation at 65°C in pure paraffin, heads were embedded and cut in 8 μm serial frontal sections. After H&E staining, images were captured using an OLYMPUS B × 50 Microscope and analyzed with the ImageJ software. Brain tissue loss was quantified as described (Sarantseva et al., 2009). The area occupied by vacuoles with a diameter of at least 3 μm was divided by the total area of the section and expressed as percentage of area loss. At least eight brains per genotype were analyzed.

Genetic Screen

To perform the genetic screen, the G4 > Aβ42 line was mated with Df lines from the Bloomington Df kit (Cook et al., 2012; Cook, 2016) to generate *elav*^{c155} [Gal4]; [UAS] Aβ42/+> Df/+ (G4 > Aβ42/Df). The experimental design consisted of three stages (**Figure 1**). In stage I, G4 > Aβ42/Df lines were analyzed at 5 and 18 days post-eclosion (d.p.e) to find a modified phenotype as compared to G4 > Aβ42. Genetic controls included G4>+ and +>Aβ42. Those Df lines that showed a difference of at least 50% in negative geotaxis only at 18 d.p.e in a single biological experiment were selected. In stage II, each chromosomal deletion; *elav*^{c155} [Gal4]; Df/+ (G4 > Df) was assessed to rule out that it did not affect negative geotaxis in the absence of Aβ42 expression. Three independent biological experiments were performed comparing G4 > Aβ42 with G4 > Aβ42/Df to select the Df lines that reached statistical significance. Deleted genes were queried for the identification of human orthologs with expression in the adult CNS (see below). If the deletion was large and included more than 10 human orthologs, overlapping deletions were analyzed as in stage II to reduce the number of candidates. Deletions with less than 10 human orthologs were selected for analysis with RNAi or mutant lines in stage III.

Bioinformatic Analysis of Deficiency Lines

Genomic deletions were queried in Bloomington Stock web page[1]. The corresponding gene list was obtained from FlyBase[2] (Attrill et al., 2016) using the GBrowse function and the Hit List tool. Each gene was searched for its human ortholog with the highest weighted score using the *Drosophila* RNAi Screen Center (DRSC) Integrative Ortholog Prediction Tool (DIOPT)

[1]http://www.flystocks.bio.indiana.edu/Browse/df/dfkit.php
[2]http://www.flybase.org

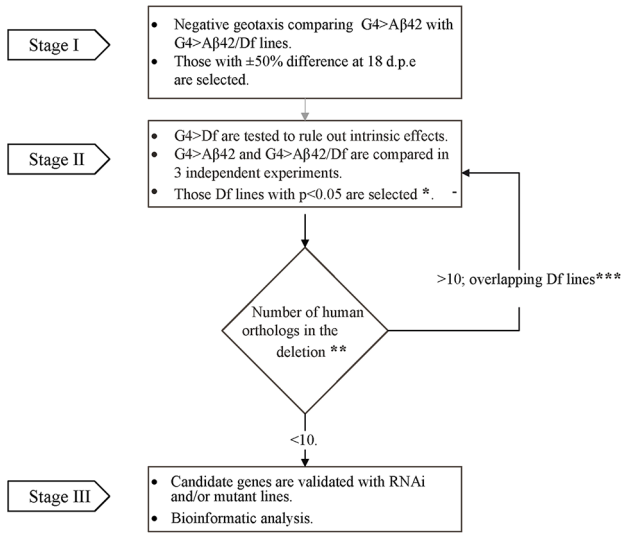


FIGURE 1 | Flow chart illustrating the overall strategy and steps of the modifier genetic screen. In stage I, G4 > amyloid β peptide 1-42 (Aβ42) line was compared to each of the G4 > Aβ42/Deficiency (Df) lines in a single negative geotaxis experiment. *In stage II, those G4 > Aβ/Df lines selected in stage I were examined in three independent biological experiments for statistical significance at 18 days post eclosion (d.p.e), (one-way ANOVA followed by least significant difference (LSD) Fisher's test $p < 0.05$). **Human orthologs were defined as those with the highest score according to *Drosophila* RNAi Screen Center (DRSC) integrative ortholog prediction tool (DIOPT). Depending on the number of deleted orthologs (> or ≤ 10), Df lines were selected for stage III or back to stage II analysis with overlapping deletions to narrow down the number of candidates (***Overlapping deletions were compared in three independent biological experiments).

from the DRSC[3]. Fly gene expression was searched in NCBI web page[4] and the RNA-seq Profile provided by FlyBase on the gene query subtitle expression data[5]. Gene products, their known functions, patterns of expression in humans, protein-protein interactions and association with human diseases were obtained from UNIPROT[6], Genecards[7] and OMIM[8] databases.

Preparation of cDNA Samples and Quantitative Real-Time PCR

RNA from 35 fly heads was extracted with the TriZol reagent (Invitrogen) according to manufacturer's instructions. cDNA was generated from 3 μg of RNA, previously treated with DNAse (Promega) using the SuperScript III system (Invitrogen). SYBR-Green quantitative real-time PCR (qRT-PCR) was performed using KAPA SYBR_FAST Universal 2X qPCR Master Mix. Reactions were run in a Stratagene Mx3005P cycler (Agilent Technologies) and analyzed by the calibration curve method. For *CG11796* primers 5′AAAGGAACCAAACCTGAA GC 3′ (forward) and 5′ATCCCTGATAGCCAAGTGGT 3′ (reverse) were used. *RPL32* was amplified for normalization using the following primers: 5′ATGCTAAGCTGTCGCACA AATG 3′ (forward) and 5′GTTCGATCCGTAACCGATGT 3′ (reverse).

Statistical Analysis

Results are presented as the mean ± SEM of at least three independent biological experiments unless otherwise stated. Data were analyzed by repeated measures (RM) two-way ANOVA with *post hoc* Bonferroni's test, RM one-way ANOVA followed by Least Significant Difference (LSD) Fisher's test or Student's *t* test using the Prism® Graphpad 6 software. Wilcoxon non-parametric test were used when indicated. The level of significance was set at $p < 0.05$.

RESULTS

G4 > Aβ42 Line Shows a Moderate and Age-Dependent Toxic Phenotype

A transgenic line with constitutive, pan-neuronal expression of Aβ42 maintained at 25°C was examined as a candidate for the screen. Western blots of fly head homogenates showed a ∼4.5 kDa band consistent with detergent-soluble Aβ42 correctly targeted and cleaved in the secretory pathway. A minor band consistent with SDS-resistant Aβ42 oligomers was also seen. Between 5 and 18 d.p.e there was a robust 3-fold increase of Aβ42 levels (**Figures 2A,B**). Negative geotaxis was not impaired in 5-day-old flies as compared with controls, strongly suggesting that there were no developmental effects upon the CNS due to Aβ42 expression. In 18-day-old flies, a significant decrease in climbing ability (∼50%) was apparent only in Aβ42-expressing animals as compared to genetic controls, G4>+ and +>Aβ42 (**Figure 2C**). Microscopic examination of the brains of affected flies revealed very mild vacuolization and negative ThS staining (see below). Therefore, this line showed age-dependent Aβ42 accumulation and CNS neurotoxicity, and the magnitude of the functional decline was optimal for the search of enhancers and suppressors. In addition, the accretion of non-fibrillar Aβ42 suggests that toxicity was induced by soluble oligomers, as proposed for AD. Taken together, these features and experimental conditions made this Aβ42 transgenic line highly suitable for a forward genetic screen.

Identification of Df Lines that Modify Age-Dependent Aβ42 Toxicity

One hundred and ninety-nine lines with defined deletions from the 2nd, 3rd and 4th chromosomes, accounting for approximately 6300 genes, were tested in the first stage of the screen. Negative geotaxis of G4 > Aβ42 line was compared to G4 > Aβ42/Df lines at 5 and 18 d.p.e. **Figure 3** shows actual examples of the three possible outcomes: Df 29667 had no modifying effect, Df 27917 worsened and Df 7681 rescued Aβ42-induced climbing dysfunction. At this stage, 73 G4 > Aβ42/Df lines showed a difference in climbing ability of at least 50% when compared to G4 > Aβ42 and

[3]http://www.flyrnai.org/cgi-bin/DRSC_orthologs.pl
[4]http://www.ncbi.nlm.nih.gov/gene
[5]http://flybase.org/reports/FBgn0036992.html
[6]http://www.uniprot.org/
[7]http://www.genecards.org/
[8]http://www.omim.org/

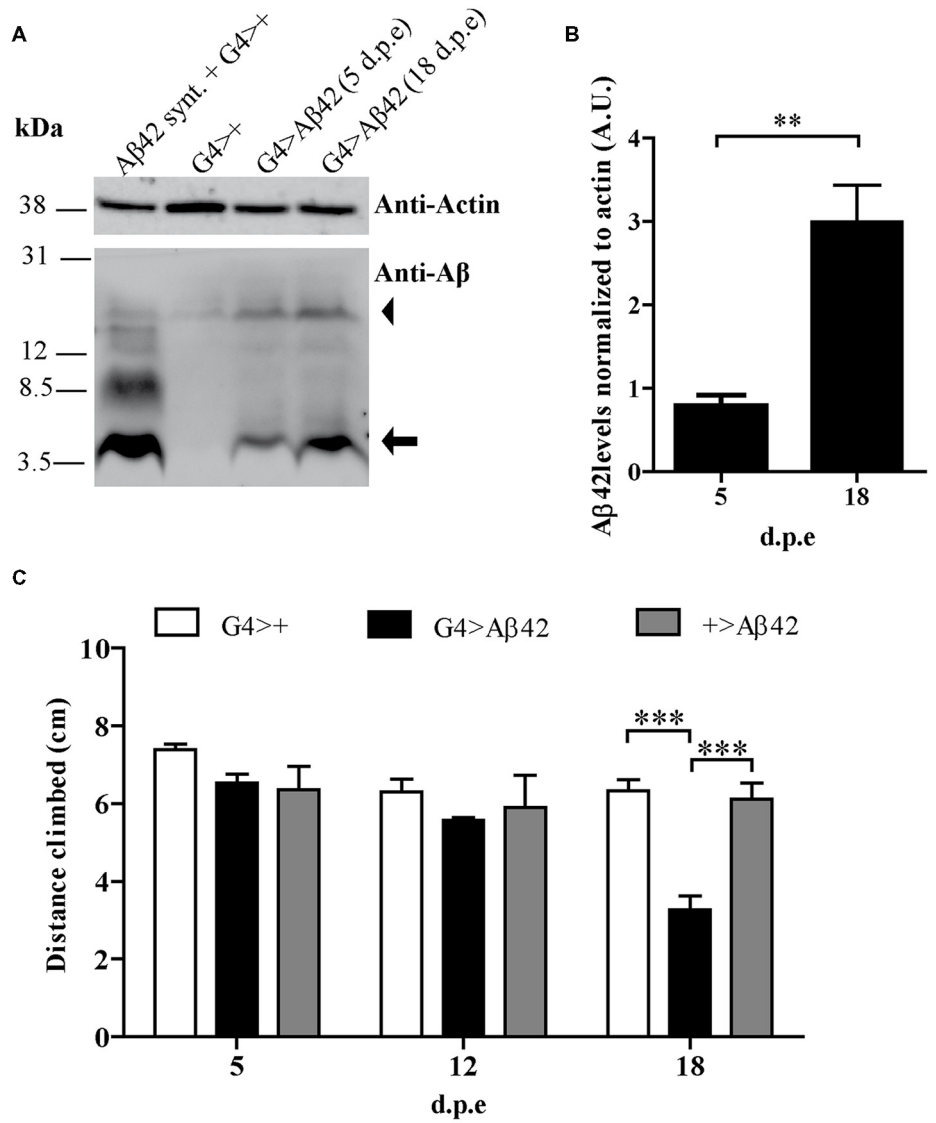


FIGURE 2 | (A) Representative Western blot of fly brain homogenates at 5 and 18 d.p.e showing Aβ42 expression detected with anti-Aβ monoclonal antibody 6E10. The 4.5 kDa band (arrow) indicates Aβ42 correctly processed in the secretory pathway. The arrowhead indicates a band consistent with sodium dodecyl sulfate (SDS)-resistant Aβ42 oligomers. A G4>+ brain homogenate was spiked with synthetic Aβ1-42 (Aβ42 Synt.) for electrophoretic mobility control. Membrane was cut above the 31 kDa marker and probed with anti-actin for normalization. **(B)** Quantification of Aβ42 levels relative to actin in arbitrary units (A.U.). Bars represent the mean ± SEM from three independent experiments; $^{**}p < 0.01$ (Student's *t*-test). **(C)** Pan-neuronal Aβ42-expressing flies (G4 > Aβ42) showed climbing impairment at 18 d.p.e as compared with genetic controls (G4>+ and +>Aβ42). Bars represent the mean ± SEM from at least three independent biological experiments; $^{***}p < 0.001$ (repeated measures [RM] two-way ANOVA followed by Bonferroni's *post hoc* test).

such differences were only seen in aged animals. These lines were selected and analyzed in stage II and six lines met statistical criteria to be considered as positive hits. Df lines 24392, 27369, 27372, 27404 and 27917 worsened negative geotaxis while line 7681 reduced Aβ42 toxicity to a full rescue of the phenotype (**Figure 4**). In the absence of Aβ42 expression, Df lines showed no intrinsic effect and none of the enhancer Df lines induced climbing impairment in G4 > Aβ42 line at 5 d.p.e, ruling out a possible acceleration of Aβ42 toxicity (not shown). Within these six Df lines, 36 *Drosophila* genes with human orthologs remain to be tested to identify enhancers and 14 genes to pin point suppressors of Aβ42 neurotoxicity. Interestingly, 14 out of 15 enhancer and six out of seven suppressor Df lines described in a previous screen based on negative geotaxis (Liu et al., 2015) were selected in stage I of our screen but did not reach statistical significance in stage II and were not further analyzed.

