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Original Article

Retrogenetic models of working memory: Preliminary multi-group analysis

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Key Words: Cognitive aging - Cognitive abilities - Working memory - Retrogenesis

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Abstract

Aim: The aim of the present study was the qualitative comparison of working memory capacity of young children and older adults through the investigation of the latent structure stability or change in Working Memory capacity (WM) in childhood and aging, using Multiple Group Confirmatory Factor Analysis (MGCFA). **Method**: The sample consisted of 62 kindergarten and 56 elementary school students (age range: 4-8 years) and 52 young-old adults and 54 old-old adults (age range: 60-94 years). Adults were asked to complete the Mini-Mental State Examination (MMSE) and the Geriatric Depression Scale-15 (GDS-15) as screening tests. The children were examined via the Raven Colored Progressive Matrix (CMP) test for the same reason. WM was examined via four measures of Working Memory Test Battery for Children (WMTB-C). **Results**: MGCFA applied to the data of the kindergarten students' subsample, elementary school students' subsample, young-old and old-old adults' subsamples as well as of older adults with low (0-9 years of education) educational level. Initially, through MGCFA, four "models" were confirmed, one for each age-related subsample, and they were different from each other. However, when the same method was applied

exclusively to young-old and old-old adults with low educational level, the models that emerged were similar to the kindergarten students' model. **Conclusion**: When we "keep" the educational level equal (low) for all, the hypothesis of retrogenesis is confirmed. Cognitive reserve appears to be protective, keeping differentiated WM's components in every age group other than that of kindergarten students. The results support the "retrogenetic" hypothesis, mainly due to the finding of a delay in WM components' development in the group of kindergarten students, and their dedifferentiation in the low-educated young-old and old-old adults.

Introduction

Researchers in the field of cognitive aging seem to agree that on average, cognitive function is reduced by aging. Among the cognitive abilities that seem to be most affected by aging-related processes is fluid intelligence which refers to the ability to reason and think flexibly. Fluid intelligence has to do with the processing of new, non-familiar information, and has a biological background [1-3]. Researchers have also demonstrated that in the adult population WM and fluid intelligence are closely linked [4, 5]. WM is the mental lab that holds and processes information for short periods of time while performing complex cognitive tasks. The model of WM was proposed by Baddeley and Hitch, and has so far been revised and enriched on a number of occasions. The original model was composed of three main components; the central executive which acts as supervisory system and controls the flow of information from and to its slave systems: the phonological loop and the visuospatial sketchpad. The phonological loop stores verbal content, whereas the visuospatial sketchpad caters to visuospatial data. In 2000 Baddeley added a third slave system to his model, the episodic buffer. It is considered a limited capacity system that provides temporary storage of information capable by conjoining information from the subsidiary systems, and long-term memory, into a single episodic representation [6,7}. In children the relationship between WM and fluid intelligence has been investigated to a lesser degree [8]. However, existing research on this issue, generally accepts that WM and fluid intelligence are significantly related but different cognitive structures [8, 9]. Theories of information processing consider that developmental differences in fluid intelligence reflect differences in processing speed or WM [10].

Moreover, findings in gerontological research have indicated that the collapse of intelligence in dementia patients causes retrogression to childhood and/or appears to reverse Piaget's developmental stages 11-13]. As stated by the retrogenic models, there is an inverse and progressive pattern of functional and cognitive decline observed in Alzheimer's disease (AD) patients compared to the developmental acquisition of these capacities in children. Retrogenesis has been defined as the process by which degenerative mechanisms reverse the order of cognitive abilities' acquisition in normal development [14-16].

The findings regarding the retrogenic models suggest that comparisons should be made between the cognitive ability of these two groups of population, namely the developing children and the retrograding older adult people. In order for a comparison between children's and older adults' cognitive ability to be correctly conducted, the administration of the same screening instruments to the two groups should be available [17,18,13].

Aim and hypotheses of the study

Based on the aforementioned theory and research, the aim of the present study was the qualitative comparison of working memory capacity of young children and older adults through the investigation of the latent structure stability or change in WM in childhood and aging, using Multiple Group Confirmatory Factor Analyses (MGCFA). WM was examined via four measures of Working Memory Test Battery for Children (WMTB-C) [19] which represent subtests of visuospatial processing and retention, and verbal processing and retention. Comparing pair-wise the four groups of our sample (first- to second- grade elementary school students with young-old adults and kindergarten students with old-old adults), the latent structure in the four tests was expected to differ between first- to second- grade elementary school students and young-old adults, on the one hand, and kindergarten students and old-old adults, on the other (Hypothesis 1). In specific, we expected to find similar latent structure in these four tests for first- to second-grade elementary school students and young-old adults (Hypothesis 1a), a similar latent structure in these four tests, for kindergarten students and old-old adults (Hypothesis 1b), and at the same time different from that confirmed for first- to second- grade elementary school students and young-old adults.

Method

Participants and Procedure

The whole sample consisted of four groups of individuals: a group of kindergarten students, a group of first- to second- grade elementary school students, a group of young-old adults, and a group of old-old adults. The first group comprised 62 kindergarten students 4 to 6 years old (mean age = 58.98 months, age range: 48-71 months). Of the 62 participants, 29 were boys (46.8%) and 33 were girls (53.2%). The second group included 56 first- to second- grade elementary school students 6 to 8 years old (mean age = 84.30 months, age range: 72-96 months). Of the 56 participants, 29 were boys (51.8%) and 27 were girls (48.2%). All the children were attending regular classrooms, without a history of learning difficulties (based on the school records and student reports) in five preschool institutions (three public and two private) and three public primary schools of medium and low socioeconomic status, in the city of Thessaloniki (Greece). All the young participants, additionally to the four tests of WMTB-C, completed the Raven's Coloured Progressive Matrices [CPM; 20] in order for a brief estimate of their overall cognitive functioning to be provided. In collaboration with the school committees, parents completed an individual-demographics form. Children's testing in the four tests of WMTB-C and CPM was performed in their school environment. No time limit was assigned for the completion of the tests and all young participants were informed that they were free to withdraw from testing at any time.

Given that the four tests of WMTB-C were also intended for use with older adults, two groups of older adults were tested. In specific, one group comprised of 52 young-old adults (mean age = 67.11 years, age range: 60-74 years). Of the 52 participants, 26 were men (50.0%) and 26 were women (50.0%). The second group of older adults included 54 old-old adults (mean age = 79.57 years, age range: 75-94 years). Of the 54 participants, 26 were men (48.1%) and 28 were women (51.9%).

Exclusion criteria for both groups were history of neurological conditions or psychiatric diseases, alcohol or drug abuse, severe head trauma, profound visual impairments, and verbal incomprehension. Moreover, all the adult participants additionally completed the Greek version of the Mini Mental State Examination [MMSE; 21, 22] and the Geriatric Depression Scale–15 [GDS-15; 23, 24]. All the participants had MMSE scores between 25 and 30 points and between 0 and 6 points in GDS-15 and therefore no one was excluded from our adult sample. All the participants were community dwelling adults recruited by the researchers through seniors' centers. They were residents of Thessaloniki, Athens, Alexandroupoli (a town in the province of East Macedonia in Greece) and Ptolemaida (a town in the province of West Macedonia in Greece). Participants were examined at an individual basis, either at the center recruited, or in their own home. No time limit was assigned for the completion of the examination and the participants were informed that they were free to withdraw from testing at any time. All adult participants completed an individual – demographics form. It should be noted that the subsample of older adults was characterized by an overrepresantation (66.9%) of persons with 9 years of formal education or fewer (Low educational level).

The authors assert that all procedures contributing to this work comply with the ethical standards on the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All participants participated voluntarily in the study. They (adult participants) or their parents (children) were informed about the procedure and the aim of the study, and subsequently they or their parents provided their written consent for participation. We should also point out that the research has been approved by the Ministry of Education, Research and Religious Affairs of Greece.

Instruments

Four tasks from the WMTB-C [19], based on Baddeley's model of WM [6, 7], were used to assess WM for all participants. In particular, we used the Greek translation and adaptation of the following memory tasks [25], which were administrated in the series presented, to assess Simple Verbal Retention (Verbal short-term memory), Simple Visuospatial Retention (Visuospatial short-term memory), Advanced Verbal Processing (Verbal WM) and Advanced Visuospatial Processing (Visuospatial WM), respectively.

Forward Digit Span (Verbal short-term memory)

The test involves the presentation of spoken sequences of digits that the child is asked to recall in correct serial order. Lists of digits, which were constructed randomly from the digits ranging from 1 to 9, are spoken by the tester at the rate of one digit per second. Following a practice session, a maximum of six lists is presented at each length. List length is increased by one if the child recalls four lists at that length correctly. If the first four trials are correct, the child is credited with correct recall of all six lists at that length, and the next list length commences. Testing commences with single-digit lists and continues until three lists of a particular length are recalled incorrectly. The number of lists correctly recalled is scored. Score range: 0-54.

Block Recall (Corsi Forwards Task; Visuospatial short-term memory)

In the block recall test, the child views nine cubes located randomly on a board. The test administrator taps a sequence of blocks, and the child's task is to repeat the sequence in the same order. Testing begins with a single block tap and increases by one additional block following the span procedure and scoring outlined above. Score range: 0-54.

Backward Digit Span (Advanced Verbal Processing)

The backward digit recall test is identical to the digit recall test in all respects except that the child is required to recall the sequence of spoken digits in reverse order. Practice trials are given in order to ensure that the participant understands the concept of "reverse". Score range: 0-36.

Backward Block Recall (Corsi Backwards Task; Advanced Visuospatial Processing)

The backward block recall test is identical to the block recall test in all respects except that the child is required to repeat the sequence of blocks in reverse order. Score range: 0-48. It should be noted that algorithm [26] from the R statistics program was used to create the "Backward Block Recall" answer sheet.

Statistical analysis

Data analysis was conducted in EQS version 6.1 [EQS 6.1; 27]. Specifically, structural equation modeling (SEM) on covariance matrices was used. A robust maximum likelihood estimation procedure was performed due to small sample size and data kurtosis. The specific SEM technique that was applied to the data was Multi-group Confirmatory Factor Analysis (MGCFA). Regarding the confirmation of a structural model, a non-significant level of Goodness of Fit index $\chi 2$ that is $p > .05$ is indicative of a good fit of the model to the data. In addition, when the value of Root Mean Square Error of Approximation (RMSEA) is < 0.05, it is also an indication of the good fit of the model to the data. RMSEA values ranging from 0.06 to 0.08 indicate a reasonable and therefore acceptable approximation error. RMSEA value is relatively "expanded" in cases of small sample size ($n<100$) and that is reflected in confidence interval range (90% CI). This means that RMSEA should be considered as a model fit index, however, with caution [28]. Comparative Fit Index (CFI) examines whether the data fit a hypothesized measurement model compared to the basic model. Values that are greater than 0.90 indicate adequate fit of the model to the data, whereas values close to 1.00 indicate a good fit.

Results

Before proceeding with analyses, it should be noted that the tasks used for the assessment of WM have not been fully standardized in the Greek population and for this reason the scores used (correct trials and potential of WM) are the raw scores of all measurements.

As we were interested in the investigation of the latent structure qualitative changes in WM, from age-group to age-group, we proceeded with MGCFA.

Testing latent structure of WM's tasks in the total sample

Based on the performance in WM tasks, a multi-group structural model was confirmed for the four age groups: $\chi 2$ (2) = 3.20, p > .05, CFI = .999, SRMR = .031, RMSEA = .052 (90% CI: .000-.152). More specifically, based on the verified model, WM of kindergarten students seems to maintain its four components (see Figure 1). In the subgroup of elementary school students, no model was confirmed. It appears that elementary school students function primarily with simple verbal retention (see Figure 2). No model was verified in the subgroup of the young-old adults, too. It appears that the young-old adults function primarily with advanced verbal processing (see Figure 3). Finally, based on the verified model, WM of old-old adults consists of simple visuospatial retention and advanced verbal processing (see Figure 4).