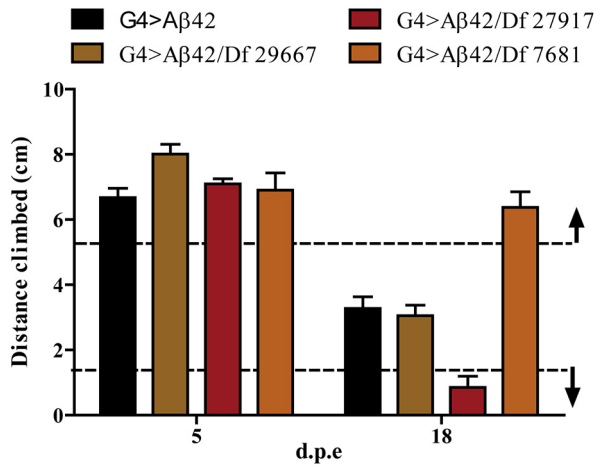


FIGURE 3 | Negative geotaxis assay of G4 > Aβ42 compared to G4 > Aβ42/Df at 5 and 18 d.p.e. The graphic shows examples of the three possible outcomes according to the quantitative criterion of at least a 50% difference in negative geotaxis (dashed lines): Df 29667 had no modifier effect, Df 27917 worsened and Df 7681 improved the climbing ability of Aβ42-expressing flies at 18 d.p.e. Bars represent the mean ± SEM from a single biological experiment (five technical repeats) and therefore, at this stage of the screen, no statistical analyses were performed.

Specific Reduction of *CG11796* Expression Enhances Aβ42 Toxicity

Thus far, three out of the five enhancer Df lines that passed stage II have been partially analyzed in stage III. The enhancer Df line 27372 included the deletion of *CG17249* whose human ortholog is *PRCC*. We used a line carrying a Piggy Bac transposon in the 3′ region of *CG17249* to assess toxicity. A significant enhancement in Aβ42 neurotoxicity was observed in mutant hemizygous flies (**Figure 5A**). Although unlikely, the 3′ insertion may compromise the expression of neighboring genes and therefore, RNAi experiments are required to validate *CG17249*. Df lines 27917 and 27369 also worsened negative geotaxis in the presence of Aβ42 and the overlapping chromosomal segment included *CG11796* whose human ortholog is *HPD* encoding HPPD, a key enzyme involved in tyrosine catabolism. To determine if a reduced expression of *CG11796* was capable of enhancing Aβ42 toxicity, we used two independent approaches: a mutant line in which a Mi[Mic] transposon was inserted in the *CG11796* gene and a specific RNAi with pan-neuronal expression using the *elav* promoter. These lines had no impairment in negative geotaxis as compared with control flies despite the reduction of *CG11796* mRNA. Yet, in the presence of pan-neuronal Aβ42 expression, *CG11796* downregulation in both the RNAi and mutant lines induced a significant enhancement of Aβ42 toxicity, similar to the overall effect of the chromosomal deletions detected at stages I-II of the screen (**Figures 5B,C**). The specificity of the RNAi was assessed by qRT-PCR from fly heads, which showed a strong reduction of *CG11796* mRNA of approximately 85% in G4 > CG11796^{RNAi} and 55% in CG11796^{Mut}, similar to the expected ∼50% mRNA reduction in Df line 27917 (**Figure 5D**).

Reduction of *CG11796* Expression Promotes the Accumulation of Non-Fibrillar Aβ42

Aβ42 levels were analyzed in the brains of flies with partial Df of *CG11796* at 18 days of age, when the toxic phenotype was detected. Confocal immunofluorescence showed extensive intraneuronal perinuclear Aβ accumulation which was ∼2-fold higher in both *CG11796* hemizygous mutant and CG11796^{RNAi} as compared with flies expressing Aβ42 alone (**Figure 6**). Western blots of head homogenates showed a 70%–80% increase in the Aβ monomer band in *CG11796* mutant and RNAi lines, consistent with the immunofluorescence results (**Figure 7**). The increment of Aβ abundance was not accompanied by ThS staining, indicating that a partial Df of *CG11796* expression promoted the accumulation of non-fibrillar Aβ species (**Figure 8**). Instead, the pattern of immunostaining and detergent solubility suggest the accretion of intraneuronal oligomeric Aβ which concurs with a higher neurotoxicity in CG11796^{Mut} and CG11796^{RNAi} flies. To assess neurodegeneration further, the extent of vacuolization in the brain was determined for each genotype. As mentioned above, there was a mild though significant increase of vacuolization in flies expressing Aβ42 as compared with their genetic controls. Yet, tissue loss did not increase in Aβ42-transgenic flies expressing *CG11796* mutant or RNAi (**Figure 9**). Together, these results strongly suggest that a partial Df of the HPD ortholog promotes the accumulation of toxic Aβ42 oligomers in the CNS leading to cellular dysfunction without histologically detectable neuronal loss.

DISCUSSION

The finding of proteins that modulate Aβ neurotoxicity in animals with a complex CNS such as *Drosophila* may impact on AD research in several ways. First, by providing novel players in the cellular mechanisms by which Aβ promotes synaptic dysfunction and neuronal death. Second, changes in the levels or activity of those proteins may be validated in human samples including post-mortem tissue and, more relevant, in biological fluids as potential biomarkers. Third, in the long range, it may open therapeutic strategies alternative to the current ones mostly aimed at Aβ and tau. Previous modifier screens in the fly have yielded interesting candidates that modulate wild-type Aβ toxicity in the eye, upon life span, or negative geotaxis induced by an aggressive Aβ mutant (Cao et al., 2008; Tan et al., 2008; Rival et al., 2009; Liu et al., 2015). Our screen was designed to search for modifiers in a context of neurotoxicity more related to what may occur in sporadic AD, including pan-neuronal expression of wild-type Aβ42 and age-dependent accumulation with no detectable behavioral impairment in young animals. Moreover, the Aβ42 transgenic line had a rather mild phenotype at ∼3 weeks of age, with little neuronal loss and the accumulation of detergent-soluble, non-fibrillar species of Aβ, avoiding features that are found in late stages of AD.

The discrepancies between our results and those reported by Liu et al. (2015) may be due to criteria for defining positive hits and the use in their study of AβE22G driven to

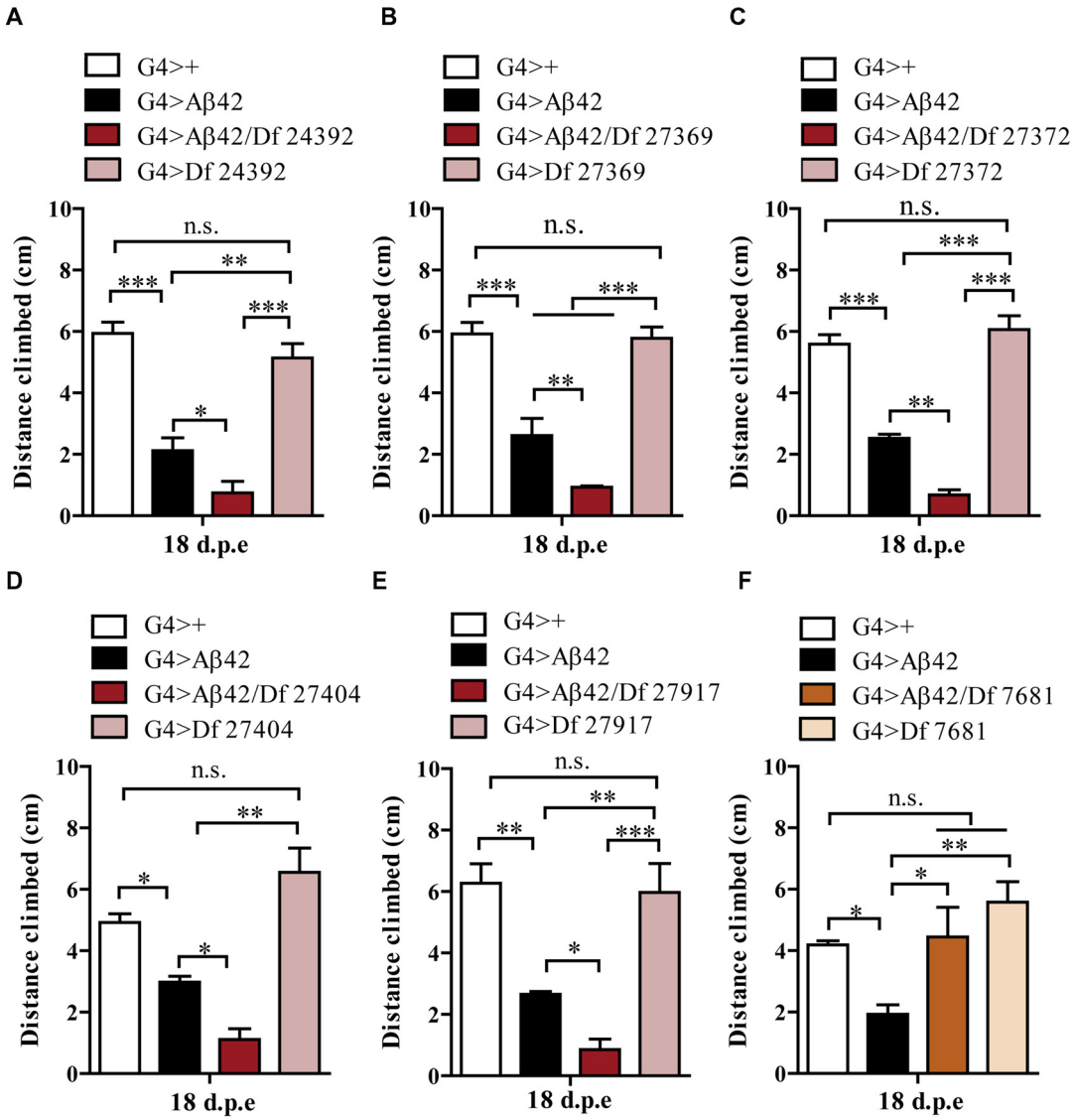


FIGURE 4 | Negative geotaxis assay of G4>+, G4 > Aβ42, G4 > Aβ42/Df and G4 > Df at 18 d.p.e. (A) Df 24392; **(B)** Df 27369; **(C)** Df 27372; **(D)** Df 27404; and **(E)** Df 27917, worsened the Aβ42-induced phenotype. **(F)** Df 7681 improved the climbing ability of Aβ42-expressing flies. Df lines had no effect in the absence of Aβ expression. Bars represent the mean ± SEM from at least three independent biological experiments; $^{*}p < 0.5$, $^{**}p < 0.01$, $^{***}p < 0.001$ (RM one-way ANOVA followed by LSD Fisher's test).

specific interneurons that relay to thoracic muscles instead of pan-neuronal wild-type Aβ42. Noteworthy, in both studies Df line 7681 was a strong suppressor, suggesting that one or more genes in homozygosity within this deletion are necessary for Aβ to impair geotaxis behavior, independent of Aβ species and type of neurons involved.

A limitation of our study was its restriction to the effect of gene deletions and therefore, likely dependent on lower than physiological levels of the encoded proteins. Those genes that modulate Aβ42 toxicity through overexpression would be missed with our strategy.

So far, two genes have passed stage III of our screen whose human orthologs are *PRCC* and *HPD*. While *PRCC* requires a final validation step with RNAi, *HPD* was unambiguously identified. The function of ppPRCC is largely unknown although early studies suggest that it may have a role in pre-mRNA splicing (Skalsky et al., 2001). A search for ppPRCC protein-protein interactions revealed association with peptidylprolyl isomerase-like 2 (Ppil2), a chaperone with putative ubiquitin ligase activity (Hatakeyama et al., 2001; Pushkarsky et al., 2005; Hegele et al., 2012). Thus, a possible role of a ppPRCC-Ppil-2 complex in protein folding, transport and degradation warrants

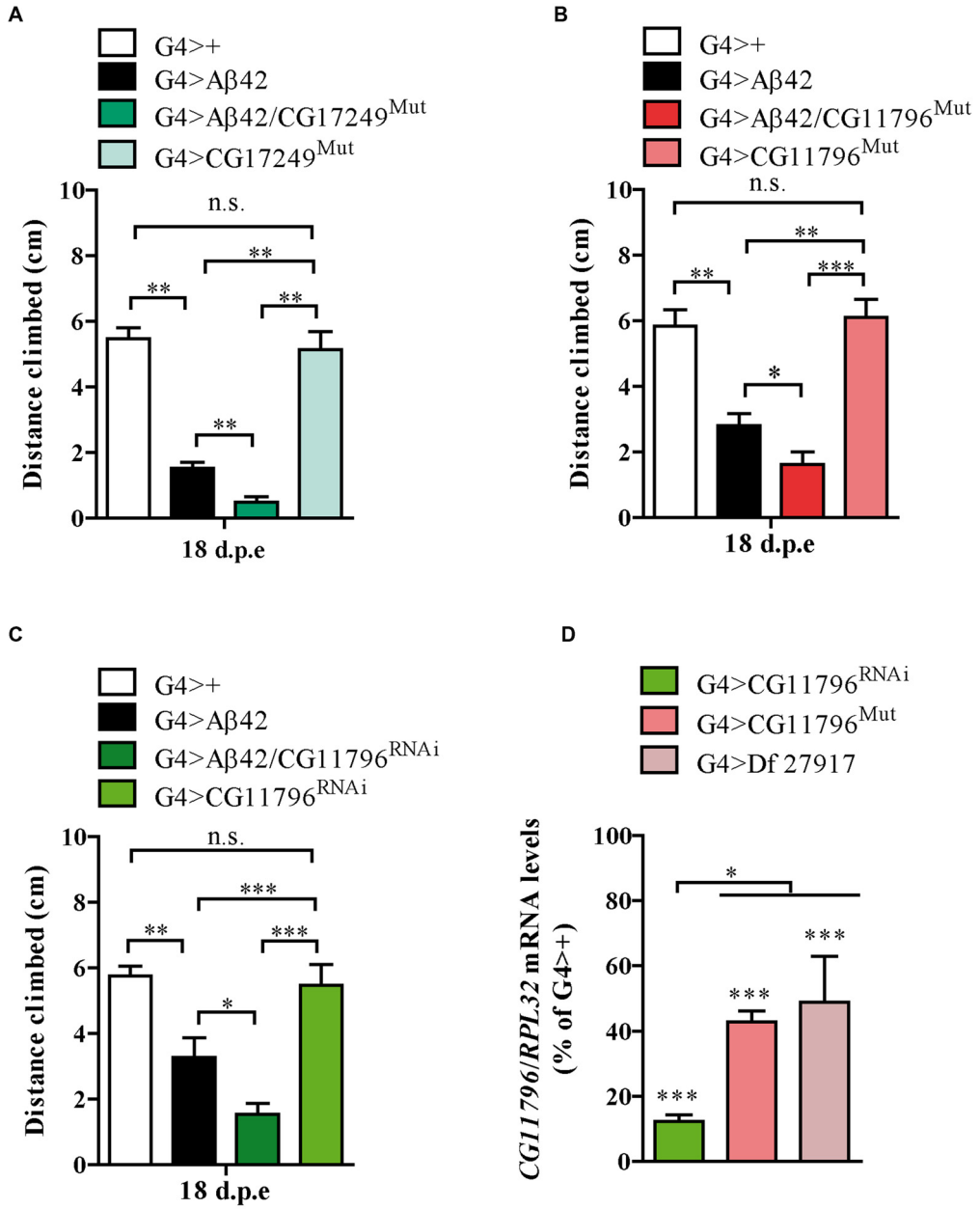


FIGURE 5 | Mutants and RNAi of candidate genes enhance Aβ toxicity. (A) *CG17249* hemizygous mutant (human ortholog, *PRCC*); **(B)** *CG11796* hemizygous mutant (human ortholog, *HPD*) and **(C)** *CG11796* RNAi. Mutant and RNAi lines had no effect in the absence of Aβ42 expression. Bars represent mean ± SEM from at least three independent biological experiments; $*p < 0.5$; $**p < 0.01$; $***p < 0.001$ (one-way ANOVA followed by LSD Fisher's test). **(D)** Quantification of *CG11796* endogenous mRNA showed a ∼40%–50% reduction in Df 27917 and CG11796^{Mut} lines, while for CG11796^{RNAi} a ∼ 85% reduction was observed. Brain samples were taken from 5 day-old flies and *RPL32* mRNA was used for normalization in each quantitative real-time PCR (qRT-PCR) assay. $***p < 0.001$ for Df 27917, CG11796^{Mut} and CG11796^{RNAi} as compared to G4>+. $*p < 0.05$ (RM one-way ANOVA followed by LSD Fisher's test from three independent biological experiments).

further study in the context of Aβ neurotoxicity. *HPD* encodes a highly conserved protein that catalyzes the conversion of 4-hydroxyphenylpyruvate to homogentisate, the second step in the tyrosine degradation pathway. Mutations in *HPD* cause the rare diseases Tyrosinemia type 3 and Hawkinsiuria. Tyrosinemia type 3 is autosomal recessive; patients show mental retardation and elevated levels of tyrosine and its derivatives in blood and urine due to HPPD Df (reviewed in Scott, 2006). Hawkinsinuria

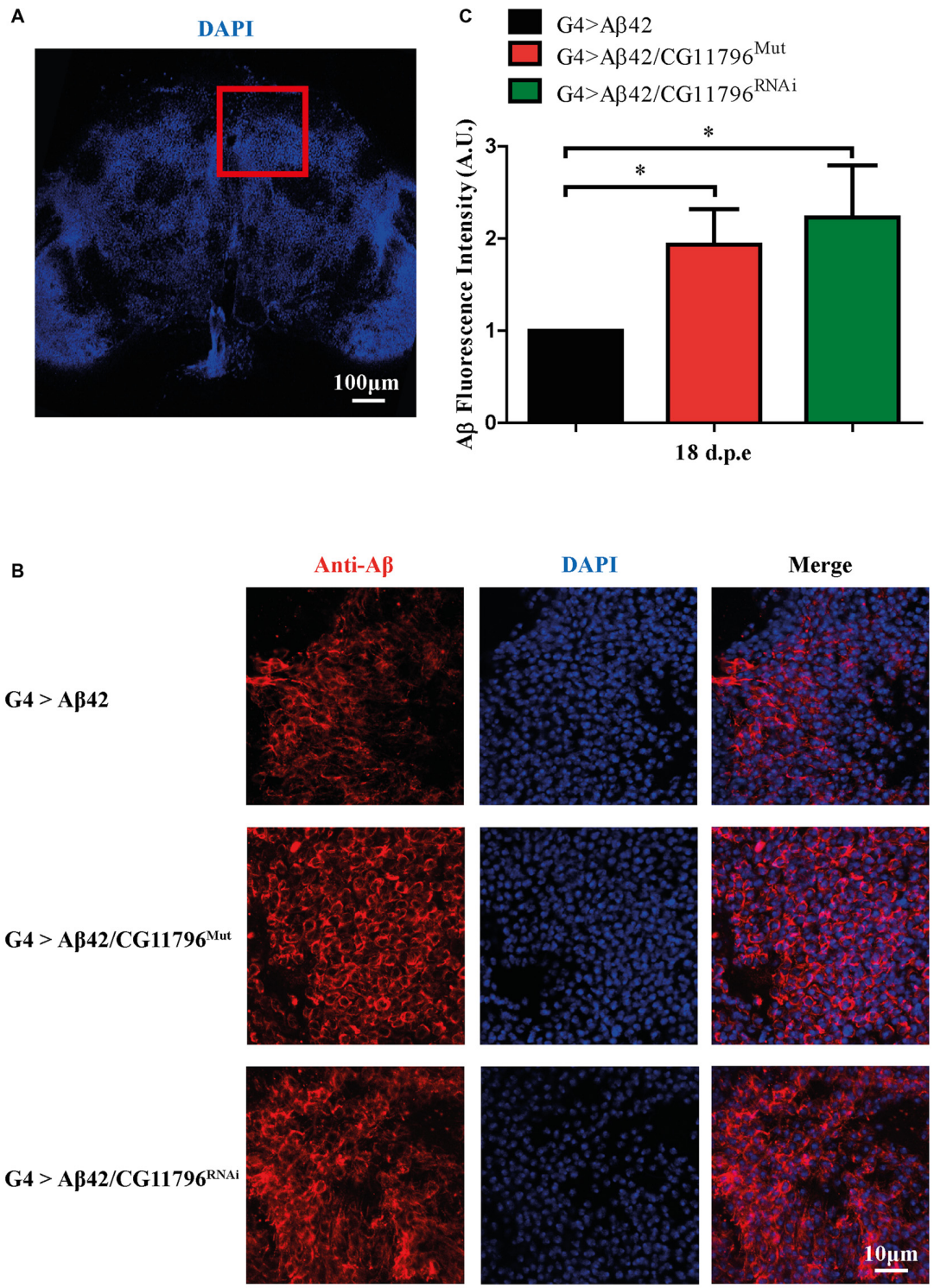


FIGURE 6 | Immunofluorescence of Aβ deposits in the brains of transgenic lines. (A) Representative image of a brain section at low magnification stained with DAPI. The red square depicts the region used for quantification in each genotype. Scale bar = 100 µm. **(B)** Representative images of the selected region as in panel **(A)** showing from left to right: anti-Aβ, DAPI nuclear staining and the merge of both signals. Scale bar = 10 µm. Genotypes G4 > Aβ42, G4 > Aβ42/CG11796^{Mut} and G4 > Aβ42/CG11796 ^{RNAi} are shown. **(C)** Quantification of Aβ fluorescence intensity normalized to G4 > Aβ42 in A.U. showing the increment induced by *CG11796* mRNA reduction. Bars represent the mean-ratio ± SEM of three independent experiments; *$p < 0.05$ (Wilcoxon test).

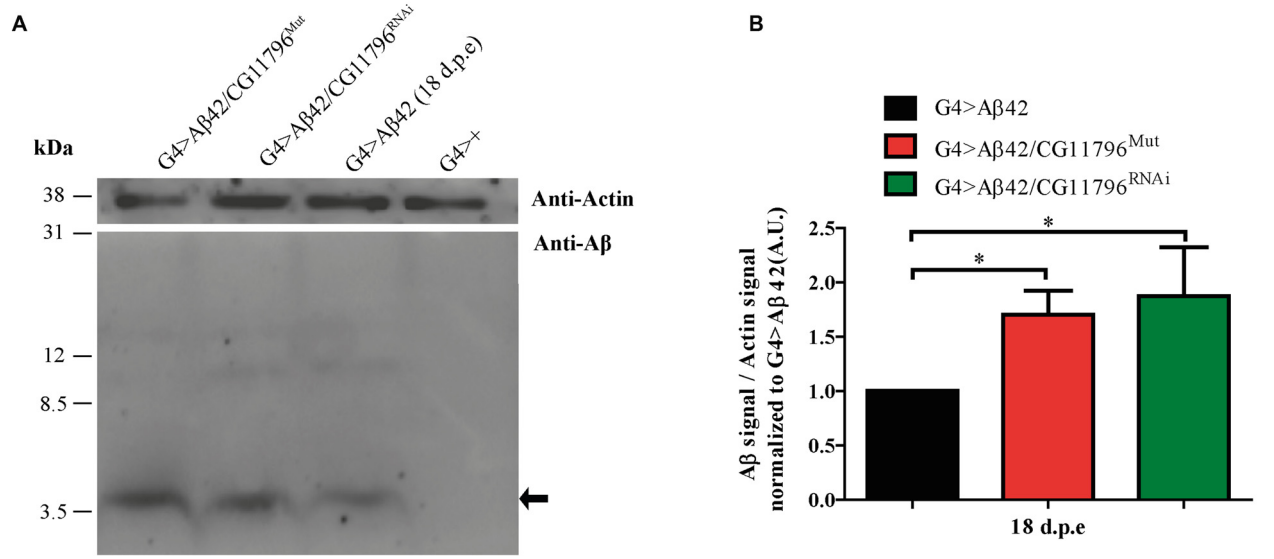


FIGURE 7 | Western blot of Aβ accumulation in the brain of *CG11796* RNAi and mutant lines. (A) Representative Western blot of fly brain homogenates in RIPA buffer at 18 d.p.e showing Aβ42 expression detected with monoclonal antibody 6E10. The arrow indicates Aβ42 monomers. Membranes were cut above the 31 kDa marker and probed with anti-actin for normalization. **(B)** Quantification of Aβ42 levels relative to actin in A.U. normalized to G4 > Aβ42 showing the increase of Aβ42 in *CG11796* mutant and RNAi lines. Bars represent the mean-ratio ± SEM of three independent experiments; $^*p < 0.05$ (Wilcoxon test).