WM factorial structure in the kindergarten students

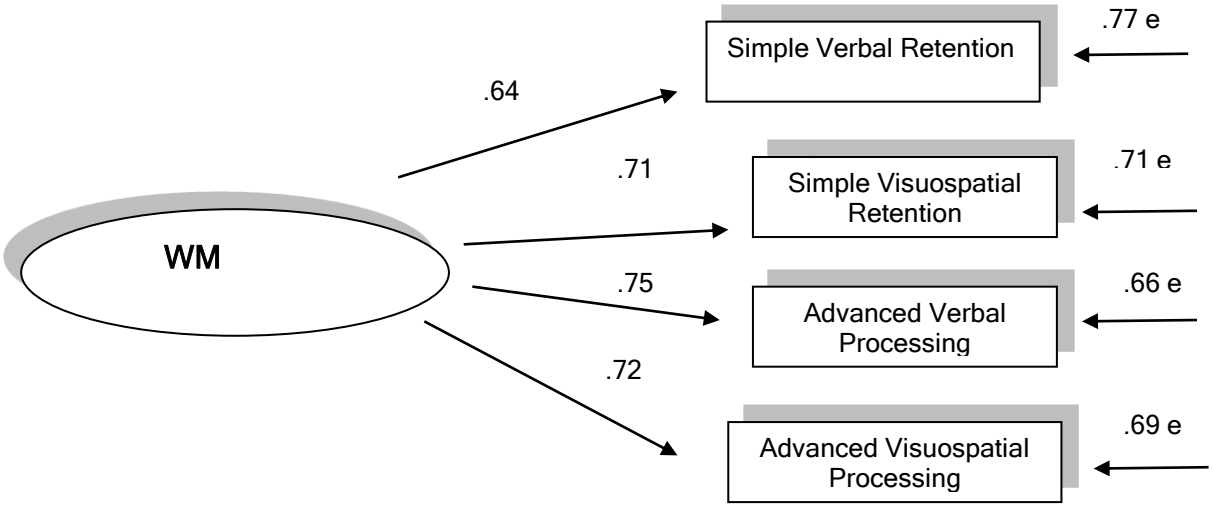

Figure 1. The uni-factorial structure of WM in the kindergarten students group based on the verified MGCFA model

*All loadings drawn indicate significant associations (p <.05). ** e = measurement error

WM component in the elementary school students

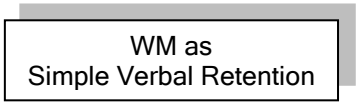

Figure 2. Simple Verbal Retention (verbal short-term memory) as the main WM component in the group of elementary students.

WM component in the young-old adults

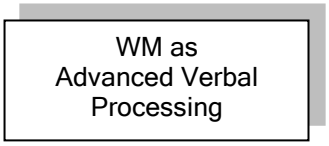

Figure 3. Advanced Verbal Processing (verbal WM) revealed as the main WM component of the young-old adult group.

WM factorial structure in the old-old adults

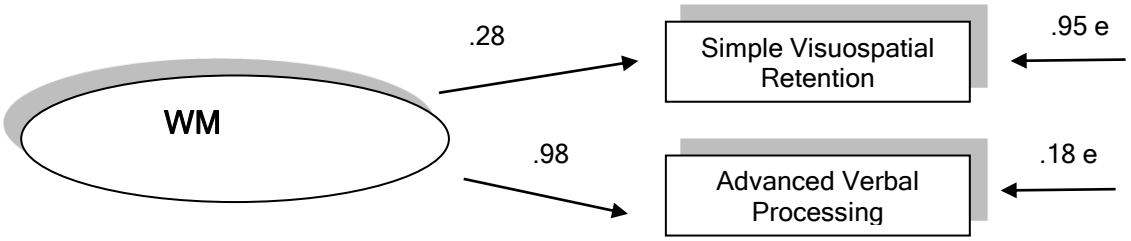

Figure 4. The uni-factorial structure of WM in the subgroup of old-old adults based on the verified MGCFA model

*All loadings drawn indicate significant associations (p <.05). ** e = measurement error

The latent structure of WM in the subsamples of low-educated young-old and old-old adults

Based on the performance in WM tasks, a multi-group structural model was verified in the subgroups of low-educated young-old and old-old adults: $\chi 2$ (9) = 6.42, p > .05, CFI = 1.000, SRMR = .085, RMSEA = .000 (90% CI: .000-.103). More specifically, based on the verified model, WM of both low-educated young-old (see Figure 5) and low-educated old-old adults (see Figure 6) is loaded by all observed variables. It is worth-mentioning that these models are similar to the one verified in the subgroup of kindergarten students.

WM factorial structure in the low-educated young-old adults

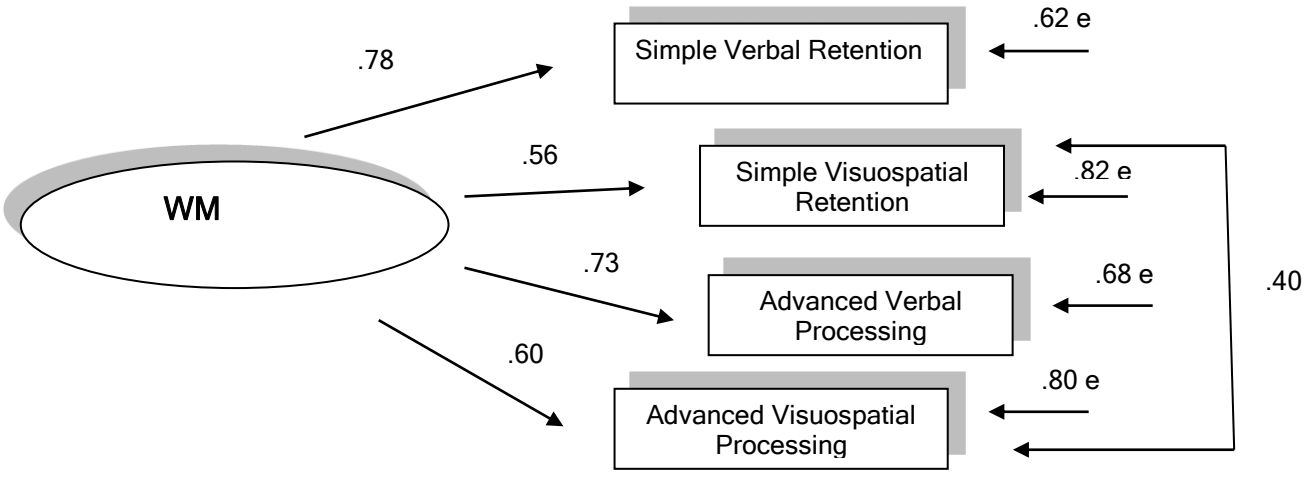

Figure 5. The uni-factorial structure of WM in the subgroup of low-educated young-old adults based on the verified MGCFA model

*All loadings drawn indicate significant associations (p <.05). ** e = measurement error

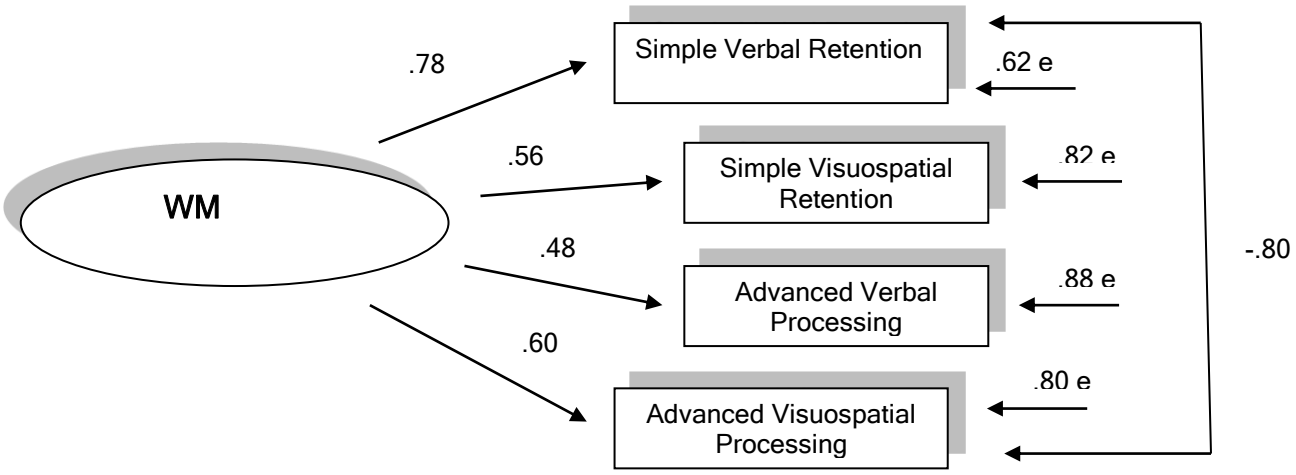

Figure 6. The uni-factorial structure of WM in the subgroup of low-educated old-old adults based on the verified MGCFA model

*All loadings drawn indicate significant associations (p <.05). ** e = measurement error

Discussion

This study aimed to investigate the latent structure stability or change in WM in childhood and aging, using MGCFA. For all the subgroups of our sample the results of MGCFA appear to support the existence of four different "models", one for each subgroup and at the same time different from each other. Therefore, our initial hypothesis (H1) that the latent structure of WM differs between elementary school students and young-old adults, on the one hand, and kindergarten students and old-old adults, on the other hand, based on the above findings, was not verified.

In particular, in the subgroup of kindergarten students, based on the verified model, WM seems to consist of the four theoretically suggested components. However, due to the low performance of this subgroup in quantitative measurements, we have come to the conclusion that WM in this subgroup has not been differentiated yet in its specific components. Baddeley and Hitch's WM model [6,7] was constructed on the basis of evidence from studies of adult participants. The modular structure of working memory evident in adults may not be in place at earlier stages of development. It has been argued that younger children's performance may be supported by more domain-general systems that become increasingly differentiated as knowledge and skills are developing. Thus, although modular systems may represent the end point of child development, they do not necessarily characterize the intermediate stages [29-31]. For example, it is possible that performance by very young children on tasks known to tap either the phonological loop or the visuospatial sketchpad in adults may reflect the operation of less highly specialized working memory subsystems such as the central executive. The fractionated modular system characterizing adult working memory function may emerge only later in development, once specialized domain-specific skills and knowledge structures have been constructed [32].

Moreover, although Baddeley did not connect the central executive to any specific brain region, it is well known that prefrontal cortex is a key area for executive functions [33-35] and the last part of the brain that is fully developed.

In the subgroup of elementary school students, which appears to function primarily with simple verbal retention, no model was verified. Beyond the normal development and organization of the prefrontal cortex, which continues throughout childhood and adolescence, a complementary explanation for this finding may be the fact that European languages are more dependent on verbal but not visual short-term memory [36]. Some aspects of culture that are closely related to education can be closely linked to cognitive functioning. The Greek educational system generally provides a basis for the development of verbal skills [37]. The way of teaching in Greek schools where the teacher's textbook and the oral explanation of the curriculum are mainly used by teachers, in the absence of experiments or visual material, is likely to lead children to rely heavily on their vocabulary to understand each type of information. Moreover, the Greek alphabetical system mainly favors the use of phonological strategies. Research has shown that literary writing systems, such as Chinese, are very different from alphabetic systems in their cognitive demands. That is, literary systems are much more demanding than alphabetical systems in visual processing and the creation of connections between concepts and representations. It is argued that the learning of the Chinese literary system has a lasting impact on visual processing that extends from the basic processing mechanisms of information related to speed and control of processing to visuospatial working memory and spatial logic [38].

In the subgroup of young-old adults, which appears to process information primarily with advanced verbal processing, no model was verified. This result shows that adults generally function with “processing (as a main aspect of WM)”, probably due to the full functioning of the specialized subsystems of WM, the maturation of the brain structures that support them, experience, crystallized intelligence etc. Adults function especially with verbal processing, which is unilaterally strengthened from childhood, mainly through the Greek educational system. They do not choose to use visuospatial processing because, in general, the Greeks have a low level of visuospatial capacity due to the fact that they do not practice it in their daily lives. Overall, cognitive processes and cognitive functions are increasingly differentiated from one another, and this differentiation guides mental development because it allows the creation of ever more interconnected, abstract and flexible inference processes and knowledge structures [39, 40].