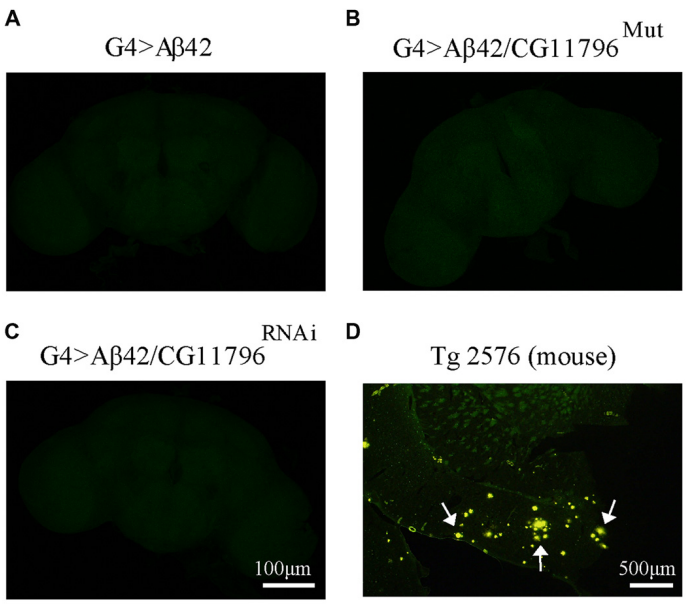


FIGURE 8 | Negative thioflavine S (ThS) staining of Aβ42 transgenic flies brains. (A–C) Representative images of ThS staining of fly brains at 18 d.p.e from G4 > Aβ42, G4 > Aβ42/CG11796^{Mut} and G4 > Aβ42/CG11796^{RNAi} showing no detection of amyloid fibrils. Scale bar = 100 μm. **(D)** A brain section of transgenic mouse Tg2576 showing ThS-positive plaques (arrows) is shown for comparison. Scale bar = 500 μm.

is autosomal dominant and characterized by metabolic acidosis and urinary excretion of "hawkinsin", a cyclic amino acid derived from quinolacetic acid produced by mutant HPPD (Brownlee et al., 2010). The mechanisms underlying mental retardation in Tyrosinemia are not known, yet an increase of acetylcholinesterase activity and energy metabolic impairment have been postulated (Ferreira et al., 2012, 2015). In addition, high tyrosine levels may reduce the activity of thiol-dependent

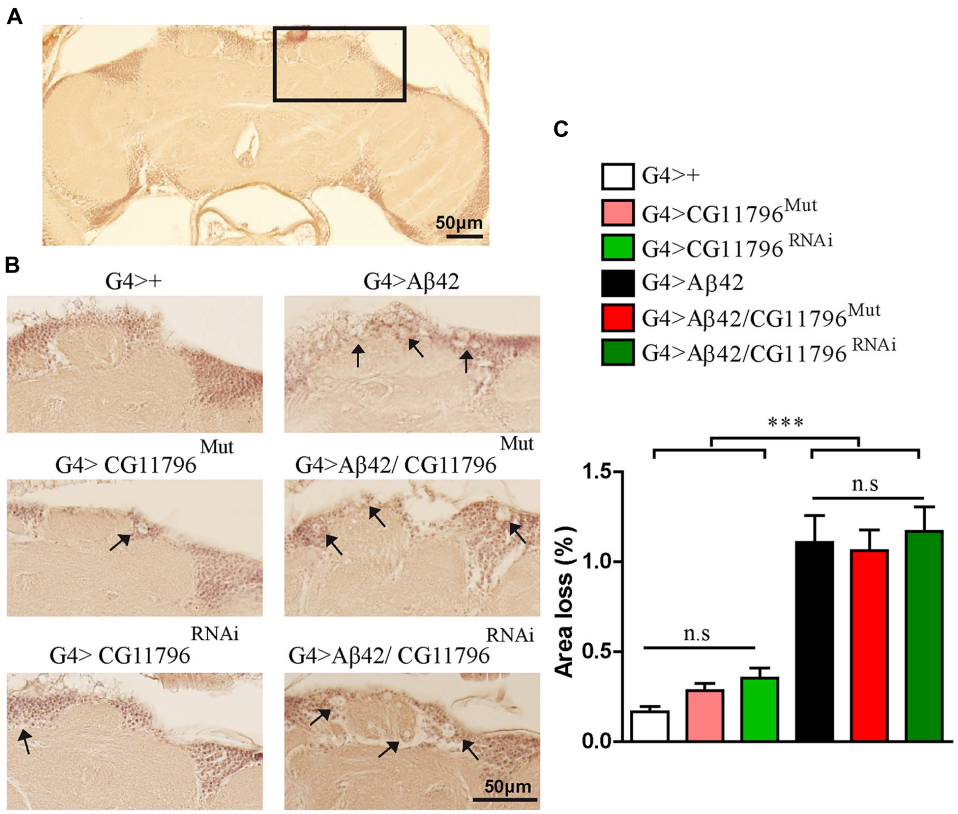


FIGURE 9 | Brain vacuolization in Aβ-expressing lines alone and in a background of *CG11796* Df. (A) Representative whole brain section of G4>+ stained with H&E used for tissue loss analysis by bright-field microscopy. The rectangle demarcates a typical area with a high number of neuronal bodies. **(B)** The region depicted in **(A)** is shown for each genotype. Arrows indicate vacuoles with a diameter of at least 3 μm. Scale bar = 50 μm. **(C)** Quantification of tissue loss in hemi-brains was calculated as the percentage of the section area occupied by vacuoles. Flies expressing Aβ42 showed increased vacuolization as compared to control flies G4>+. No differences were found in G4 > Aβ42/CG11796^{Mut} and G4 > Aβ42/CG11796^{RNAi} compared with G4 > Aβ42. ***$p < 0.001$ (one way ANOVA followed by Tukey's *post hoc* test).

creatine kinases (CK) leading to misbalance of a key ATP buffering and shuttling system (Wallimann et al., 2011; de Andrade et al., 2012). Interestingly, CK activity is reduced in AD brains as compared to age-matched controls and Aβ induces a reduction of CK activity in cultured neurons (Aksenov et al., 1998, 2000; David et al., 1998). Consistent with these findings, creatine accumulates in old transgenic mice expressing a mutant APP and in the hippocampus of AD patients (Gallant et al., 2006). Our finding that the partial Df of *HPD* ortholog promoted the accumulation of oligomeric Aβ42 provides a likely explanation for the worsening of age-dependent geotaxis performance. Yet, such degree of Aβ accumulation seems to be sufficient to impact negatively upon neuronal function without inducing gross neuropathological changes up to 18 d.p.e. With regard to possible mechanisms for Aβ accretion in the context of lower HPPD expression, the reduction in CK activity as a consequence of high tyrosine levels may accelerate Aβ aggregation or impair its clearance due to lower ATP availability and oxidative stress (Meyer et al., 2006). Moreover, Aβ42 oligomers induce oxidative stress (Butterfield et al., 2013) leading to a vicious cycle in disease progression. Alternatively, the possibility that a partial Df of HPPD is more directly involved in Aβ accumulation deserves further investigation. Inhibitors of HPPD such as nitisinone are used to treat patients with hereditary Tyrosinemia type 1 in which downstream metabolites of HPPD activity accumulate and are highly toxic to the kidney and liver (Mayorandan et al., 2014; Zeybek et al., 2015). Long-term outcome of patients under nitisinone treatment show a high frequency of progressive cognitive impairment that has been related with chronically elevated tyrosine levels (Masurel-Paulet et al., 2008; Thimm et al., 2012). Early reports on tyrosine levels in the cerebrospinal fluid of AD as compared with controls remain controversial (Degrell et al., 1989; Martinez et al., 1993) and there are no studies on the levels and/or activity of HPPD in AD. In light of our results regarding Aβ accumulation, such studies may be relevant to better understand the complex pathogenesis of AD.

In summary, our work describes the first genetic screen to search for modifiers of wild-type Aβ42 neurotoxicity in the CNS of *Drosophila* by exploring age-dependent alterations in a complex behavior. So far, this strategy has led us to identify candidate genes that warrant further research to determine their significance in sporadic AD.

AUTHOR CONTRIBUTIONS

LFB-C, MSM and NIB performed the experiments, analyzed results, drafted and revised the manuscript; MFC and LM designed the work, analyzed data, interpreted the results and revised the manuscript. EMC designed the work, analyzed data, interpreted the results and wrote the article.

FUNDING

This work was supported by grants from the Alzheimer's Association (IIRG 11-205127 to EMC), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT2013-0318 (to EMC), PICT2013-1382 (to MFC) and CONICET-PIP0378 (to LM).

ACKNOWLEDGMENTS

We thank Cecilia Rotondaro, Agata Fernández-Gamba and Luciana Pujol Lereis for their contributions to this work. Aβ42 transgenic fly was kindly provided by Dr. Mary Konsolaki (Rutgers University). Bloomington *Drosophila* Stock Center and the Vienna *Drosophila* RNAi Center provided the flies stocks. LFB-C fellowship is supported by ANPCyT; MSM and NIB fellowships are supported by CONICET. MFC, LM and EMC are Principal Investigators supported by CONICET.

REFERENCES

Aksenov, M., Aksenova, M., Butterfield, D. A., and Markesbery, W. R. (2000). Oxidative modification of creatine kinase BB in Alzheimer's disease brain. *J. Neurochem.* 74, 2520–2527. doi: 10.1046/j.1471-4159.2000.0742520.x

Aksenov, M. Y., Aksenova, M. V., Markesbery, W. R., and Butterfield, D. A. (1998). Amyloid β-peptide (1–40)-mediated oxidative stress in cultured hippocampal neurons. Protein carbonyl formation, CK BB expression and the level of Cu, Zn and Mn SOD mRNA. *J. Mol. Neurosci.* 10, 181–192. doi: 10.1007/bf02761773

Attrill, H., Falls, K., Goodman, J. L., Millburn, G. H., Antonazzo, G., Rey, A. J., et al. (2016). FlyBase: establishing a gene group resource for *Drosophila melanogaster*. *Nucl. Acids Res.* 44, D786–D792. doi: 10.1093/nar/gkv1046

Bennett, D. A., Schneider, J. A., Arvanitakis, Z., Kelly, J. F., Aggarwal, N. T., Shah, R. C., et al. (2006). Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology* 66, 1837–1844. doi: 10.1212/01.WNL.0000219668.47116.e6

Bouleau, S., and Tricoire, H. (2015). *Drosophila* models of Alzheimer's disease: advances, limits and perspectives. *J. Alzheimers Dis.* 45, 1015–1038. doi: 10.3233/JAD-142802

Brownlee, J. M., Heinz, B., Bates, J., and Moran, G. R. (2010). Product analysis and inhibition studies of a causative Asn to Ser variant of 4-hydroxyphenylpyruvate dioxygenase suggest a simple route to the treatment of Hawkinsinuria. *Biochemistry* 49, 7218–7226. doi: 10.1021/bi1008112

Butterfield, D. A., Swomley, A. M., and Sultana, R. (2013). Amyloid β-peptide (1–42)-induced oxidative stress in Alzheimer disease: importance in disease pathogenesis and progression. *Antioxid. Redox Signal.* 19, 823–835. doi: 10.1089/ars.2012.5027

Cacace, R., Sleegers, K., and Broeckhoven, C. V. (2016). Molecular genetics of early-onset alzheimer disease revisited. *Alzheimers Dement.* 12, 733–748. doi: 10.1016/j.jalz.2016.01.012

Campion, D., Flaman, J. M., Brice, A., Hannequin, D., Dubois, B., Martin, C., et al. (1995). Mutations of the presenilin I gene in families with early-onset alzheimer's disease. *Hum. Mol. Genet.* 4, 2373–2377. doi: 10.1093/hmg/4.12.2373

Cao, W., Song, H.-J., Gangi, T., Kelkar, A., Antani, I., Garza, D., et al. (2008). Identification of novel genes that modify phenotypes induced by Alzheimer's β-amyloid overexpression in *Drosophila*. *Genetics* 178, 1457–1471. doi: 10.1534/genetics.107.078394

Cook, K. (2016). *Bloomington Drosophila Stock Center at Indiana University.* Bloomington: Indiana University. Available online at: http://flystocks.bio.indiana.edu/Browse/df/dfkit.php. [Accessed on May 13, 2016].

Cook, R. K., Christensen, S. J., Deal, J. A., Coburn, R. A., Deal, M. E., Gresens, J. M., et al. (2012). The generation of chromosomal deletions to provide extensive coverage and subdivision of the *Drosophila melanogaster* genome. *Genome Biol.* 13:R21. doi: 10.1186/gb-2012-13-3-r21

Crowther, D. C., Kinghorn, K. J., Miranda, E., Page, R., Curry, J. A., Duthie, F. A. I., et al. (2005). Intraneuronal Aβ, non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease. *Neuroscience* 132, 123–135. doi: 10.1016/j.neuroscience.2004.12.025

David, S., Shoemaker, M., and Haley, B. E. (1998). Abnormal properties of creatine kinase in Alzheimer's disease brain: correlation of reduced enzyme activity and active site photolabeling with aberrant cytosol-membrane partitioning. *Brain. Res. Mol.* 54, 276–287. doi: 10.1016/s0169-328x(97)00343-4

de Andrade, R. B., Gemelli, T., Rojas, D. B., Funchal, C., Dutra-Filho, C. S., Wannmacher, C. M., et al. (2012). Tyrosine impairs enzymes of energy metabolism in cerebral cortex of rats. *Mol. Cell. Biochem.* 364, 253–261. doi: 10.1007/s11010-012-1225-y

De Strooper, B., and Karran, E. (2016). The cellular phase of Alzheimer's disease. *Cell.* 164, 603–615. doi: 10.1016/j.cell.2015.12.056

Degrell, I., Hellsing, K., Nagy, E., and Niklasson, F. (1989). Amino acid concentrations in cerebrospinal fluid in presenile and senile dementia of Alzheimer type and multi-infarct dementia. *Arch. Gerontol. Geriatr.* 9, 123–135. doi: 10.1016/0167-4943(89)90033-2

Fernandez-Funez, P., de Mena, L., and Rincon-Limas, D. E. (2015). Modeling the complex pathology of Alzheimer's disease in *Drosophila*. *Exp. Neurol.* 274, 58–71. doi: 10.1016/j.expneurol.2015.05.013

Ferreira, G. K., Carvalho-Silva, M., Gomes, L. M., Scaini, G., Teixeira, L. J., Mota, I. T., et al. (2015). The characterization of neuroenergetic effects of chronic L-tyrosine administration in young rats: evidence for striatal susceptibility. *Metab. Brain Dis.* 30, 215–221. doi: 10.1007/s11011-014-9615-3

Ferreira, G. K., Carvalho-Silva, M., Gonçalves, C. L., Vieira, J. S., Scaini, G., Ghedim, F. V., et al. (2012). L-tyrosine administration increases acetylcholinesterase activity in rats. *Neurochem. Int.* 61, 1370–1374. doi: 10.1016/j.neuint.2012.09.017

Finelli, A., Kelkar, A., Song, H.-J., Yang, H., and Konsolaki, M. (2004). A model for studying Alzheimer's Aβ42-induced toxicity in *Drosophila melanogaster*. *Mol. Cell. Neurosci.* 26, 365–375. doi: 10.1016/j.mcn.2004.03.001

Gallant, M., Rak, M., Szeghalmi, A., Del Bigio, M. R., Westaway, D., Yang, J., et al. (2006). Focally elevated creatine detected in amyloid precursor protein (APP) transgenic mice and Alzheimer disease brain tissue. *J. Biol. Chem.* 281, 5–8. doi: 10.1074/jbc.C500244200

Gargano, J. W., Martin, I., Bhandari, P., and Grotewiel, M. S. (2005). Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in *Drosophila*. *Exp. Gerontol.* 40, 386–395. doi: 10.1016/j.exger.2005.02.005

Hatakeyama, S., Yada, M., Matsumoto, M., Ishida, N., and Nakayama, K. I. (2001). U box proteins as a new family of ubiquitin-protein ligases. *J. Biol. Chem.* 276, 33111–33120. doi: 10.1074/jbc.m102755200

Hegele, A., Kamburov, A., Grossmann, A., Sourlis, C., Wowro, S., Weimann, M., et al. (2012). Dynamic protein-protein interaction wiring of the human spliceosome. *Mol. Cell* 45, 567–580. doi: 10.1016/j.molcel.2011.12.034

Jackson, G. R. (2008). Guide to understanding *Drosophila* models of neurodegenerative diseases. *PLoS Biol.* 6:e53. doi: 10.1371/journal.pbio.0060053

Kandimalla, R. J. L., Prabhakar, S., Binukumar, B. K., Wani, W. Y., Gupta, N., Sharma, D. R., et al. (2011). Apo-E4 allele in conjunction with Aβ42 and tau

in CSF: biomarker for Alzheimers disease. *Curr. Alzheimer Res.* 8, 187–196. doi: 10.2174/156720511795256071

Kandimalla, R. J. L., Wani, W. Y., Binukumar, B. K., and Gill, K. D. (2012). siRNA against presenilin 1 (PS1) down regulates amyloid β42 production in IMR-32 cells. *J. Biomed. Sci.* 19:2. doi: 10.1186/1423-0127-19-2

Karran, E., Mercken, M., and De Strooper, B. (2011). The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat. Rev. Drug Discov.* 10, 698–712. doi: 10.1038/nrd3505

Khan, S., and Bloom, G. S. (2016). Tau: the center of a signaling nexus in Alzheimer's disease. *Front. Neurosci.* 10:31. doi: 10.3389/fnins.2016.00031

Lenz, S., Karsten, P., Schulz, J. B., and Voigt, A. (2013). *Drosophila* as a screening tool to study human neurodegenerative diseases. *J. Neurochem.* 127, 453–460. doi: 10.1111/jnc.12446

Lim, J. Y., Ott, S., and Crowther, D. C. (2016). *Drosophila melanogaster* as a model for studies on the early stages of Alzheimer's disease. *Methods Mol. Biol.* 1303, 227–239. doi: 10.1007/978-1-4939-2627-5_13

Liu, H., Han, M., Li, Q., Zhang, X., Wang, W. A., and Huang, F. D. (2015). Automated rapid iterative negative geotaxis assay and its use in a genetic screen for modifiers of Aβ42-induced locomotor decline in *Drosophila*. *Neurosci. Bull.* 31, 541–549. doi: 10.1007/s12264-014-1526-0

Maarouf, C. L., Daugs, I. D., Kokjohn, T. A., Walker, D. G., Hunter, J. M., Kruchowsky, J. C., et al. (2011). Alzheimer's disease and non-demented high pathology control nonagenarians: comparing and contrasting the biochemistry of cognitively successful aging. *PLoS One* 6:e27291. doi: 10.1371/journal.pone.0027291

Martinez, M., Frank, A., Diez-Tejedor, E., and Hernanz, A. (1993). Amino acid concentrations in cerebrospinal fluid and serum in Alzheimer's disease and vascular dementia. *J. Neural Transm. Park. Dis. Dement. Sect.* 6, 1–9. doi: 10.1007/bf02252617

Masurel-Paulet, A., Poggi-Bach, J., Rolland, M. O., Bernard, O., Guffon, N., Dobbelaere, D., et al. (2008). NTBC treatment in tyrosinaemia type I: long-term outcome in French patients. *J. Inherit. Metab. Dis.* 31, 81–87. doi: 10.1007/s10545-008-0793-1

Mayorandan, S., Meyer, U., Gokcay, G., Segarra, N. G., de Baulny, H. O., van Spronsen, F., et al. (2014). Cross-sectional study of 168 patients with hepatorenal tyrosinaemia and implications for clinical practice. *Orphanet. J. Rare Dis.* 9:107. doi: 10.1186/s13023-014-0107-7

Meyer, L. E., Machado, L. B., Santiago, A. P., da-Silva, W. S., De Felice, F. G., Holub, O., et al. (2006). Mitochondrial creatine kinase activity prevents reactive oxygen species generation: antioxidant role of mitochondrial kinase-dependent ADP re-cycling activity. *J. Biol. Chem.* 281, 37361–37371. doi: 10.1074/jbc.m604123200

Moloney, A., Sattelle, D. B., Lomas, D. A., and Crowther, D. C. (2010). Alzheimer's disease: insights from *Drosophila melanogaster* models. *Trends Biochem. Sci.* 35, 228–235. doi: 10.1016/j.tibs.2009.11.004

Moran, M. T., Tare, M., Kango-Singh, M., and Singh, C. (2013). Homeotic gene teashirt (tsh) has a neuroprotective function in amyloid-β 42 mediated neurodegeneration. *PLoS One* 8:e80829. doi: 10.1371/journal.pone.0080829

Newman, M., Musgrave, I. F., Musgrave, F. I., and Lardelli, M. (2007). Alzheimer disease: amyloidogenesis, the presenilins and animal models. *Biochim. Biophys. Acta* 1772, 285–297. doi: 10.1016/j.bbadis.2006.12.001

Perez-Nievas, B. G., Stein, T. D., Tai, H. C., Dols-Icardo, O., Scotton, T. C., Barroeta-Espar, I., et al. (2013). Dissecting phenotypic traits linked to human resilience to Alzheimer's pathology. *Brain* 136, 2510–2526. doi: 10.1093/brain/awt171

Prüßing, K., Voigt, A., and Schulz, J. B. (2013). *Drosophila melanogaster* as a model organism for Alzheimer's disease. *Mol. Neurodegener.* 35, 1–11. doi: 10.1186/1750-1326-8-35

Pushkarsky, T., Yurchenko, V., Vanpouille, C., Brichacek, B., Vaisman, I., Hatakeyama, S., et al. (2005). Cell surface expression of CD147/EMMPRIN is regulated by cyclophilin 60. *J. Biol. Chem.* 280, 27866–27871. doi: 10.1074/jbc.M503770200

Rival, T., Page, R. M., Chandraratna, D. S., Sendall, T. J., Ryder, E., Liu, B., et al. (2009). Fenton chemistry and oxidative stress mediate the toxicity of the β-amyloid peptide in a *Drosophila* model of Alzheimer's disease. *Eur. J. Neurosci.* 29, 1335–1347. doi: 10.1111/j.1460-9568.2009.06701.x

Sarantseva, S., Timoshenko, S., Bolshakova, O., Karaseva, E., Rodin, D., Schwarzman, A. L., et al. (2009). Apolipoprotein E-mimetics inhibit neurodegeneration and restore cognitive functions in a transgenic *Drosophila* model of Alzheimer's disease. *PLoS One* 4:e8191. doi: 10.1371/journal.pone.0008191

Scheltens, P., Blennow, K., Breteler, M. M. B., de Strooper, B., Frisoni, G. B., Salloway, S., et al. (2016). Alzheimer's disease. *Lancet* 338, 505–517. doi: 10.1016/s0140-6736(15)01124-1

Scott, C. R. (2006). The genetic tyrosinemias. *Am. J. Med. Genet. C Semin. Med. Genet.* 142C, 121–126. doi: 10.1007/978-1-4614-6430-3_279-1

Shulman, J. M., Imboywa, S., Giagtzoglou, N., Powers, M. P., Hu, Y., Devenport, D., et al. (2014). Functional screening in *Drosophila* identifies Alzheimer's disease susceptibility genes and implicates Tau-mediated mechanisms. *Hum. Mol. Genet.* 23, 870–877. doi: 10.1093/hmg/ddt478

Skalsky, Y. M., Ajuh, P. M., Parker, C., Lamond, A. I., Goodwin, G., and Cooper, C. S. (2001). PRCC, the commonest TFE3 fusion partner in papillary renal carcinoma is associated with pre-mRNA splicing factors. *Oncogene.* 20, 178–187. doi: 10.1038/sj.onc.1204056

Steffener, J., and Stern, Y. (2012). Exploring the neural basis of cognitive reserve in aging. *Biochim. Biophys. Acta* 1822, 467–473. doi: 10.1016/j.bbadis.2011.09.012

Tan, L., Schedl, P., Song, H.-J., Garza, D., and Konsolaki, M. (2008). The toll→NFkB signaling pathway mediates the neuropathological effects of the human Alzheimer's Aβ42 Polypeptide in *Drosophila*. *PLoS One.* 3:e3966. doi: 10.1371/journal.pone.0003966

Thimm, E., Richter-Werkle, R., Kamp, G., Molke, B., Herebian, D., Klee, D., et al. (2012). Neurocognitive outcome in patients with hypertyrosinemia type I after long-term treatment with NTBC. *J. Inherit. Metab. Dis.* 35, 263–268. doi: 10.1007/s10545-011-9394-5

Wallimann, T., Tokarska-Schlattner, M., and Schlattner, U. (2011). The creatine kinase system and pleiotropic effects of creatine. *Amino Acids* 40, 1271–1296. doi: 10.1007/s00726-011-0877-3

Zeybek, A. C., Kiykim, E., Soyucen, E., Cansever, S., Altay, S., Zubarioglu, T., et al. (2015). Hereditary tyrosinemia type 1 in Turkey: twenty year single-center experience. *Pediatr. Int.* 57, 281–289. doi: 10.1111/ped.12503

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

GENERAL COMMENTARY
published: 03 August 2016
doi: 10.3389/fnagi.2016.00182

Commentary: XBP-1 Is a Cell-Nonautonomous Regulator of Stress Resistance and Longevity

Gabriela Martínez[1, 2, 3, 4], *Claudia Duran-Aniotz*[1, 2, 3], *Felipe Cabral-Miranda*[1, 2, 3] *and Claudio Hetz*[1, 2, 3, 5, 6*]

[1] Center for Geroscience, Brain Health and Metabolism, Santiago, Chile, [2] Biomedical Neuroscience Institute, Faculty of Medicine, University of Chile, Santiago, Chile, [3] Program of Cellular and Molecular Biology, Institute of Biomedical Sciences, University of Chile, Santiago, Chile, [4] Center for Integrative Biology, Universidad Mayor, Santiago, Chile, [5] Buck Institute for Research on Aging, Novato, CA, USA, [6] Department of Immunology and Infectious diseases, Harvard School of Public Health, Boston, MA, USA

Keywords: aging, proteostasis cell stress and aging, unfolded protein response (UPR), cell-nonautonomous, proteostasis deficiencies, protein misfolding and disease, protein misfolding disease

A commentary on

XBP-1 Is a Cell-Nonautonomous Regulator of Stress Resistance and Longevity
by Taylor, R. C., and Dillin A. (2013). Cell 153, 1435–1447. doi: 10.1016/j.cell.2013.05.042

OPEN ACCESS

Edited by:
Agustin Ibanez, Instituto de Neurología Cognitiva, Argentina

Reviewed by:
Diego Ruano, University of Seville, Spain
Emmanuel Moyse, François Rabelais University, France

***Correspondence:**
Claudio Hetz
clahetz@med.uchile.cl

Received: *23 May 2016*
Accepted: *14 July 2016*
Published: *03 August 2016*

Citation:
Martínez G, Duran-Aniotz C, Cabral-Miranda F and Hetz C (2016) Commentary: XBP-1 Is a Cell-Nonautonomous Regulator of Stress Resistance and Longevity. Front. Aging Neurosci. 8:182. doi: 10.3389/fnagi.2016.00182

The life expectancy in the world's population is increasing, highlighting the need of better understanding of the cellular and molecular pathways that drive the aging process. Because aging is the major risk factor to develop neurodegenerative conditions such as Alzheimer's and Parkinson's disease, the number of patients affected is constantly increasing, representing a major social and economic problem. Importantly, abnormal protein aggregation is a transversal pathological event of most aging-related brain diseases, suggesting that the ability of neurons to handle alterations in the proteome is specifically altered (Kaushik and Cuervo, 2015). Several hallmarks of aging have been identified at the cellular and molecular level (Lopez-Otin et al., 2013; Kennedy et al., 2014), highlighting alterations in protein homeostasis or proteostasis. In fact, studies in simple model organisms indicate that the buffering capacity of the proteostasis network (PN) is reduced during aging (Douglas and Dillin, 2010; Mardones et al., 2015). The PN can be decomposed in different interrelated sub-networks including mechanisms responsible for protein synthesis, translation, folding, trafficking, quality control, secretion, and degradation (Balch et al., 2008). Sustained dysfunction of one or more components of the PN may translate into cell dysfunction and even proteotoxicity (**Figure 1**).