In the subgroup of old-old adults, WM consists of simple visuospatial retention and advanced verbal processing. Advanced verbal processing was found to decrease statistically significantly, from young-old to old-old adults, in the quantitative analyses. Since this decline is observed, it is very likely that old-old adults use simple visuospatial retention, which is useful for their navigation, as a compensational mechanism. The neurobiological decline associated with aging is well documented in the research literature and explains why older adults have worse performance than young people in neurocognitive testing. However, not all older people show lower performance. Some manage to perform just as well as younger people. This “unexpected” differentiation has been scientifically investigated, revealing that older people with higher performance use the same areas of the brain as young people do, but they also use other areas that neither young people nor other older people do. Researchers have come to the conclusion that the use of new cognitive resources reflects a compensation strategy [41,42]. Studies show

that the brain reaches this functional solution by activating different nerve pathways, so areas are increasingly activated in both hemispheres. In particular, the HAROLD model [43] argues that with the progress of time, the brain also employs the corresponding unilateral brain regions required to perform a cognitive test, which at a younger age remained "silent", in order to cope with the cognitive decline. According to the CRUNCH model [44], compensation arises not only from the increased activation of specialized brain structures but also from the strategic recruitment of alternative brain regions.

The inability to highlight a common latent structure of the four WM assessing tools in specific subgroups of our sample -for which we expected to detect a similar factorial structure- led us to the application of the same method (MGCFA), exclusively to the subgroups of low-educated young-old and old-old adults. Since in both models emerged, WM found to receive loads from all four observed variables - components, we reached to the conclusion that these models are qualitatively similar to that of the subgroup of kindergarten students. One possible explanation for this finding is that during childhood, general ability gradually evolves into more specific abilities, while more specific abilities encounter biological constraints that tend to cause blurring of their boundaries, i.e., dedifferentiation, at the advanced age [45-49]. The "Case of Age Differentiation", introduced by Garrett [50], argued that as children grow up during childhood, the organization of intelligence moves from a general ability to a group of less closely related abilities. Balinsky [45] examined the case of age differentiation throughout life and observed that the structure of cognitive abilities shifted back to a general factor at the advanced age. On the basis of age differentiation and Balinsky's work [45], it was argued that the cognitive structure differs in late childhood and early adolescence, remains in a relatively diverse organization throughout adulthood, and then dedifferentiates again in old age [51]. A similar age-related trajectory of WM seems to be revealed in this study but only when older age is associated with low educational level. Hence, it appears that experience and crystallized knowledge can function protectively into very old age, against dedifferentiation. The high negative correlation observed in the confirmed model in the subgroup of old-old adults between simple retention and advanced visuospatial processing shows just how distinct the two subsystems are, both at the level of retention (short term memory) versus processing (cognitive control) and at the level of the type of information (verbal versus visuospatial) that they hold.

To summarize, the aforementioned findings confirm Hypothesis 1b and support the existence of a similar latent structure of the four WM assessment tools in the subgroup of kindergarten students and this of old-old adults, when only the low-educated remained in this subgroup and the middle- and high-educated participants were removed. The subgroup of elementary school students appears to use basically verbal storage as opposed to the subgroup of young-old adults that seems to use both simple visuospatial retention and advanced verbal processing. However, the subgroup of low-educated young-old adults appears to use all of WM components examined in the present study. Therefore, our assumption for a similar latent structure of the four tools in the subgroups of elementary school students and young-old adults (Hypothesis1a) was not confirmed either as regards all the sample of the last group or the low-educated participants especially. Inversely, it appears that low-educated young-old adults display a similar pattern of dedifferentiation with the old-old adult group.

Therefore, when we keep the educational level equal (low) for all, the hypothesis of

retrogenesis seems to be confirmed. Cognitive reserve appears to be protective, keeping differentiated WM components in every age group other than that of kindergarten students. The results support the "retrogenetic" hypothesis, mainly due to the finding of delay in the development of WM in the group of kindergarten students and its potential decline / regression in the subgroups of the low-educated older adults.

Limitations and future directions

Our research findings must be interpreted with several limitations in mind. A key limitation was the relatively small sample size, especially as regards each of the four age subgroups. In addition, the research plan was cross-sectional, a disadvantage in detecting age differences without the involvement of confounding variables.

Therefore, longitudinal research is needed in a larger number of participants and cohorts which are representative of the population of interest, in order to test for different models of WM structure. In the context of such a large-scale research, it would be advisable to use more and perhaps different psychometric tools in assessing WM and this would provide us with a complete and in-depth picture of its function in the age groups of our interest.

The authors declare that they have no conflicts of interest.

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Original Article

Differences between professional and non-professional drivers with cognitive disorders

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Abstract

The study describes the driving habits of people with cognitive disorders and previous professional driving experience. A similar study has not been mentioned in the literature. **Methods:** From a total of 639 drivers who participated in the research, 153 participants were selected based on their answer on an extensive driving questionnaire. They were asked whether they had a professional driving license. Forty-three participants (28.1%) said "Yes", 110 participants (71.9%) said "No". Out of the 153 participants, 55 (35.9%) were diagnosed with Alzheimer's disease (AD), 44 (28.8%) with Mild Cognitive Impairment (MCI), and 54 (35.3%) were healthy. Additionally, 31 professional drivers were compared to 31 non-professional drivers (N=62) on a short driving questionnaire. The distribution of the population according to the diagnosis was: 18 (29%) with MCI (N=9 professionals, N=9 non-professionals), 30 (48.4%) with AD (N=15 professionals, N=15 non-professionals), 14 (22.6%) healthy (N=7 professionals, N=7 non-professionals). Professional and non-professional drivers were randomly selected to match in terms of diagnosis, age, gender and years of education. The AD diagnosis was based on the NINCDS-ARDRA criteria while the MCI diagnosis was based on the Petersen and Winblad criteria. Healthy older adults were examined across the same neuropsychological battery. **Results:** The extensive driving questionnaire showed that more non-professional AD drivers (21.9%) had not renewed their license compared to professionals (p=0.048). More non-professional MCI drivers (91.7%) travelled fewer kilometers compared to professionals (p=0.029). Taking both MCI (27.6%) and AD patients (63.2%) together, more non-professional drivers always avoided driving in unfamiliar areas compared to professionals, MCI (p=0.045) and AD (p=0.026). Finally, more non-professional AD drivers (80.0%) avoided driving when it snowed compared to professionals (p=0.34). The

short driving questionnaire showed that healthy non-professional drivers almost always (85.7%) avoided turning into difficult intersections compared to professional drivers (14.3%) (p=0.001). **Conclusions:** People with cognitive disorders and previous professional experience were better at driving than the inexperienced ones.

Introduction

Dementia is an acquired brain syndrome characterized by deterioration from a previous level of cognitive function entailing failure of two or more cognitive domains (memory, executive functions, attention, language, judgment, visual-perceptual and visual-spatial abilities) [1]. The term dementia has been replaced by the term *major neurocognitive disorder* which features deficits in the cognitive domain and which aims at reducing stigma [2].

In the USA, every minute, every day, a new adult is diagnosed with Alzheimer's disease (AD), while more than 5.5 million people aged 65 live with the disease. By 2050, the number will have increased to 14 million. Moreover, in the USA, AD is the most costly disease and represents the majority of dementias (60%-80%) affecting the lives of patient families, friends, and caregivers [3]. AD patients live between 8 and 10 years following the diagnosis and therefore need long-term care and support [4]. The distribution of new cases of dementia (incidence) is 4.9 million (49% of the total) in Asia, 2.5 million (25%) in Europe, 1.7 million (18%) in the USA and 0.8 million (8%) in Africa [5].

In Greece, studies on the prevalence of dementia, depression, and Mild Cognitive Impairment (MCI) are few and vary significantly as regards the epidemiological method, geographical areas, dietary habits and lifestyle. A recent (2017) door-to-door research explored the prevalence of dementia, depression, and MCI in a rural population of Greece. The prevalence of dementia in Crete was 9.2% for those aged over 60. MCI was more common than dementia. High rates of depression may be related to low education [6].

Low educational level has been reported as a risk factor probably because it is associated with poor quality of life and harmful health habits [7]. Researchers believe that people with more years of education have built a "cognitive reserve" [8], which is enhanced through intellectual and social activities and hobbies [9]. Combined with studies on "cognitive reserve" [10], it is suggested that social interaction can help build "cognitive reserve". More research is needed to understand how the engagement in social and intellectual activities influences biological processes which reduce the risk of dementia [11].

Occupational therapy (OT) can be effective for patients with dementia. It supports the functionality of the person with dementia, assessing factors such as skills, work position, past interests and hobbies, as well as current daily routine, taking into account the stage of dementia. The most important role of OT is to find the best adjustment between the environment and the patient, task requirements (when it is performed) and patient cognitive capacity. Its main objective is to improve the ability of dementia patients to perform Activities of Daily Living (ADL) such as driving. Through OT, patients gain independence to participate in social activities, while caregivers are relieved of their burden, since their self-confidence and ability to address behavior problems of dementia patients are increased [12].

Occupational therapists (OTs) play an important role in supporting patients with cognitive disorders and their caregivers through all the phases of the disease. They study the individual and the environment, while performing task analysis and skill analysis through the healing relationship during the care [13]. Scientific knowledge along with the use of habits and routine tasks, such as driving, render OTs suitable to work with such a patient population as well as with their caregivers [14].

In cognitive disorders, maintaining the ability to carry out daily and complex activities, such as driving, is important because individuals value them as part of their identity and independence [15]. Research has shown that autobiographical memory is interwoven with human emotions and that there is a direct correlation between psychological well-being and the satisfaction that one takes from life. Moreover, it has been hypothesized that autobiographical memory bears purposefulness and meaningfulness to someone's life [16].

The early stages of AD affect not only cognitive but also complex functions, namely executive function, attention, response speed and immediate problem solution which may occur on the road [17]. Also, MCI patients may experience a functional impairment in the Instrumental ADLs (IADLs) [18], which are more demanding for cognitive functioning, such as driving.

Driving may facilitate the participation of older adults in the community [19]. It allows them to move whenever they want, so that they can participate in social and recreational activities and have access to various services when necessary [20].

Driving and moving are included in the use of means of transport which, together with telephone use, financial management, and medication taking, belong to the IADLs [21, 22]. Movement is defined as travelling by public or private means of transport, including driving, walking, cycling and accessing all means of transport [23]. The above constitute a subgroup of transport functions [24].

Assessing the ability of people with dementia to continue driving is becoming a common clinical problem. Due to the increasing proportion of older adults with dementia and the risks of accidents, it is becoming necessary to evaluate the ability to continue or to stop driving [25]. When cognitive functioning starts to fail, it is safety which is of primary importance. The ability to move with orientation is evaluated along with patient protection in cases which might threaten their safety [26]. A clinically meaningful way to evaluate the driving ability of AD and MCI people is a combination of clinical examination, neuropsychological tests, driving simulator and assessment in real driving conditions [27].

Whether older adults should continue using their driving licenses or not is related to their functional problems concerning driving behavior, cognitive and perceptual skills as well as driving skills. Recommending withdrawing the driving license may have a significant impact on the lives of seniors, which is why further research is needed to avoid, whenever possible, its negative consequences [28]. Although it is accepted that cognitive disorders may impact on driving, there are no published guidelines to answer the question until when patients with mild AD and MCI are safe to drive. However, most guidelines consider that patients with moderate to severe AD are no t suitable for driving [29]. Ceasing to drive depends on the people themselves and their family, which results in accident risk, endangering the lives of patients and the people around them [30].

OTs can use their judgment, which is based on the observation of patients daily activities, concerning their functional ability. In combination with evaluation tests, they can recommend

driving continuation, or suggest interventions in vehicle equipment or advocate driving cessation [31]. Medical and social health models may help older adults to drive safely, but also help those who have lost that ability by designing a smooth stop [32].