Around 30% of the total proteome is synthetized at the endoplasmic reticulum (ER), an essential compartment involved in calcium handling, lipid synthesis among other functions. Different physiological and pathological stimuli can alter the function of this organelle, resulting in the accumulation of misfolded proteins. Importantly ER stress has been proposed as a central driver of several neurodegenerative conditions (Hetz and Mollereau, 2014). ER stress triggers the activation of the unfolded protein response (UPR), a central homeostatic pathway that orchestrates cells adaptation (Hetz et al., 2015). Studies in *Caenorhabditis elegans* and rats indicate that the activity of the UPR is drastically ablated during aging (Paz Gavilan et al., 2006; Naidoo et al., 2008; Ben-Zvi et al., 2009; Gavilan et al., 2009; Taylor and Dillin, 2013). The UPR is mediated by three main stress sensors located at the ER membrane including ATF6, PERK, and IRE1 (Ron and Walter, 2007). In brief, activation of IRE1 controls to the expression of the transcription factor XBP1s, leading to the upregulation of genes related with protein quality control, folding, ERAD, among other targets

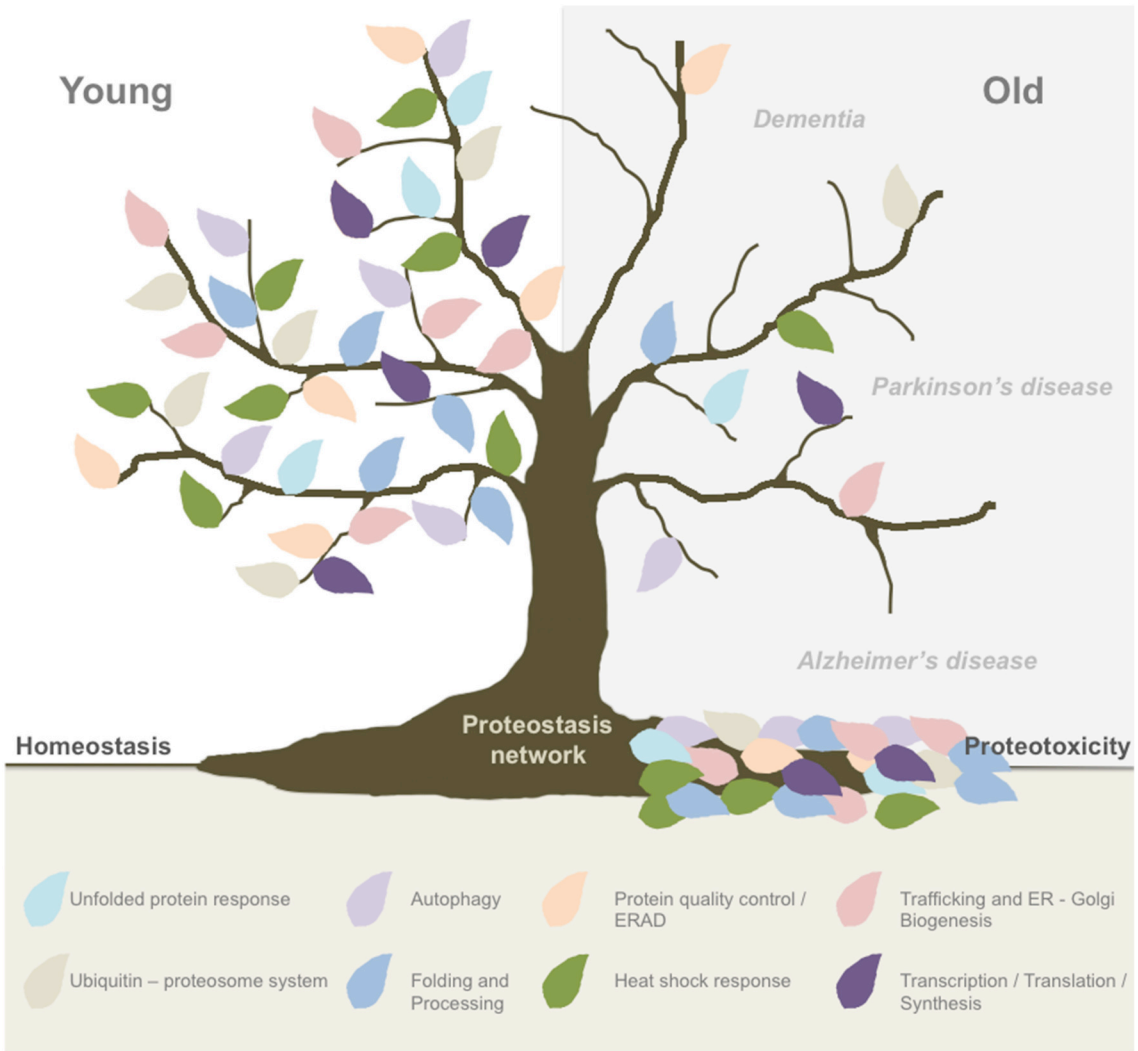


FIGURE 1 | Global proteostasis network impairment during aging. Aging is the main risk factor to develop most neurodegenerative conditions and new evidence has pointed out to a progressive decline in the buffering capacity of the proteostasis network (PN) to handle cellular stress. The PN is formed by different interrelated sub-networks including mechanisms responsible for protein translation, folding, synthesis, protein quality control, trafficking, secretion, and degradation (ERAD, proteasome, autophagy). Proteostasis breakdown during aging may result in proteotoxicity and the development of neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

(Hetz et al., 2015). PERK phosphorylates eIF2α; inhibiting the translation of proteins into the ER, in addition to induce the expression of the transcription factor ATF4 regulating genes involved in the antioxidant response, amino acid metabolism and folding. Under irreversible ER stress ATF4 is essential to trigger apoptosis. ATF6 encodes a transcription factor in its cytosolic domain that upon processing is realized to control gene expression. Altogether, the activation of the UPR enforces adaptive mechanisms to sustain proteostasis or trigger cell demise when protein misfolding cannot be mitigated determining cell fate.

Several studies in model organisms have uncovered the significance of UPR signaling to the aging process. IRE1 is the only ER stress sensor expressed in yeast and contributes to lifespan extension (Labunskyy et al., 2014), consistent with the fact that UPR activation in this organism is a relevant feature involved in the health span control triggered by caloric restriction (Choi et al., 2013). Similarly, genetic modifications that enhance the activity of the UPR improve replicative lifespan in *Saccharomyces cerevisiae* (Cui et al., 2015). Studies in *C. elegans* demonstrated that ablating the expression of XBP1 reduces life expectancy, associated with altered FOXO and insulin/IGF-1 signaling, a canonical aging pathway (Henis-Korenblit et al., 2010). Importantly, another report indicated that the ectopic expression of XBP1s in neurons has a significant effect in increasing lifespan in *C. elegans* (around 30%), representing one of the strongest aging modulator described so far in this specie (Taylor and Dillin, 2013). In *D. melanogaster*, the occurrence

of ER stress and chronic inflammation alters the stem cell pool in the gut, affecting intestinal homeostasis during aging (Wang et al., 2014). Unexpectedly, a recent study indicated that chronic PERK signaling limits lifespan by controlling intestinal homeostasis, having important consequences to organismal health (Wang et al., 2015). In mammals, it was reported that the capacity to response to ER stress and activate IRE1 is attenuated in macrophages during aging, increasing the susceptibility to apoptosis (Song et al., 2013). Accordantly, aged rats present more pro-apoptotic UPR components as opposed to adaptive mediators such as BIP, calnexin, and PDI after ER stress induction (Paz Gavilan et al., 2006; Naidoo et al., 2008). In contrast, during the aging process B cells, osteoclasts, adipocyte tissue, the retina, and muscle experience elevated levels of ER stress and UPR activation (Chalil et al., 2015; Ghosh et al., 2015; Lenox et al., 2015; Baehr et al., 2016; Kannan et al., 2016). These observations suggest that aging maybe associated with accumulative damage to the ER rather than an attenuation of UPR responses. However, the role of ER proteostasis impartment in mammalian aging needs to be functionally defined.

The UPR is emerging as a key player in the integration of systemic responses to handle proteostasis alterations at the whole organism, governed by the central nervous system (Sun et al., 2012; Taylor and Dillin, 2013). In addition to regulate the intrinsic capacity of the cell to respond to ER stress, activation of IRE1 in neurons engages an organismal reaction to promote stress resistance and longevity on a cell-nonautonomous manner (Taylor and Dillin, 2013). Interestingly, the activation of XBP1s in neurons *per se* was irrelevant to sustain organismal homeostasis, suggesting that the nervous system operates as a global adjustor of proteostasis, where the effectors in terms of enforcing aging resistance operate in the periphery, highlighting the intestine. Importantly, other studies have shown a similar mode of control for the heat shock response and the innate immunity in *C. elegans* (reviewed in Mardones et al., 2015). Similarly, in flies activation of PERK engages cell-nonautonomous responses in the gut during aging (Wang et al., 2015). The concept cell-nonautonomous UPR was recently validated in mammals, where the expression of XBP1s in the hypothalamus propagates signals to the periphery (i.e., the liver) to adjust energy metabolism (Williams et al., 2014). However, the specific mechanism of proteostasis control in mammals and the neuronal circuits mediating the propagation of UPR signals between cells remain to be determined. Importantly, in *C. elegans* the propagation of ER stress signals to the periphery depends on neurotransmitters, suggesting that signaling mechanisms may mediate the activation of UPR-like responses in the targeted tissue probably on a stress-independent manner (Taylor and Dillin, 2013). In this line, we recently reported that XBP1s has a novel function in controlling synaptic plasticity and behavior in mammals, where growth factors like BDNF can engage the pathway (Martinez et al., 2016).

Although several studies are placing the ER PN as a relevant adjustor of organismal aging in several species, its actual impact to human aging remains to be established. Many important questions need to be solved in this emerging field: Why is the UPR buffering capacity attenuated during aging? How does the nervous system control organismal proteostasis? Is there a connection between ER stress and aging in protein misfolding disorders affecting the nervous system? Can we exploit the control of cell-nonautonomous UPR as a therapeutic strategy to delay aging? Importantly, recent studies suggest that oxidative damage could directly modify UPR stress sensors, ablating adaptive responses (Nakato et al., 2015). In addition, the redox status of the ER is altered during aging in *C. elegans*, suggesting that intrinsic physiological alterations to this subcellular compartment may underlay the reduced capacity of the pathway to handle proteostasis alterations when cells get old (Kirstein et al., 2015). Several novel drugs are available to fine-tune the UPR and reduce ER stress levels (Hetz et al., 2013), which promises new avenues to intervene brain aging which may reduce the risk to develop neurodegenerative diseases, improving health span.

AUTHOR CONTRIBUTIONS

GM: conceptualization, editing, and writing of the manuscript. CD: editing of manuscript, FC: editing of manuscript. CH: conceptualization, editing, and writing of the manuscript.

ACKNOWLEDGMENTS

This work is funded by Millennium Institute No. P09-015-F, and FONDAP 15150012, the Frick Foundation 20014-15, ALS Therapy Alliance 2014-F-059, Muscular Dystrophy Association 382453, CONICYT-USA 2013-0003, Michael J. Fox Foundation for Parkinson's Research – Target Validation grant No. 9277, COPEC-UC Foundation 2013.R.40, Ecos-Conicyt C13S02, FONDECYT no. 1140549, Office of Naval Research-Global (ONR-G) N62909-16-1-2003 and ALSRP Therapeutic Idea Award AL150111 (CH). FONDECYT No. 3150637 (GM) and FONDECYT No. 3140466 (CD).