Driving is closely linked to the identity and independence of older adults [33]. Literature review has revealed no research on the driving ability of professional drivers with dementia. On the other hand, a recent study has reported that people with dementia and previous professional experience demonstrate better driving behavior than inexperienced individuals [34, 35].

The purpose of this study was to investigate whether there is a significant difference in the driving performance between AD and MCI drivers with and without previous professional driving experience.

Methods

Participants

Participants came from the wider region of Northern Greece (Thessaloniki) and from the wider region of Attica (Athens). In Thessaloniki, they were examined at the outpatient clinic of Memory and Dementia, 3rd University Department of Neurology of the "G. Papanikolaou" University General Hospital, at the Greek Association of Alzheimer's Disease and Related Disorders, and at the **Hellenic Institute of Transport (HIT)**, Center for Research and Technology Hellas (**CERTH)**, In Athens, they were examined at the "Nestor" Psychogeriatric Association, at three Open Care Centers for the Elderly (KAPI) in the municipality of Zografou, at the NGO "IASIS" Day Care Unit for people with dementia and at the "Mission" Alzheimer Disease Day Center Unit.

A total of 639 drivers were examined: 307 participants were assessed on an extensive driving questionnaire, while 285 participants were assessed on a short driving questionnaire for caregivers. The selected participants were derived from the extensive and the short driving questionnaires; they were over 65 years old; they were divided into three diagnostic groups (healthy, MCI, AD). People who fell under the category "other dementias" were excluded, because we were interested in examining the traits of the three diagnostic groups.

Out of the 639 participating drivers, 153 were selected based on their answer on the extensive driving questionnaire. They were asked whether they had a professional driving license. Forty-three participants (28.1%) said "Yes", 110 (71.9%) said "No". Fifty-five participants (35.9%) were diagnosed with AD, 44 (28.8%) were diagnosed with MCI, and 54 (35.3%) were healthy.

Additionally, 31 professional drivers were compared to 31 non-professional drivers (N=62) on the short driving questionnaire. The distribution of the population according to the diagnosis was: 18 (29%) with MCI (N=9 professionals, N=9 non-professionals), 30 (48.4%) with AD (N=15 professionals, N=15 non-professionals), 14 (22.6%) healthy older adults (N=7 professionals, N=7 non-professionals). Professional and non-professional drivers were randomly selected to match in terms of diagnosis, age, gender and years of education.

Data collection took place between December 2012 and September 2016, when the study was completed.

Diagnosis

The AD diagnosis of the participants was based on the NINCDS-ARDRA [36] criteria, while the MCI diagnosis on the Petersen and Winblad [2, 3, 37] ones; participants with other dementias were excluded. In order to support the AD and MCI diagnosis, neurological, neuropsychological and neuropsychiatric assessments, medical/social history, neuroimaging and blood tests were conducted.

Assessment measures

All participants were evaluated on: the Mini Mental State Examination (MMSE) [38, 39], the Clock-drawing Test [40-42], the Functional Rating Scale for Symptoms of Dementia (FRSSD) [43], the Geriatric Depression Scale (GDS) [44-46], Hamilton's Depression Rating Scale (HDRS) [47], the Functional Cognitive Assessment Scale (FUCAS) [48, 49], the Montreal Cognitive Assessment (MoCA) [50, 51], and the Neuropsychiatric Inventory (NPI) [52-54]. This battery was employed as a routine for the staging of AD and MCI. Healthy older adults were also evaluated on the same battery.

The above tests were chosen for their psychometric properties (validity, reliability) and their standardization for the Greek population. Furthermore, they seem to be acceptable for measurements in the international literature.

Extensive driving questionnaire

A combination of two driving ability assessment questionnaires for older adults mentioned in the literature was used in the present study: the AGed people Integration, mobility, safety and quality of Life Enhancement through living (AGILE) Questionnaire [55] and the Driving Questionnaire for patients with dementia [56].

The newly created driving questionnaire included 33 questions with 52 sub-questions. Specifically, we sought information on the following topics: personal information, opinions concerning training and assessment related to age, physical and cognitive abilities, and driving habits.

Before the administration of the questionnaire, both patients and healthy older adults granted their written informed consent.

Short driving questionnaire

The Short Driving Questionnaire [57] was created after the first results from the extensive driving questionnaire, where important findings emerged for 15 questions for people with AD aged 65 and above (see APPENDIX).

Statistical analysis

Statistical analysis included descriptive statistics and mono-factorial analysis. The normality of the data was evaluated with the Shapiro-Wilk test. Normality assumptions were rejected and as a result non-parametric tests were preferred for data analysis. For the independence between qualitative variables, the χ^2 control was used. For the comparison of independent measurements between the two groups, the Mann-Whitney statistical control was used. P values (P values) less

than 0.05 were considered statistically significant. Statistical analysis was performed with the SPSS 24.0 statistical analysis software (IBM Inc., Armonk, NY).

Results

Extensive driving questionnaire

Demographics		Diagnosis							
		Healthy N(%): 54 (35.3%)		**MCI N(%):** 44(28.8%)		**AD N(%):** 55(35.9%)		**Total N(%):** 153 (100%)	**p**
Age	«65-74»	30 (55.6%)		16 (36.4%)		17(30.9%)		63 (41.2%)	0.085
	«75-84»	23 (42.6%)		25 (56.8%)		34(61.8%)		82 (53.6%)	
	«>=85»	1 (1.9%)		3 (6.8%)		4 (7.3%)		8 (5.2%)	
Gender	**Male**	38 (70.4%)		33 (75.0%)		48(87.3%)		119(77.8%)	0.092
	Female	16 (29.6%)		11 (25.0%)		7(12.7%)		34 (22.2%)	
Professional Driver	**NO**	42 (77.8%)		34 (77.3%)		34(61.8%)		110(71.9%)	0.115
	YES	12 (22.2%)		10 (22.7%)		21(38.2%)		43 (28.1%)	

Table 1. *Professional driver, diagnosis and age groups.*

Healthy individuals were significantly more educated than the other two groups (p=0.003). Also, healthy non-professional drivers were proportionately more educated than professional ones (p=0.012). AD or MCI professional drivers were significantly less educated (8.93±4.88) than non-professionals (11.77±5.26) (p=0.003). There was no significant difference on the MMSE between professional and non-professional drivers (p=0.751). In all three diagnostic groups there were no women drivers with a professional license, HC (p=0.011), MCI (p=0.038) and AD (p=0.026).

The results further showed that more non-professional AD drivers (21.9%) had not renewed their license compared to professionals (p=0.048). More non-professional MCI drivers (91.7%) travelled fewer kilometers compared to professionals (p=0.029). Taking both MCI (27.6%) and AD patients (63.2%) together, more non-professional drivers always avoided driving in unfamiliar areas compared to professionals, MCI (p=0.045) and AD (p=0.026). Finally, more non-professional AD drivers (80.0%) avoided driving when it snowed compared to professionals (p= 0.34).

Short driving questionnaire

Demographics

Professional driver, diagnosis and age groups						p
Diagnosis				Professional Driver		
				NO N(%): 31 (50 %)	YES N(%): 31 (50%)	Total N(%):62 (100%)
HealthyN(%): 14 (22.6%)	Age	65-74		5	5	10	.720
				(35.7%)	(35.7%)	(71.4%)	
		75-84		2	2	4	
				(14.3%)	(14.3%)	(28.6%)	
	Total			7	7	14	
				(50.0%)	(50.0%)	(100.0%)	
MCI N(%): 18 (29.00%)	Age	65-74		5	5	10	.681
				(27.8%)	(27.8%)	(55.6%)	
		75-84		4	4	8	
				(22.2%)	(22.2%)	(44.4%)	
	Total			9	9	18	
				(50.0%)	(50.0%)	(100.0%)	
AD N(%): 30 (48.4%)	Age	65-74		8	8	16	.642
				(26.7%)	(26.7%)	(53.3%)	
		75-84		7	7	14	
				(23.3%)	(23.3%)	(46.7%)	
	Total			15	15	30	
				(50.0%)	(50.0%)	(100.0%)	
Total N(%): 62 (100%)	Age	65-74		18	18	36	
				(29.0%)	(29.0%)	(58.1%)	
		75-84		13	13	26	
				(21.0%)	(21.0%)	(41.9%)	
	Total			31	31	62	
				(50.0%)	(50.0%)	(100.0%)	

Table 2. *Professional driver, diagnosis and age groups.*

Professional driver and gender						p
Diagnosis				Professional Driver		
				No N(%): 31 (50 %)	Yes N(%): 31 (50 %)	Total N(%): 62 (100%)
HealthyN(%) : 14(22.6%)	Gender	Male		7	7	14	
				(50.0%)	(50.0%)	(100.0%)	
	Total			7	7	14	
				(50.0%)	(50.0%)	(100.0%)	
MCI N(%): 18 (29.00%)	Gender	Male		8	8	16	1.000
				(44.4%)	(44.4%)	(88.9%)	
		Female		1	1	2	
				(5.6%)	(5.6%)	(11.1%)	
	Total			9	9	18	
				(50.0%)	(50.0%)	(100.0%)	
AD N(%): 30 (48.4%)	Gender	Male		14	14	28	1.000
				(46.7%)	(46.7%)	(93.3%)	
		Female		1	1	2	
				(3.3%)	(3.3%)	(6.7%)	
	Total			15	15	30	
				(50.0%)	(50.0%)	(100.0%)	
Total N(%): 62 (100%)	Gender	Male		29	29	58	
				(46.8%)	(46.8%)	(93.5%)	
		Female		2	2	4	
				(3.2%)	(3.2%)	(6.5%)	
	Total			31	31	62	
				(50.0%)	(50.0%)	(100.0%)	

Table 3. *Professional driver and gender.*

Marital status						p
Diagnosis				Professional Driver NO N(%): 31 (50 %)	Professional Driver YES N(%): 31 (50 %)	Total N(%): 62 (100%)	
Healthy N(%): 14 (22.6%)	Marital status	Married		6	6	12	.769
				(42.9%)	(42.9%)	(85.7%)	
		Divorced		1	1	2	
				(7.1%)	(7.1%)	(14.3%)	
	Total			7	7	14	
				(50.0%)	(50.0%)	(100.0%)	
MCI N(%): 18 (29.00%)	Marital status	Married		8	9	17	.500
				(44.4%)	(50.0%)	(94.4%)	
		Divorced		1	0	1	
				(5.6%)	(0.0%)	(5.6%)	
	Total			9	9	18	
				(50.0%)	(50.0%)	(100.0%)	
AD N(%): 30 (48.4%)	Marital status	Married		14	13	27	.500
				(46.7%)	(43.3%)	(90.0%)	
		Widow/-er		1	2	3	
				(3.3%)	(6.7%)	(10.0%)	
	Total			15	15	30	
				(50.0%)	(50.0%)	(100.0%)	
Total N(%): 62 (100%)	Marital status	Married		28	28	56	
				(45.2%)	(45.2%)	(90.3%)	
		Divorced		2	1	3	
				(3.2%)	(1.6%)	(4.8%)	
		Widow/-er		1	2	3	
				(1.6%)	(3.2%)	(4.8%)	
	Total			31	31	62	
				(50.0%)	(50.0%)	(100.0%)	

Table 4. Marital status

RETIRED						p
Diagnosis				Professional Driver No N(%): 31 (50 %)	Professional Driver Yes N(%): 31 (50 %)	Total N(%): 62 (100%)	
Healthy N(%): 14 (22.6%)	Retired	Yes		7	6	13	.299
				(50.0%)	(42.9%)	(92.9%)	
		No		0	1	1	
				(0.0%)	(7.1%)	(7.1%)	
	Total			7	7	14	
				(50.0%)	(50.0%)	(100.0%)	
MCI N(%): 18 (29.00%)	Retired	Yes		9	9	18	
				(50.0%)	(50.0%)	(100.0%)	
	Total			9	9	18	
				(50.0%)	(50.0%)	(100.0%)	
AD N(%): 30 (48.4%)	Retired	Yes		15	15	30	
				(50.0%)	(50.0%)	(100.0%)	
	Total			15	15	30	
				(50.0%)	(50.0%)	(100.0%)	
Total N(%): 62 (100%)	Retired	Yes		31	30	61	
				(50.0%)	(48.4%)	(98.4%)	
		No		0	1	1	
				(0.0%)	(1.6%)	1.6%	
	Total			31	31	62	
				(50.0%)	(50.0%)	(100.0%)	

Table 5. Retired.

Among the healthy group, there was no difference in the **years of education** between non-professional (mean=10.57, SD=4.577) and professional drivers (mean=10.57, SD=4.577, p=1.000). On the **MMSE**, there was no difference between non-professional (mean=29.1667, SD=.75277) and professional drivers, (mean=29.1667, SD=.98319, p=0.932).

Among the MCI group, there was no difference in the **years of education** between non-professional (mean=9.67, SD=3.640) and professional drivers (mean=9.33, SD= 4.123, p=0.893). On the **MMSE**, there was no difference between non-professional (mean=26.6667, SD=1.93649) and professional drivers (mean=27.3333, SD=2.39792, p=0.503). On the **FUCAS**, there was no difference between non-professional (mean=44.00, SD=.000) and professional drivers (mean=42.50, SD=1.000, p=0.114). On the **FRSSD**, there was no difference between non-professional (mean=3.50, SD=.707) and professional drivers (mean=3.75, SD=3.500, p=1.000).

Among the AD group, there was no difference in the **years of education** between non-professional (mean=7.40, SD=3.738) and professional drivers (mean=7.73, SD=3.390, p=0.783). On the **MMSE**, there was no difference between non-professional (mean=17.8000, SD=6.06159) and professional drivers (mean=20.6000, SD=4.46894, p=0.190). On the **FUCAS**, there was no difference between non-professional (mean=57.11, SD=10.612) and professional drivers (mean=56.80, SD=15.135, p=0.623). On the **FRSSD**, there was no difference between non-professional (mean=9.40, SD=3.978) and professional drivers (mean=10.67, SD=4.397, p=0.315).

Driving avoidance

Avoiding driving into difficult intersections						
Diagnosis			Professional Driver			p
			NO N(%): 31 (50 %)	YES N(%): 31 (50 %)	Total N(%): 62 (100%)	
Healthy N(%): 14 (22.6%)	Avoid turning into difficult intersections	Never	1	7	8	.001
			(7.1%)	(50.0%)	(57.1%)	
		Always	6	0	6	
			(42.9%)	(0.0%)	(42.9%)	
	Total		7	7	14	
			(50.0%)	(50.0%)	(100.0%)	
MCI N(%): 18 (29.00%)	Avoid turning into difficult intersections	Never	6	8	14	.257
			(33.3%)	(44.4%)	(77.8%)	
		Always	3	1	4	
			(16.7%)	(5.6%)	(22.2%)	
	Total		9	9	18	
			(50.0%)	(50.0%)	(100.0%)	
AD N(%): 30 (48.4%)	Avoid turning into difficult intersections	Never	7	10	17	.269
			(23.3%)	(33.3%)	(56.7%)	
		Always	8	5	13	
			(26.7%)	(16.7%)	(43.3%)	
	Total		15	15	30	
			(50.0%)	(50.0%)	(100.0%)	

Total N(%): 62 (100%)	Avoid turning into difficult intersections	Never		14	25	39	
				(22.6%)	(40.3%)	(62.9%)	
		Always		17	6	23	
				(27.4%)	(9.7%)	(37.1%)	
	Total			31	31	62	
				(50.0%)	(50.0%)	(100.0%)	

Table 6. *Avoiding driving into difficult intersections.*

Healthy non-professional drivers almost always avoided turning into difficult intersections compared to professional drivers (chi-square=10,500, df=1, p=0.001). There is independence concerning the diagnosis between non-professional and professional drivers in the following questions: Do you travel the same kilometers as in the past? Have family members recommended that you stopped driving? Do you take medications which may cause driving problems? Is your attention easily distracted from driving? For example, in urban centers, do you have difficulty concentrating on driving after having driven for over half an hour? Do you have difficulty concentrating on driving when someone is talking to you while you drive? Are you unable to react quickly when needed? Do you avoid driving in urban areas? Do you avoid driving on motorways? Do you avoid driving unaccompanied? Do you avoid driving under time pressure? Is the average speed slower today than when you were 45 years old? Do you avoid driving in unfamiliar areas?

Discussion

In order to test our research hypothesis, that is, whether there is a difference between AD and MCI drivers with and without previous professional driving experience, an extensive driving questionnaire was administered to people aged at least 65 years, with dementia, and holders of a professional driving license.

Out of the 153 participants who were asked whether they had a professional driving license, 43 (28.1%) said “Yes”, 110 (71.9%) said “No”; 55 (35.9%) were diagnosed with AD, 44 (28.8%) were diagnosed with MCI, and 54 (35.3%) were healthy; the latter were the control group. In total, 119 (77.8%) men and 34 (22.2%) women were examined. Very few women in Greece drive at this age. The results showed that more non-professional AD drivers (21.9%) had not renewed their license compared to professionals (p=0.048). More non-professional MCI drivers (91.7%) travelled fewer kilometers compared to professionals (p=0.029). Taking both MCI (27.6%) and AD patients (63.2%) together, more non-professional drivers always avoided driving in unfamiliar areas compared to professionals, MCI (p=0.045) and AD (p=0.026). Finally, more non-professional AD drivers (80.0%) avoided driving when it snowed compared to professionals (p=0.34) [34].

The results from the short driving questionnaire showed that healthy non-professional drivers almost always avoided turning into difficult intersections compared to professional drivers (chi-square=10.500, df=1, p=0.001).

Literature review has shown that the driving ability of professional drivers with dementia has not been investigated before our research. This constitutes a limitation since we cannot extensively discuss and compare our findings with other studies. For the moment, we can only discuss strategies and driving behavior of people with cognitive disorders in general.

Four hundred and seventy-three drivers (473) aged 55-64, 65-74, and over 74 were assessed in a major European study on habits, accidents, compensatory driving behavior, and their attitude to the reassessment of the driving license due to age. It was found that the use of compensatory strategies and adaptation techniques was higher among older adults. The reassessment of driving ability due to age was limited to medical examinations, with the risk of false evaluations, which could be reduced with the human-centered approach of OT. The usefulness of this approach, apart from complying with the principles of OT, is that it focuses on the appropriateness of driving. Older adult drivers are given the opportunity to assess for themselves whether they are able to cope with their functional deficiencies on a more realistic basis as regards driving [58]. There is no information about professional drivers.

A very recent study (2017) sought to identify prediction factors for driving discontinuation in dementia patients. A total of 779 dementia patients from 9 Australian memory clinics were examined. Patients and caregivers were examined for the severity of dementia, while their cognitive functions, neuropsychiatric symptoms, and drug use were evaluated. Their follow-up took place at regular intervals over a three-year period. Out of the 247 patients who were still driving at the beginning of the study, 147 (59.5%) stopped driving during the study. The variables which predicted driving discontinuation included older age, female gender, severity of dementia, and deterioration of cognitive functions. These findings confirm that the above features can predict future driving situation. In addition, they underline the value of regular assessment of dementia patients through the usual tests to determine the course of the disease and the possible prognosis [59]. No recording about professionals drivers.

A recent research (2017) employed a standard driving methodology to compare the differences in the driving behavior of a small sample of healthy older adults (N=10) and AD patients (N=10) over a year. As expected, considering the small sample size, there were no statistically significant differences between the two groups, but AD patients were less likely to drive at night and had less aggressive behavior, such as hard braking and sudden acceleration [60].

Based on our study, we conclude that patients with cognitive disorders and previous professional experience display better driving behavior than professionally inexperienced ones. This conclusion constitutes the added value of our research for scientific literature considering that similar studies have not been published. Further research with a larger sample of individuals with cognitive disorders and previous professional driving experience would be useful.

Appendix

Short Driving Questionnaire

(The questionnaire was administered as part of a doctoral thesis held at the Medical School of the Aristotle University of Thessaloniki.)

Our goal was to develop a new driving assessment and training methods for aged drivers.

Please note that all information will be kept entirely anonymous and for statistical purposes only. All information will have no effect on your driving license, nor will it be related in any way with you.

Completing the questionnaire will take about 10'.

Gender	❒ *M*	❒ *F*
Marital Status	❒ *Married* ❒ *Divorced* ❒ *Widow/-er* ❒ *Single*	*Which category is/was your driving license valid for?* ❒ *A (Motorcycle)* ❒ *B (4-wheel Motor Vehicle)* ❒ *C (Truck)* ❒ *D (Bus)* ❒ *E (Special Trailer)*
Retired	❒ *YES*	❒ *NO*
(Previous) Profession: *Date of Birth:* *Age:* *Years of Education:*		*Have you renewed your driving license?* ❒ *YES* ❒ *NO* *Do you travel the same kilometers as in the past and why?* ❒ *YES* ❒ *NO*

1. Do your family members complain about the way you drive?
   YES NO
2. Do your family members recommend that you stop driving?
   YES NO
3. Do you take medications which cause problems while driving?
   YES NO
4. Is your attention easily distracted from driving, for example, in complex areas, such as urban centers?
   YES NO
5. Is it difficult to concentrate on driving after having driven for more than half an hour?
   YES NO
6. Is it difficult to concentrate on driving when someone is talking to you while you drive?
   YES NO
7. Is it difficult to react quickly when necessary?
   YES NO
8. Do you avoid driving in urban areas?
   YES NO
9. Do you avoid driving on motorways?
   YES NO
10. Do you avoid driving in the rush hour?
    YES NO
11. Do you avoid driving in unknown areas?
    YES NO
12. Do you avoid turning into difficult intersections?
    YES NO

13. Do you avoid driving without a co-driver (spouse, etc)?
YES NO

14. Do you avoid driving under time pressure?
YES NO

15. Is the average speed slower today than when you were 45 years old?
YES NO

Thank you in advance for your invaluable time and assistance.

Date

Researcher Signature Participant Signature

The authors declare that they have no conflicts of interest.

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Original Article

Naringin nanoparticles against neurodegenerative processes: A preliminary work

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Abstract

It is well established that during Alzheimer disease (AD), gradual loss of neuronal networks occurs in the brain, consequently, affecting cognition and memory tasks of the patients. Among other causative factors, oxidative stress induces changes that are eventually accompanied by an irreversible disruption of synaptic connectivity and death of neurons. Moreover, aging and oxidative stress cause alterations to the blood brain barrier, leading to increased permeability, which are thought to further aggravate the underlying pathology. Up to date, no effective treatment is available to Alzheimer's disease patients. Lately, scientific efforts are focusing on exploiting the antioxidant properties that natural polyphenol agents such as flavonoids possess and their potential beneficial effect against neurodegenerative diseases. For that reason, the current investigation, aims at developing more effective flavonoid agents by encapsulating naringin into modified PEG 3000 Silica nanoparticles before its use at cellular level. Overall, our findings suggest an enhanced protective capacity of naringin pegylated nanoparticles against A β amyloid linked oxidative stress mediated neurodegeneration in primary rat neuronal and glial hippocampal cultures for a certain incubation period. The functional biological reactivities of the novel flavonoid nanoparticles were in line with their physicochemical features and reflect the a) differential nature of the structural assemblies of the new nanoparticles, thereby distinguishing them from other polymeric and liposomal drug carriers, and b) significance and impact of PEG chemistry in the synthetic assembly of the nanocarriers. The ability of the employed nanoparticles to entrap a relatively high dose of otherwise insoluble drugs and their biological activity highlight their potential as brain targeting therapeutics.

Introduction

Alzheimer's disease (AD) is associated with several deleterious molecular cascades that lead to neuronal dysfunction and can be attributed to a variety of genetic and environmental factors [1,2] including oxidative stress [3,4]. Indeed, oxidative stress, which is characterized by an imbalance between production and inactivation or inhibition of the formation of active forms of Reactive Oxygen Species (ROS) [5,6], is considered a major contributor to cell neurodegeneration [7].

Generally, neurons, in contrast to glial cells, have low antioxidant defense, which makes them particularly vulnerable toxic insults and injuries [8,9]. Therefore, treatment from oxidative stress has always been a substantial problem for the early prevention of neurodegeneration and, at the same time, a research challenge for the diagnosis and possible treatment of the disease [10].