REFERENCES

Baehr, L. M., West, D. W., Marcotte, G., Marshall, A. G., De Sousa, L. G., Baar, K., et al. (2016). Age-related deficits in skeletal muscle recovery following disuse are associated with neuromuscular junction instability and ER stress, not impaired protein synthesis. *Aging (Albany. NY).* 8, 127–146. doi: 10.18632/aging.100879

Balch, W. E., Morimoto, R. I., Dillin, A., and Kelly, J. W. (2008). Adapting proteostasis for disease intervention. *Science* 319, 916–919. doi: 10.1126/science.1141448

Ben-Zvi, A., Miller, E. A., and Morimoto, R. I. (2009). Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14914–14919. doi: 10.1073/pnas.0902882106

Chalil, S., Jaspers, R. T., Manders, R. J., Klein-Nulend, J., Bakker, A. D., and Deldicque, L. (2015). Increased endoplasmic reticulum stress in mouse osteocytes with aging alters Cox-2 response to mechanical stimuli. *Calcif. Tissue Int.* 96, 123–128. doi: 10.1007/s00223-014-9944-6

Choi, K. M., Kwon, Y. Y., and Lee, C. K. (2013). Characterization of global gene expression during assurance of lifespan extension by caloric restriction

in budding yeast. *Exp. Gerontol.* 48, 1455–1468. doi: 10.1016/j.exger.2013.10.001

Cui, H. J., Liu, X. G., McCormick, M., Wasko, B. M., Zhao, W., He, X., et al. (2015). PMT1 deficiency enhances basal UPR activity and extends replicative lifespan of *Saccharomyces cerevisiae*. *Age (Dordr).* 37, 9788. doi: 10.1007/s11357-015-9788-7

Douglas, P. M., and Dillin, A. (2010). Protein homeostasis and aging in neurodegeneration. *J. Cell Biol.* 190, 719–729. doi: 10.1083/jcb.201005144

Gavilan, M. P., Pintado, C., Gavilan, E., Jimenez, S., Rios, R. M., Vitorica, J., et al. (2009). Dysfunction of the unfolded protein response increases neurodegeneration in aged rat hippocampus following proteasome inhibition. *Aging Cell* 8, 654–665. doi: 10.1111/j.1474-9726.2009.00519.x

Ghosh, A. K., Garg, S. K., Mau, T., O'Brien, M., Liu, J., and Yung, R. (2015). Elevated endoplasmic reticulum stress response contributes to adipose tissue inflammation in aging. *J. Gerontol. A Biol. Sci. Med. Sci.* 70, 1320–1329. doi: 10.1093/gerona/glu186

Henis-Korenblit, S., Zhang, P., Hansen, M., McCormick, M., Lee, S. J., Cary, M., et al. (2010). Insulin/IGF-1 signaling mutants reprogram ER stress response regulators to promote longevity. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9730–9735. doi: 10.1073/pnas.1002575107

Hetz, C., Chevet, E., and Harding, H. P. (2013). Targeting the unfolded protein response in disease. *Nat. Rev. Drug Discov.* 12, 703–719. doi: 10.1038/nrd3976

Hetz, C., Chevet, E., and Oakes, S. A. (2015). Proteostasis control by the unfolded protein response. *Nat. Cell Biol.* 17, 829–838. doi: 10.1038/ncb3184

Hetz, C., and Mollereau, B. (2014). Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. *Nat. Rev. Neurosci.* 15, 1–19. doi: 10.1038/nrn3689

Kannan, S., Dawany, N., Kurupati, R., Showe, L. C., and Ertl, H. C. (2016). Age-related changes in the transcriptome of antibody-secreting cells. *Oncotarget* 7, 13340–13353. doi: 10.18632/oncotarget.7958

Kaushik, S., and Cuervo, A. M. (2015). Proteostasis and aging. *Nat. Med.* 21, 1406–1415. doi: 10.1038/nm.4001

Kennedy, B. K., Berger, S. L., Brunet, A., Campisi, J., Cuervo, A. M., Epel, E. S., et al. (2014). Geroscience: linking aging to chronic disease. *Cell* 159, 709–713. doi: 10.1016/j.cell.2014.10.039

Kirstein, J., Morito, D., Kakihana, T., Sugihara, M., Minnen, A., Hipp, M. S., et al. (2015). Proteotoxic stress and ageing triggers the loss of redox homeostasis across cellular compartments. *EMBO J.* 34, 2334–2349. doi: 10.15252/embj.201591711

Labunskyy, V. M., Gerashchenko, M. V., Delaney, J. R., Kaya, A., Kennedy, B. K., Kaeberlein, M., et al. (2014). Lifespan extension conferred by endoplasmic reticulum secretory pathway deficiency requires induction of the unfolded protein response. *PLoS Genet.* 10:e1004019. doi: 10.1371/journal.pgen.1004019

Lenox, A. R., Bhootada, Y., Gorbatyuk, O., Fullard, R., and Gorbatyuk, M. (2015). Unfolded protein response is activated in aged retinas. *Neurosci. Lett.* 609, 30–35. doi: 10.1016/j.neulet.2015.10.019

Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. *Cell* 153, 1194–1217. doi: 10.1016/j.cell.2013.05.039

Mardones, P., Martinez, G., and Hetz, C. (2015). Control of systemic proteostasis by the nervous system. *Trends Cell Biol.* 25, 1–10. doi: 10.1016/j.tcb.2014.08.001

Martinez, G., Vidal, R. L., Mardones, P., Serrano, F. G., Ardiles, A. O., Wirth, C., et al. (2016). Regulation of memory formation by the transcription factor XBP1. *Cell Rep.* 14, 1382–1394. doi: 10.1016/j.celrep.2016.01.028

Naidoo, N., Ferber, M., Master, M., Zhu, Y., and Pack, A. I. (2008). Aging impairs the unfolded protein response to sleep deprivation and leads to proapoptotic signaling. *J. Neurosci.* 28, 6539–6548. doi: 10.1523/JNEUROSCI.5685-07.2008

Nakato, R., Ohkubo, Y., Konishi, A., Shibata, M., Kaneko, Y., Iwawaki, T., et al. (2015). Regulation of the unfolded protein response via S-nitrosylation of sensors of endoplasmic reticulum stress. *Sci. Rep.* 5:14812. doi: 10.1038/srep14812

Paz Gavilan, M., Vela, J., Castano, A., Ramos, B., del Rio, J. C., Vitorica, J., et al. (2006). Cellular environment facilitates protein accumulation in aged rat hippocampus. *Neurobiol. Aging* 27, 973–982. doi: 10.1016/j.neurobiolaging.2005.05.010

Ron, D., and Walter, P. (2007). Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* 8, 519–529. doi: 10.1038/nrm2199

Song, Y., Shen, H., Du, W., and Goldstein, D. R. (2013). Inhibition of x-box binding protein 1 reduces tunicamycin-induced apoptosis in aged murine macrophages. *Aging Cell* 12, 794–801. doi: 10.1111/acel.12105

Sun, J., Liu, Y., and Aballay, A. (2012). Organismal regulation of XBP-1-mediated unfolded protein response during development and immune activation. *EMBO Rep.* 13, 855–860. doi: 10.1038/embor.2012.100

Taylor, R. C., and Dillin, A. (2013). XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. *Cell* 153, 1435–1447. doi: 10.1016/j.cell.2013.05.042

Wang, L., Ryoo, H. D., Qi, Y., and Jasper, H. (2015). PERK limits Drosophila lifespan by promoting intestinal stem cell proliferation in response to ER Stress. *PLoS Genet.* 11:e1005220. doi: 10.1371/journal.pgen.1005220

Wang, L., Zeng, X., Ryoo, H. D., and Jasper, H. (2014). Integration of UPRER and oxidative stress signaling in the control of intestinal stem cell proliferation. *PLoS Genet.* 10:e1004568. doi: 10.1371/journal.pgen.1004568

Williams, K. W., Liu, T., Kong, X., Fukuda, M., Deng, Y., Berglund, E. D., et al. (2014). Xbp1s in Pomc neurons connects ER stress with energy balance and glucose homeostasis. *Cell Metab.* 20, 471–482. doi: 10.1016/j.cmet.2014.06.002

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

OPINION
published: 29 November 2016
doi: 10.3389/fnagi.2016.00280

Cognitive Intervention As an Early Non-pharmacological Strategy in Alzheimer's Disease: A Translational Perspective

Sarah W. Gehres[1†], Andreia Rocha[1†], Antoine Leuzy[2], Cássio M. Loss[1], Giordano G. Viola[3] and Eduardo R. Zimmer[1,4]*

[1] Department of Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [2] Department NVS, Center for Alzheimer Research, Division of Translational Alzheimer Neurobiology, Karolinska Institutet, Stockholm, Sweden, [3] Department of Physiology, Federal University of Sergipe, São Cristóvão, Brazil, [4] Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Keywords: Alzheimer's disease, cognitive intervention, cognitive reserve, dementia, environmental enrichment

Brain amyloid-β (Aβ) accumulation is currently considered the main causative pathophysiological event in Alzheimer's disease (AD) (Hardy and Higgins, 1992; Karran et al., 2011). Importantly, this process is thought to precede the onset of AD clinical symptoms by more than two decades, indicating that early therapeutic strategies prior to symptomatology offer the best chance of success. In line with this, there is growing attention being paid to the concept of cognitive reserve (CR) (Stern et al., 1994; Stern, 2002). CR concept is based on extensive epidemiological data indicating that those with higher lifetime levels of social, physical, and cognitive engagement have a lower risk of developing dementia despite the presence of brain pathology (Fratiglioni et al., 2004; Nithianantharajah and Hannan, 2009). Recently, cognitive intervention (CI)—such as cognitive training (Bahar-Fuchs et al., 2013), cognitive stimulation (Woods et al., 2012), and cognitive rehabilitation (Clare et al., 2003)—has emerged as a potential non-pharmacological strategy for the treatment and prevention of AD (Gates and Sachdev, 2014). Although based upon distinct theoretical constructs, these CI strategies are frequently not distinguished in clinical trials.

Clinical data are supported by compelling evidence from experimental studies, which have demonstrated that early-life exposure to environmental enrichment (EE), an experimental method of CR in animals, is able to prevent memory decline in AD-like animal models (Valero et al., 2011; Verret et al., 2013; Polito et al., 2014). However, the precise mechanisms behind this phenomenon remain elusive. Environmental enrichment for animals involves stimulating not only their senses (e.g., smell, sight, touch), but also stimulating their ability to learn and adapt when exposed to novelty and challenge, including via stimulation of innate behaviors, such as foraging and partner seeking. The same line of thinking can be applied to humans, on its own scale. This would translate into exercise, social interaction, learning new things, exploring new environments, keeping the brain active with cognitive training, i.e., challenging the brain to self-adapt to novelty.

In a recent study, published in *Frontiers in Aging Neuroscience*, Bezzina et al. (2015) reared mice harboring a human pathological double mutation in the amyloid precursor protein gene (APP, model tg2576) in standard or enriched housing conditions for 10 weeks, starting at 3 months of age (pre-Aβ plaque phase). Two weeks after EE exposure, transgenic animals housed in standard or EE cages presented similar seizure susceptibility to pentylenetetrazole (PTZ), a well-established GABA receptor antagonist. Moreover, the frequency of interictal spikes—indexed by electroencephalography (EEG)—after EE was similar between groups. In short, the authors show that EE is not capable of halting the Aβ-induced aberrant neuronal activity in the Tg2576 AD-like model. These findings indicate that preventive effects of EE on cognitive decline are not necessarily

OPEN ACCESS

Edited by:
Lucas Sedeño, Institute of Cognitive Neurology (INECO), Argentina

Reviewed by:
James C. Vickers, University of Tasmania, Australia

***Correspondence:**
Eduardo R. Zimmer
eduardo.zimmer@ufrgs.br

[†]These authors have contributed equally to this work.

Received: *09 September 2016*
Accepted: *08 November 2016*
Published: *29 November 2016*

Citation:
Gehres SW, Rocha A, Leuzy A, Loss CM, Viola GG and Zimmer ER (2016) Cognitive Intervention As an Early Non-pharmacological Strategy in Alzheimer's Disease: A Translational Perspective. Front. Aging Neurosci. 8:280. doi: 10.3389/fnagi.2016.00280

related to changes in neuronal activity. Although negative, these findings are very relevant since they help rule out abnormal neuronal activity as a mechanism by which EE exerts its beneficial impact on memory performance.

By contrast, data analyzing the effects of EE on neurogenesis in AD-like animal models have been mostly positive. EE exposure for 6 months was able to restore the impaired hippocampal neurogenesis of a triple transgenic AD-like mouse model (3xTg-AD, a model harboring human mutations in: APP (Swedish, KM670/671NL), Tau (MAPT P301L) and Presenilin 1 (PSEN1 M146V) (Rodríguez et al., 2008, 2011). The density analysis of hippocampal proliferating cells was performed by immunohistochemistry targeting the presence of phosphorylated Histone H3 (HH3), and their potential neuronal and glial phenotype by co-localizing the proliferating cells with the immature neuronal marker doublecortin (DCX), the mature neuronal marker (NeuN) or the specific astroglial marker (GFAP). This very interesting study showed a significant increase in neuronal proliferating cells (HH3/DCX or HH3/NeuN positive) in the dentate gyrus of transgenic animals housed in EE. These results, together with the findings of other experimental studies, suggest the stimulation of adult neurogenesis as a potential process by which EE prevents cognitive impairment in AD-like models (Wolf et al., 2006; Herring et al., 2009; Mirochnic et al., 2009; Lahiani-Cohen et al., 2011). In this context, recent evidence indicates that the benefitial effects of EE are more related to the reorganization of the neuronal network built by these newborn neurons than to the absolute number of newborn neurons *per se* (Vivar et al., 2016).

However, the impact of EE on Aβ levels in the brain is still largely inconclusive (Mainardi et al., 2014; Polito et al., 2014; Rodríguez et al., 2015). Male APP23, a mouse model expressing the human APP Swedish double mutation, exposed to EE starting at 3 months of age until 7 or 18 months showed a marked cognitive improvement without altering soluble Aβ (7 month-old) but reducing the number of Aβ plaques at 18 month-old. These data indicate that EE effect seems to affect Aβ in a distinct manner.

We are of the opinion that deciphering the underlying mechanisms of EE can provide important information about novel therapeutic targets. With currently available treatments for AD being palliative at best and ineffective at worst—results from drug trials targeting brain Aβ have been either inconclusive or negative—non-pharmacological interventions stimulating cognition in humans hold a measure of promise, not only for delaying symptomatic onset and the loss of cognitive functions, but also in terms of unveiling potential targets.