To this respect, flavonoids have attracted considerable interest in recent years due to their potential beneficial effects on human health [11-15]. Chronic consumption of flavonoids on a daily basis has been shown to reduce the risk of developing various forms of cancer [16-18] such as cancer of the oral cavity, pharynx, larynx, esophagus [19], stomach and colon, lungs [20,21], thyroid gland and breast cancer. The molecular mechanisms attributable to the potential anti-cancer activity of flavonoids include the inhibition of specific phases of carcinogenesis, such as, the onset, progression and proliferation of neoplastic cells or even the stage of angiogenesis [22-24]. Flavonoids including naringin (NAR) are polyphenolic compounds well-widespread in the plant kingdom [25-27]. They are classified according to their chemical structure in flavones, flavanols, flavanones, isoflavones, catechins, anthocyanins and chalcones [28]. Over 4000 flavonoids have been recognized and many of which are found in fruits, vegetables, beer, wine, and natural fruit juices [29-31].

NAR undergoes hydrolysis of the glycosidic bond and cleavage of its sugar by intestinal microflora enzymes [32,33].Hydrolysis of the glycosidic bond and removal of the sugar (Rhamnose) to the NAR molecule is considered necessary for the absorption of the corresponding aglycans from the gastrointestinal tract, since the size of the molecules and the strongly hydrophilic nature of the flavonoid glycosides does not allow their absorption [34,35]. NAR has low bioavailability and its plasma concentration is characterized by very high variability. It is excreted in the urine but also in the bile, mainly in the form of its glucuronides. The renal excretion of this flavanone begins 6 hours after administration of its corresponding flavonoid glycoside, increases to 11 hours to complete at 24 hours and does not appear be affected by the administered dose [36].

Inclusion of bioactive compounds, such as NAR, in pegylated nanoparticles represents an effective approach for controlled release, increased physical stability, protection from interactions with the environment.and enhancement of their bioactivity [37,38]. Thus, the aim of the current study is to exploit the antioxidant properties of NAR and preserve its bioactivity at cellular level by encapsulating it at a suitable nanocarrier. Furthemore, NAR pegylated nanoparticles biological profile was also examined, based on two parameters of neurodegeneration including cell loss and connectivity loss.

Methods

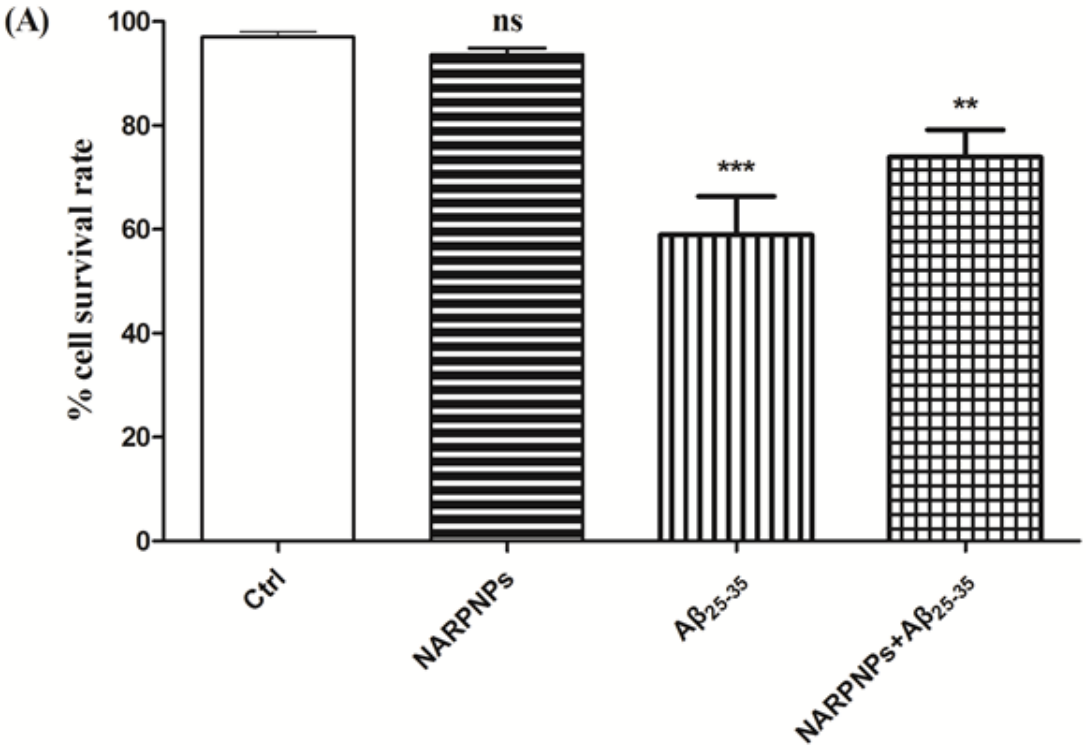
Among the popular methods to synthesize nanoparticles, sol-gel processing has proven to be particularly useful. This method was also employed in the current work and implicates an inorganic matrix formation of a nanosized level by applying at low temperature to sol gel in order to allow the encapsulation of the active molecules with biological properties. The sol-gel silicon dioxide is a degradable nanomaterial commonly employed for a) biocompatible coatings on implants and medical products, biosensors, biocatalysts, and b) as coating for controlled release of biocides, pharmaceuticals and vitamins. The main advantage of using sol-gel in the nanoparticle composition is that the resulting materials are non-toxic, biocompatible and able to act as hosts of various natural or potentially synthetic therapeutic substances for controlled release drug applications. Herein, NAR was encapsulated into modified PEG 3000 Silica nanoparticles and fully characterized using multiple physicochemical techniques including elemental analysis, zeta-potential and hydrodynamic size, FT-IR and SEM [39].

Furthermore, the ability of encapsulated NAR pegylated nanoparticles (NARPNPs) to function as neuroprotective agents was investigated using primary neuronal and glial hippocampal cells and studied against $A\beta_{25-35}$ amyloid induced oxidative stress conditions. Under the physiological state (pH 7.4), the presence of specific oxidizing agent implies loss of hippocampal cells, as well as a decrease in the number of viable cells. Cytotoxicity and effect on morphology and connectivity of neurons (synapses) were examined through various widely used in vitro methods including laser scanning microscope. Initially, tests focused on finding concentrations of toxic and neuroprotective substances as well as incubation times for nanoparticles were conducted to induce cell death levels appropriate to investigate possible protective effects by finding the IC50 concentration. This pilot study led to the selection of the incubation times and times used to obtain the experimental results that follow. Of note, survival rate was determined by counting Calcein-AM stained cells upon 12-hour incubation period allowed determination of the number of live cells, while a count of PI/LIVE/DEAD fixable dead-stained cells indicated the number of dead cells. Neuronal connectivity assessment was achieved by applying quantitative analyses (data expressed as area per neuron+SEM) of polymerised tubulin fluorescence in rat hippocampal neuronal cells only for a 3-hour incubation period. The final concentrations to insure optimized results were 30 μM and 10 μM for NARPNPs and $A\beta_{25-35}$, respectively.

Results

Despite the preliminary characteristic of the current ongoing work, upon the sol-gel methodological experimental procedure, well-charachterized monodispersed spherical rounded agglomerates nanoparticles of approximately 150 nm were achieved. The capacity of entrapment of NAR in silica pegylated NPs was measured and led to a 90% of entrapment rate. The rate of the release capacity of the hybrid nanoparticles was in congruence with the entrapment capacity and reached a 24-hour plateau showing a dose and time dependence profile.

Regarding the biological behavior of the novel nanoparticles, NAR pegylated nanoparticles protective effects were demonstrated on the survival of hippocampal cells at the concentration of 30 μM. This observation was consistent with our previous studies with analogous nanomaterials [40]. It is noteworthy that no cytotoxicity was observed in any case while incubating hippocampal cells in the presence of NAR pegylated nanoparticles alone at that concentration (**Figure 1**. A & B). Briefly, upon a 12-hour incubation time, glial viability was increased by more than 30% when treated with both 30 μM NAR pegylated nanoparticles and 15 μM $A\beta_{25-35}$ compared to the individual $A\beta_{25-35}$ cell treatment. Under the same treatment conditions, neuronal viability was only 15% suggesting that neurons are more susceptible than glial cells.



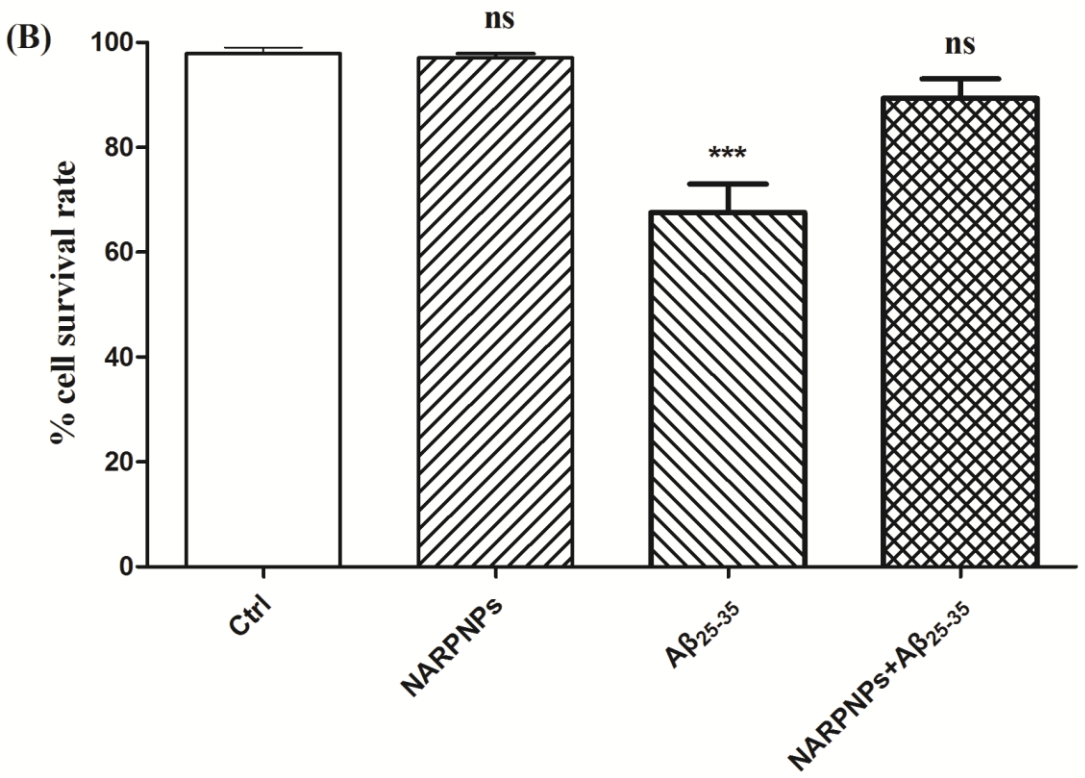


Figure 1. The protective pattern of NAR-loaded nanoparticle (NARPNPs) against specific amyloid concentration. The action of NARPNPs was assessed on the survival rate in an amyloidogenic rat primary hippocampal neuronal (A) and glial (B) environment. Experimental conditions of NARPNPs, $A\beta_{25-35}$ and NARPNPs+ $A\beta_{25-35}$, at the aforementioned concentrations, were also run. 12 h incubation period was employed for the above experimental conditions. Data are shown as means, relative to control (untreated) viability rates recorded in the absence of NARPNPs and Amyloid (in % + SEM). Statistical differences are indicated as $p < 0.05$ (ns =non-significant), $p > 0.05$ = * (significant), $p > 0.01$ = ** (highly significant) and $p > 0.001$= *** (extremely significant).

Neuronal connectivity in both aforementioned experimental treatment conditions was much improved in the presence of both NAR pegylated nanoparticles and $A\beta_{25-35}$ compared to the individual $A\beta_{25-35}$ treatment condition (Figure 2).

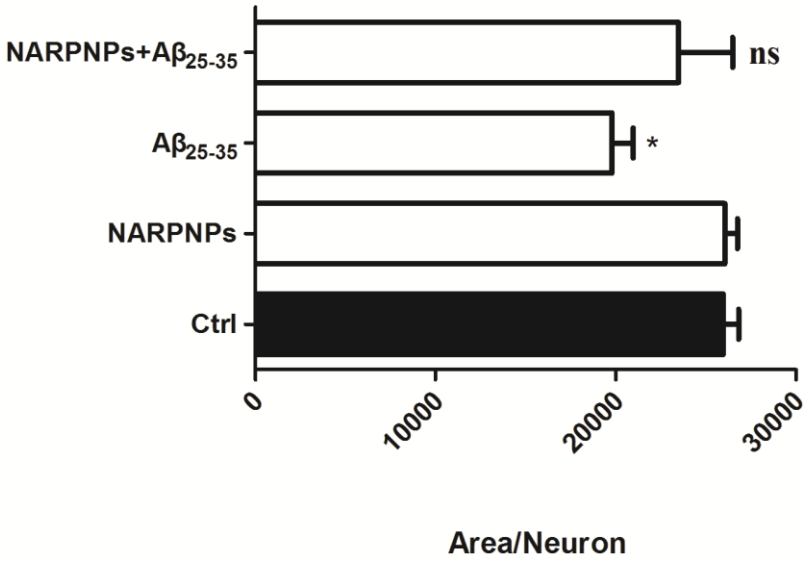


Figure 2. Neuronal connectivity under NARPNPs treatment against amyloid induced oxidative stress in rat primary hippocampal cell environment. 12-hour incubation period was employed for the above experimental conditions. Data are shown as means, relative to control (untreated cells) viability rates recorded in the absence of NAR-loaded nanoparticles (NARPNPs) and Amyloid (in % + SEM). Statistical differences are indicated as $p < 0.05$ (ns =non-significant), $p > 0.05$ = * (significant), $p > 0.01$ = ** (highly significant) and $p > 0.001$= *** (extremely significant).

Finally, protection against the oxidative damage caused by $A\beta_{25-35}$ (15 μM) is attributed to the ability of the flavonoid to block the active forms of the peptide. Further neuroprotective effects of NAR pegylated nanoparticles against the neurotoxicity of $A\beta_{25-35}$ amyloid peptide, can be linked to the presence of the polyethylene glycol chain on the surface of the nanoparticles and its ability to bind to Aβ, thus preventing it from causing lesions in the neuronal synaptic network which might lead to cell loss.

Discussion

Naringin, a potent antioxidant, is poorly soluble in water, and rapidly degraded and metabolized in the human body [41]. Despite the limitations regarding its use, NAR has been suggested to be very promising for the prevention and therapy of major chronic diseases [42,43] In fact, several epidemiological studies indicate that consumption of flavonoids is associated with a lower incidence of neurodegenerative disorders such as Alzheimer's disease (AD) [44]. Incorporation of bioactive agents in delivery systems was reported to provide higher therapeutic efficiency and reduce the side effects related to their [45]. This has led us to try to formulate functional nanocarriers, able to encapsulate the active antioxidant NAR in order to enhance its neuroprotective capacity. To this end, we synthesized pegylated silca nanoparticles using the sol-gel method and encapsulated naringin within their polymerix matrix.

The physicochemical characteristics of the naringin containing hybrid materials reflect the significance and effect of polyethylene glycol (PEG) chemistry on nanoparticle synthesis, which

suggests their potential biological activities. This was in line with previous scientific studies conducted on flavonoid PEGylated nanoparticles [46-48]. Flavonoid binding and release studies were consistent with the physicochemical characteristics and demonstrate the adequacy of hybrid nanoparticles in their ability to bind NAR, maintain the antioxidant potential of the flavonoid, and control the release of their load. It is therefore evident that loading NAR in nanovesicles offers significant advantages including: a) an improvement in bioavailability and selectivity, b) an increase in the stability of the phenolic as well as c) the ability to overcome the blood brain barrier (BBB) due to the colloidal nature of the nanoparticles. These observations are in agreement with the litterature where biopolymer nanoparticles have been proposed as effecient encapsulation that can markedly help the delivery of phenolic compounds [49-51].

The results related to the nanoparticle treatment of primary neuronal and glial cultures reveal the protective potential of the hybrid particles against oxidative stress mediated cell loss and the perservation of neuronal cell morphology. Action against neurodegeneration appears to arise from the antioxidant capability of naringin which has been enhanced by its encapsulation in pegylated nanoparticles. Indeed, NAR-loaded NPs could facilitate the sustained and targeted administration of the active flavonoid. The improved cellular morphology and preservation of neuronal network could also be attributed to the nanoparticle surface modification with PEG. As shown in previous studies, PEGylation of NPs can dramatically increase their affinity for toxic Aβ peptides, which can be absorbed at the nanoparticle surface, and leads to fewer β-sheets and less aggregation [52,53]. This favorable interaction between PEG and Aβ peptides along with the antioxidant effect of the NAR-loaded NPs could help in delaying Alzheimer's disease development.

Although the current ongoing work is in its preliminary stage, the cytotoxicity and biological activity profile of flavonoid nanoparticles in a cellular neurodegenerative environment caused by the presence of oxidizing agents (Aβ amyloid peptide) a) suggests an improved antioxidant effect in the treatment of oxidative stress; b) reveals the positive effect of nanomaterials in the loss of neuronal synaptogenesis and neurodegeneration, and c) paving the way for the development of molecular protection and prophylactic medical technology related to neuron cardiovascular diseases such as Alzheimer's disease.

Conclusion

Overall, our current work sets the stage for the development of functional nano-medicinal materials targeting prominent pathogenic mechanisms of Alzheimer's disease. Although our observations are encouraging, there were certain limitations associated with the study, including the need for further investigation of protective actions of the NAR-loaded nanoparticles. Confirmation of the upstream signal pathways related to the radical scavenging capacity of the NAR-loaded nanoparticles could provide an insight into the mechanisms underlying the neuroprotective effects observed. In addition, the ability of the NPs to cross the BBB should be validated with appropriate methods. Finally, performing systemic toxicity experiments and studying the NAR-loaded nanoparticle biological fate upon administration with regards to their absorption and pharmacokinetics should be considered in future experiments.

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The authors declare that they have no conflicts of interest.

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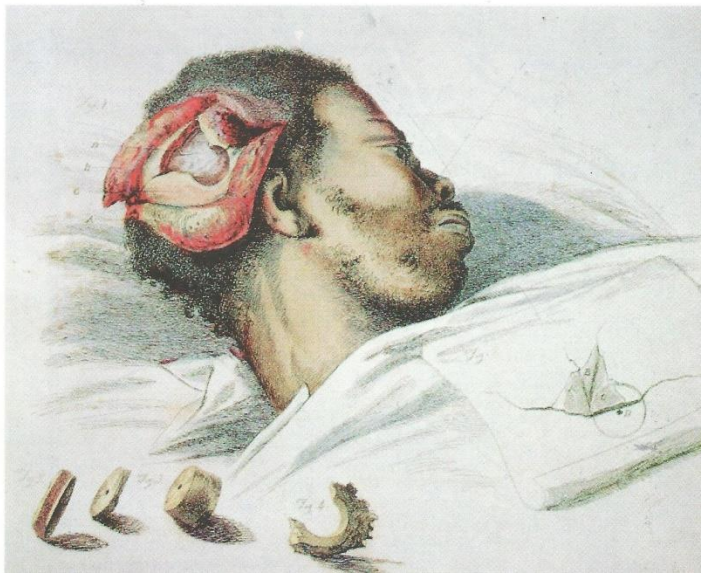

Anonymous. Cranial surgery.

Original Article

Magnetic chrysin silica nanomaterials behavior in an amyloidogenic environment

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Keywords: Alzheimer's disease - Amyloid - Oxidative stress - Chrysin - Nanoparticles

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with brain damage induced by β-amyloid and tau accumulation. One of the hallmarks of amyloidogenesis, is the aggregation of amyloid proteins into a specific cross-β sheet structure, which alters their biological activity thereby affecting neuronal connectivity and function. Despite significant progress in the management of AD over the recent years, the early diagnostic and treatment options still remain limited. Recently, great attention has been focused on the advancement of therapeutic strategies exploiting the antioxidant properties of naturally occurring compounds. Flavonoids, a major class of phytochemicals, have been found to possess a multiple range of health promoting effects, including neuroprotection. Chrysin (ChR) is a flavonoid of the flavone class with potent neuroprotective and anti-inflammatory activity. In addition, ChR improves cognitive decline by exerting anti-amyloidogenic and neurotrophic effects. Magnetic nanoparticles allow binding of drugs by entrapment on the particles, adsorption, or covalent attachment. In our study, well characterized ChR-loaded magnetic PEGylated silica nanospheres (MChRPNPs) were employed with potential enhanced protective characteristics against amyloid induced oxidative stress. The interactions of MChRPNPs with β-amyloid were demonstrated in rat hippocampal cell cultures. Overall, the findings regarding the biological activity profile of MChRPNPs in a cellular amyloidogenic environment suggest an improved specificity of antioxidant properties counteracting amyloid mediated oxidative stress reactivity.

Introduction

A severe, age-associated neurodegenerative disorder, Alzheimer disease (AD) affects a large proportion of the elderly population, with nearly half of those aged 85 afflicted with this disorder.

Recent demographic and epidemiologic data suggest that AD contributes to approximately 60-70% of all dementia cases [1,2].

Among the multitude of factors influencing the onset and progression of the disease, oxidative stress plays a pivotal role in the development of AD pathooanatomical features [3-5]. The latter include amyloid peptide (Aβ) plaque deposition, tau pathology, loss of function of presenilin (PSEN), dysfunction of the cholinergic system, disrupted calcium signaling, neuronal loss of connectivity and cell loss, resulting in cognitive impairment [6-8]. Currently, there is no available therapy against the disease [9], with the limited treatment options focusing on alleviating symptoms and delaying the disease progression. Also among the aforementioned factors contributing to AD pathology, Aβ peptides which are produced as a result of cleavage of APP found in cell membranes, is a central cause of the pathology. Its accumulation in the brain is associated with oxidative stress [10,11].

Given the fact that oxidative stress emerges as a crucial factor in neuronal cell degeneration, efforts in our lab have focused on strategies to avert upstream processes and factors contributing to it, thereby influencing pro-oxidant-antioxidant balance in cellular physiology. Key to such an approach is employment of strong antioxidant molecules originating in natural sources. Outstanding among them are natural polyphenols and flavonoids, a group of low molecular weight benzo-g-pyrone derivatives, ubiquitous in plants. This naturally occurring compounds have structural features that support excellent radical scavenging properties. The high potency, and (bio)chemical reactivity renders them viable alternatives to conventional therapeutic drugs.

Chrysin (5,7-dihydroxyflavone, ChR) is a natural flavonoid that can be derived by several plants, honey and propolis [12,13]. Recent studies demonstrate that ChR exhibits multiple biological and pharmacological activities, such as anti- inflammation, anti-oxidation, as well as immunomodulatory and vasorelaxation effects [14-17]. For instance, ChR effectively inhibits the expression of the key pro-inflammatory enzymes, such as nitric oxide synthase and cyclooxygenase-2. Moreover, ChR has potential anticancer [18], antiviral, antidiabetic [19] and anti-inflammatory properties [20-22].

It has also been postulated that ChR can protect neurons from oxidative insults and apoptosis and improve learning and memory ability [23-25]. Previous efforts, have shown that ChR can attenuate loss of dopaminergic neurons and improve motor, learning and memory functions, thereby highlighting its potential against neurodegenerative diseases such as Parkinson's [25-29]. However, evidence on the use of ChR against AD linked pathology is limited. In our study we propose that ChR may have a mitigating effect against amyloid β -induced oxidative stress.

Although, flavonoids possess remarkable therapeutic potential in promoting cognitive performance and preventing neurodegenerative disease, their use has not been fully exploited due to their numerous structurally related limitations [30-32]. In particular, the therapeutic efficacy of ChR is restricted by its low bioavailability. Therefore, in this work, ChR was used loaded in well characterized magnetic PEGylated silica nanospheres (MChRPNPs) in order to preserve this potent antioxidant and enhance its protective action(s) against amyloid mediated stress.