The take-home message from EE studies is clear, however, and translates to human disease: interventions must occur early on in the disease course, prior to substantial cognitive deficits (**Figure 1**). The question then becomes: how to translate these findings to clinical research? A promising approach is to study individuals in the so-called "preclinical" or "asymptomatic at-risk" stage of AD [please see revised definition of preclinical AD in Sperling et al. (2011) and Dubois et al. (2016)].

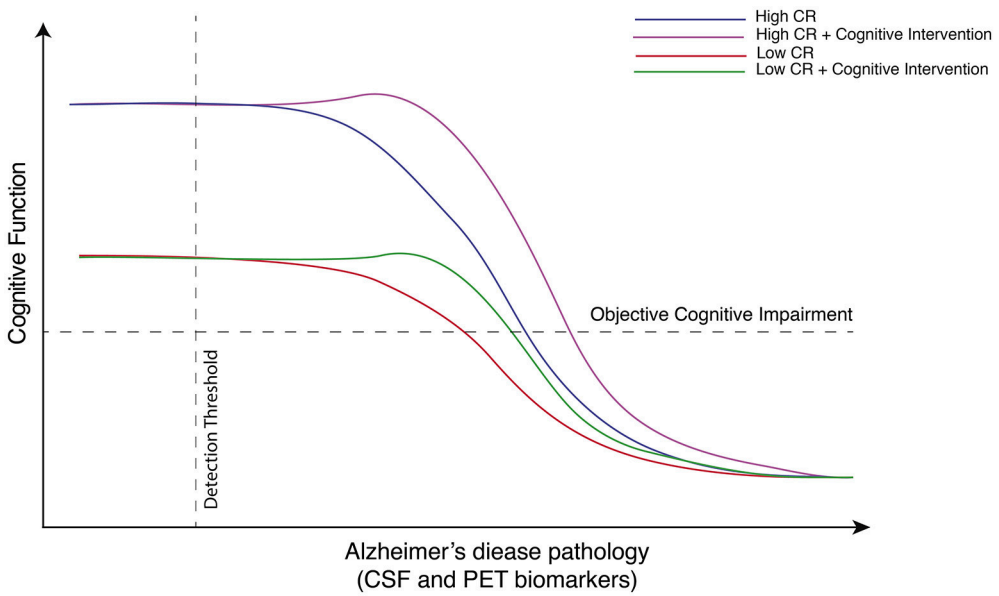


FIGURE 1 | Hypothetical model depicting the beneficial effects of cognitive intervention on cognitive function on low and high cognitive reserve individuals as a function of Alzheimer's disease neuropathology. This hypothetical model exemplifies changes in cognitive function changes over Alzheimer's disease (AD) continuum in individuals with high and low cognitive reserve (CR). When AD pathology is absent or possibly below detectable levels using current biomarkers, individuals with high CR have been shown to perform better on neurophysiological tests than individual with low CR. While cognitive intervention probably cannot ultimately arrest the progression of AD pathology, as measured by imaging or fluid based biomarkers, it can potentially delay the onset and progression of cognitive symptoms. In keeping with the observation that individuals with high CR can tolerate greater levels of pathology prior to declines in cognitive performance, the rate of decline is greater in the high CR group.

Defined by the absence of cognitive deficits despite *in situ* AD pathology—established using cerebrospinal fluid (CSF) or positron emission tomography (PET) based biomarkers—cross-sectional and longitudinal studies suggest this preclinical phase to span some 20–30 years (Jansen et al., 2015). Based on this, non-pharmacological interventions directed at CR could be encouraged in so called "amyloid-positive" individuals believed to be on the path to symptomatic AD. In this way, we refer to the concepts of primary and secondary prevention, as defined by Gates et al. (2011). Primary prevention in the form of early intervention, aimed at preclinical individuals, in order to keep them asymptomatic for as long as possible; and secondary prevention in the form of cognitive training and stimulation for those who already show subtle and early cognitive impairment [such as those in the mild cognitive impairment (MCI) phase], to slow the cognitive decline into AD dementia.

While follow-up studies with longer assessment periods are essential for more definitive data, early results from several multi-domain non-pharmacologic intervention studies—addressing diet, exercise, cognitive training and vascular risk monitoring—suggest that cognitive functioning can be maintained or even improved in at-risk elderly individuals (Ngandu et al., 2015). The incorporation of amyloid biomarkers into such intervention trials could stand as a valuable adjunct to cognitive and functional outcome measures, possibly allowing for more rapid translational estimations.

Though EE stimulation has been shown to be a promising approach for delaying disease progression in animal models mimicking early onset autosomal dominant AD, which accounts for less than 5% of cases, what about the far more common age-related (sporadic) and multifactorial form of AD? How does EE impact on sporadic models of AD? Here stands a problem in that models replicating sporadic AD are currently lacking. In our opinion, major developments in modeling the sporadic form of AD in rodents will potentially come from the incorporation of genetic variations that increase the risk of developing AD, such as the ε4 allele of apolipoprotein E (APOE) gene, or the R47H allele of the triggering receptor expressed on myeloid cells 2 (TREM2). Animal models expressing such combinations of genetic risk factors may better resemble sporadic AD and ultimately translate into more relevant disease models.

In conclusion, study designs aiming toward deciphering EE specific mechanisms are vital and can potentially drive non-pharmacological strategies. Here we make an argument that the combination of EE with models that better mimic the sporadic form of AD, as well as CSF- and PET-based biomarkers, will potentially provide important insights into AD pathophysiology and highlight novel therapeutic targets, with strong translational value.

AUTHOR CONTRIBUTIONS

SG and AR were responsible for reviewing the literature and drafting the manuscript. CL, GV and AL were responsible for revising the manuscript. EZ was responsible for the conceptualization and drafting the manuscript. All authors critically revised the final version of the manuscript.

ACKNOWLEDGMENTS

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) [460594/2014-1], the INCT for Excitotoxicity and Neuroprotection [573677/2008-5], the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) [PNPD], and the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS).

REFERENCES

Bahar-Fuchs, A., Clare, L., and Woods, B. (2013). Cognitive training and cognitive rehabilitation for persons with mild to moderate dementia of the Alzheimer's or vascular type: a review. *Alzheimers Res. Ther.* 5, 35. doi: 10.1186/alzrt189

Bezzina, C., Verret, L., Halley, H., Dahan, L., and Rampon, C. (2015). Environmental enrichment does not influence hypersynchronous network activity in the Tg2576 mouse model of Alzheimer's disease. *Front. Aging Neurosci.* 7:178. doi: 10.3389/fnagi.2015.00178

Clare, L., Woods, R. T., Moniz Cook, E. D., Orrell, M., and Spector, A. (2003). Cognitive rehabilitation and cognitive training for early-stage Alzheimer's disease and vascular dementia. *Cochrane Database Syst. Rev.* CD003260. doi: 10.1002/14651858.CD003260. [Epub ahead of print].

Dubois, B., Hampel, H., Feldman, H. H., Scheltens, P., Aisen, P., Andrieu, S., et al. (2016). Preclinical Alzheimer's disease: definition, natural history, and diagnostic criteria. *Alzheimers. Dement.* 12, 292–323. doi: 10.1016/j.jalz.2016.02.002

Fratiglioni, L., Paillard-Borg, S., and Winblad, B. (2004). An active and socially integrated lifestyle in late life might protect against dementia. *Lancet Neurol.* 3, 343–353. doi: 10.1016/S1474-4422(04)00767-7

Gates, N. J., and Sachdev, P. (2014). Is cognitive training an effective treatment for preclinical and early Alzheimer's disease? *J. Alzheimers Dis.* 42(Suppl. 4), S551–S559. doi: 10.3233/JAD-141302

Gates, N. J., Sachdev, P. S., Fiatarone Singh, M. A., and Valenzuela, M. (2011). Cognitive and memory training in adults at risk of dementia: a systematic review. *BMC Geriatr.* 11:55. doi: 10.1186/1471-2318-11-55

Hardy, J. A., and Higgins, G. A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184–185. doi: 10.1126/science.1566067

Herring, A., Ambrée, O., Tomm, M., Habermann, H., Sachser, N., Paulus, W., et al. (2009). Environmental enrichment enhances cellular plasticity in transgenic mice with Alzheimer-like pathology. *Exp. Neurol.* 216, 184–192. doi: 10.1016/j.expneurol.2008.11.027

Jansen, W. J., Ossenkoppele, R., Knol, D. L., Tijms, B. M., Scheltens, P., Verhey, F. R., et al. (2015). Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. *JAMA* 313, 1924–1938. doi: 10.1001/jama.2015.4668

Karran, E., Mercken, M., and De Strooper, B. (2011). The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nat. Rev. Drug Discov.* 10, 698–712. doi: 10.1038/nrd3505

Lahiani-Cohen, I., Lourbopoulos, A., Haber, E., Rozenstein-Tsalkovich, L., Abramsky, O., Grigoriadis, N., et al. (2011). Moderate environmental enrichment mitigates tauopathy in a neurofibrillary tangle mouse model. *J. Neuropathol. Exp. Neurol.* 70, 610–621. doi: 10.1097/NEN.0b013e318221bfab

Mainardi, M., Di Garbo, A., Caleo, M., Berardi, N., Sale, A., and Maffei, L. (2014). Environmental enrichment strengthens corticocortical interactions and reduces amyloid-beta oligomers in aged mice. *Front. Aging Neurosci.* 6:1. doi: 10.3389/fnagi.2014.00001

Mirochnic, S., Wolf, S., Staufenbiel, M., and Kempermann, G. (2009). Age effects on the regulation of adult hippocampal neurogenesis by physical activity and

environmental enrichment in the APP23 mouse model of Alzheimer disease. *Hippocampus* 19, 1008–1018. doi: 10.1002/hipo.20560

Ngandu, T., Lehtisalo, J., Solomon, A., Levälahti, E., Ahtiluoto, S., Antikainen, R., et al. (2015). A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. *Lancet* 385, 2255–2263. doi: 10.1016/S0140-6736(15)60461-5

Nithianantharajah, J., and Hannan, A. J. (2009). The neurobiology of brain and cognitive reserve: mental and physical activity as modulators of brain disorders. *Prog. Neurobiol.* 89, 369–382. doi: 10.1016/j.pneurobio.2009.10.001

Polito, L., Chierchia, A., Tunesi, M., Bouybayoune, I., Kehoe, P. G., Albani, D., et al. (2014). Environmental enrichment lessens cognitive decline in APP23 mice without affecting brain sirtuin expression. *J. Alzheimers Dis.* 42, 851–864. doi: 10.3233/JAD-131430

Rodríguez, J. J., Jones, V. C., Tabuchi, M., Allan, S. M., Knight, E. M., Laferla, F. M., et al. (2008). Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer's disease. *PLoS ONE* 3:e2935. doi: 10.1371/journal.pone.0002935

Rodríguez, J. J., Noristani, H. N., Olabarria, M., Fletcher, J., Somerville, T. D., Yeh, C. Y., et al. (2011). Voluntary running and environmental enrichment restores impaired hippocampal neurogenesis in a triple transgenic mouse model of Alzheimer's disease. *Curr. Alzheimers Res.* 8, 707–717. doi: 10.2174/156720511797633214

Rodríguez, J. J., Noristani, H. N., and Verkhratsky, A. (2015). Microglial response to Alzheimer's disease is differentially modulated by voluntary wheel running and enriched environments. *Brain Struct. Funct.* 220, 941–953. doi: 10.1007/s00429-013-0693-5

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., et al. (2011). Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 280–292. doi: 10.1016/j.jalz.2011.03.003

Stern, Y. (2002). What is cognitive reserve? Theory and research application of the reserve concept. *J. Int. Neuropsychol. Soc.* 8, 448–460. doi: 10.1017/S1355617702813248

Stern, Y., Gurland, B., Tatemichi, T. K., Tang, M. X., Wilder, D., and Mayeux, R. (1994). Influence of education and occupation on the incidence of Alzheimer's disease. *JAMA* 271, 1004–1010. doi: 10.1001/jama.1994.03510370056032

Valero, J., España, J., Parra-Damas, A., Martin, E., Rodríguez-Álvarez, J., and Saura, C. A. (2011). Short-term environmental enrichment rescues adult neurogenesis and memory deficits in APP(Sw,Ind) transgenic mice. *PLoS ONE* 6:e16832. doi: 10.1371/journal.pone.0016832

Verret, L., Krezymon, A., Halley, H., Trouche, S., Zerwas, M., Lazouret, M., et al. (2013). Transient enriched housing before amyloidosis onset sustains cognitive improvement in Tg2576 mice. *Neurobiol. Aging* 34, 211–225. doi: 10.1016/j.neurobiolaging.2012.05.013

Vivar, C., Peterson, B. D., and Van Praag, H. (2016). Running rewires the neuronal network of adult-born dentate granule cells. *Neuroimage* 131, 29–41. doi: 10.1016/j.neuroimage.2015.11.031

Wolf, S. A., Kronenberg, G., Lehmann, K., Blankenship, A., Overall, R., Staufenbiel, M., et al. (2006). Cognitive and physical activity differently modulate disease progression in the amyloid precursor protein (APP)-23 model of Alzheimer's disease. *Biol. Psychiatry* 60, 1314–1323. doi: 10.1016/j.biopsych.2006.04.004

Woods, B., Aguirre, E., Spector, A. E., and Orrell, M. (2012). Cognitive stimulation to improve cognitive functioning in people with dementia. *Cochrane Database Syst. Rev.* CD005562. doi: 10.1002/14651858.CD005562.pub2. [Epub ahead of print].

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.

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