Methods

Synthesis & physicochemical characterization

Based on hydrolytic precipitation method, magnetic nanoparticles were synthesized and modified with PEG 3000. The procedure followed was, first, to add 0.2g $FeCl_2.4H_2O$ and 0.5g $FeCl_3.6H_2O$ as well as 0.5g PEG 3000 into 75mL ddH_2O under specific experimental conditions (intense stirring at 55°C for 15min). ChR was then added at two different amounts (0.50 and 1g) to maximize incorporation into the magnetic nanoparticles. Moreover, 10mL NH_4OH (25%) was added to achieve 11 pH value in a time period of at least 8 hours. MChRPNPs were isolated from the rest of the solution and washed couple of times using 1 Molar ammonium solution and ddH_2O before drying for 72 hours at 55 °C.

The synthesized nanomaterials were characterized employing various techniques such as particle size, z-potential, FT-IR and SEM. The entrapment efficiency and the *in vitro* ChR release study were also determined through UV-Visible spectroscopy at the characteristic wavelength of ChR absorption (275 nm) [33].

Cell survival assessment upon MChRPNPs vs. Amyloid $A\beta_{25-35}$ incubation

To prepare hippocampal cultures, Sprague-Dawley rat neonates were used after cervical dislocation in accordance with the Department of Veterinary Medicine of Aristotle University regulations. Detailed cell culture experimental procedure is described in our previous work [34,35].

Upon piloting, concentrations and incubation period of interest used for MChRPNPs and $A\beta_{25-35}$ were determined at 30 μM and 10 μM, respectively for a incubation period of 30 minutes. To assess cell viability, cultures were stained using live/dead Cell Double Staining Kit without further modification of the protocol (Sigma Aldrich, UK). Images were visualized using an Axioskop 2 plus microscope (Carl Zeiss, Germany) with a 40x phase contrast water immersion objective. Images were captured using an AxioCam HRc camera, controlled by AxioVision software (Version 3.1) using the appropriate Rhodamine (for propidium iodide) and Fluorescein isothiocyanate (FITC; for calcein) filters. ImageJ software was applied for manual cell counting assessment. Each experiment was conducted in triplicate and repeated at least three times upon statistical analysis. Specifically, GraphPad Prism1 (Version 4.01; GraphPad Software, San Diego, CA, USA) was employed to evaluate the statistics. Mean viability rates and SEMs were considered for each group treatment as well as cell hippocampal type.

The statistical analysis used was One-way of variance (ANOVA), followed by Tukey's multiple comparison tests and was performed considering all group pair comparison. Three rating values were set to define the degrees of significance: *$p<0.05$ (significant), **$p<0.01$ (highly significant) and ***$p<0.001$ (extremely significant) or non-significant ($p>0.05$).

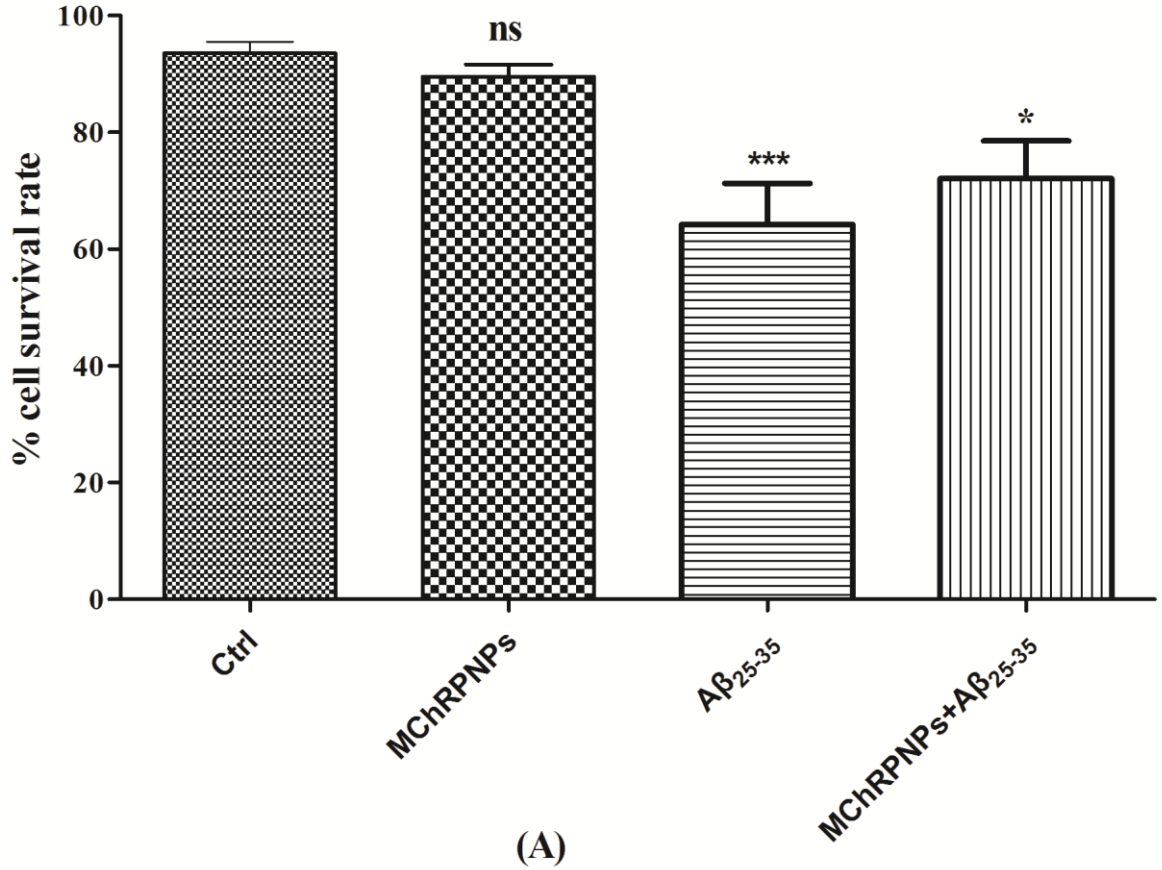
Results

Upon determining the physico-chemical characteristics of ChR-loaded magnetic PEGylated silica nanospheres, the size of the novel nanoparticles was approximately 100nm. ChR adsorption and release rates were approximately 90%, projecting the suitability for drug encapsulation of the magnetic nanospheres employed herein. Further examination of the MChRPNPs neuroprotective effect(s) on amyloid induced oxidative stress at the cellular level revealed promising results on their protective behavior against amyloid expression in the glial and neuronal cells. Specifically, the survival rate was assessed, compared to untreated experimental condition at both glial and neuronal primary cell line, in the presence of MChRPNPs and/or amyloid at specific concentration of 30 μM and 10 μM, accordingly. The data are shown at figure 1. In details, survival rate of neuronal primary cell line was 89±1% ($p>0.05$), 64±4% ($p<0.001$) and 72±3% ($p<0.05$), in the presence of MChRPNPs, $A\beta_{25-35}$ and MChRPNPs+$A\beta_{25-35}$, respectively, compared to the untreated cells survival rate which was 94±1%.

Survival rate of Glial primary cell line, was 91±1% ($p>0.05$), 64±3% ($p<0.05$) and 80±2% ($p>0.05$), in the presence of MChRPNPs, $A\beta_{25-35}$ and MChRPNPs+$A\beta_{25-35}$, respectively, compared to the untreated cells survival rate which was 94±1%.

Based on a general observation of the results, glial cells showed to be less affected that neuronal cell line as their survival rate was slightly higher than the neuronal survival rate one.

Additionally, neuronal or glial cell line survival rate of MChRPNPs experimental treated condition showed not statistically significant than their untreated cell lines. In contrary to the latter observation, $A\beta_{25-35}$ experimental treated condition exhibits lower survival rate in both neuronal and glial cell line compared to all experimental conditions.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Aβ25-35
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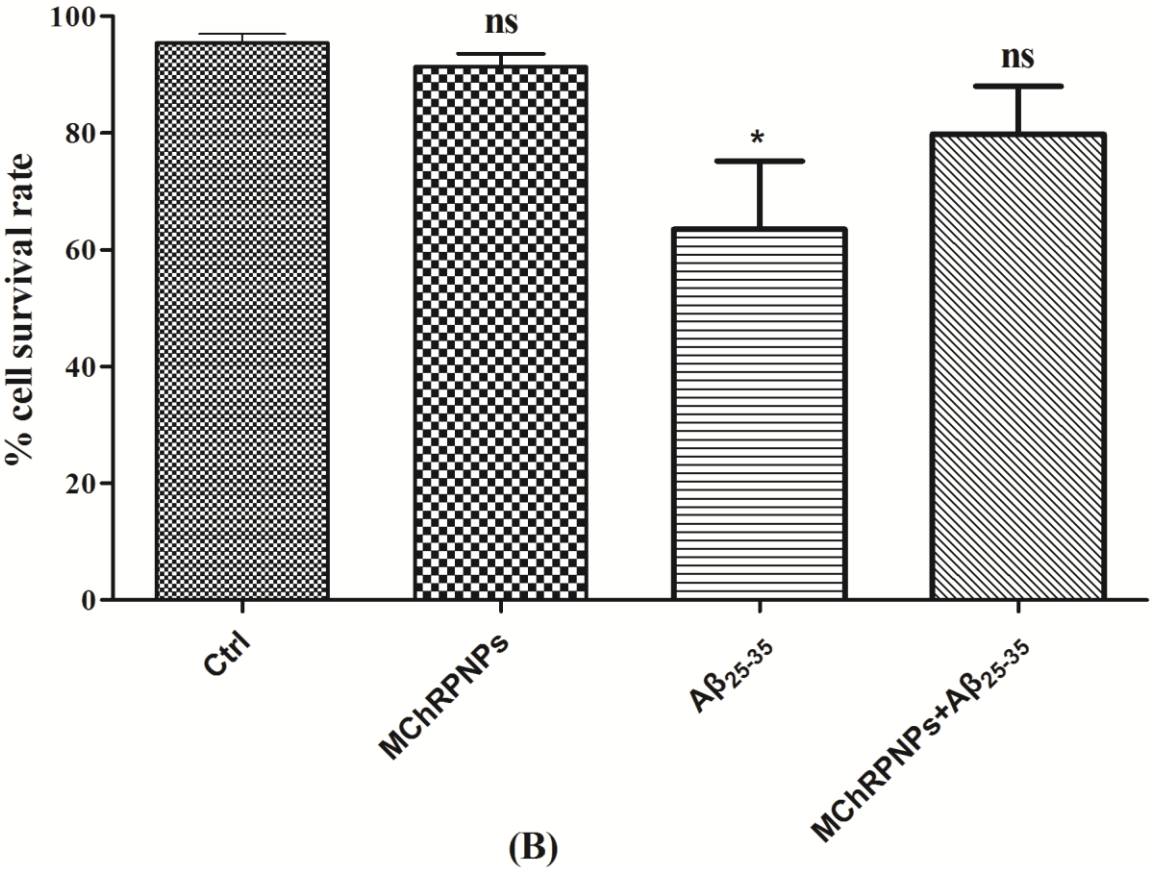
(A)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MChRPNPs
Aβ25-35
MChRPNPs+Aβ25-35

(B)

Figure 1. The protective pattern of against specific amyloid concentration. The action of MChRPNPs was assessed on the survival rate in an amyloidogenic rat primary hippocampal neuronal (A) and glial (B) environment. Experimental conditions of MChRPNPs, $A\beta_{25-35}$ and MChRPNPs+ $A\beta_{25-35}$, at the aforementioned concentrations, were also run. 3 h incubation period was employed for the above experimental conditions. Data are shown as means, relative to control (untreated) viability rates recorded in the absence of MChRPNPs and Amyloid (in % + SEM). Statistical differences are indicated as $p < 0.05$ (ns =non-significant), $p > 0.05$ = * (significant), $p > 0.01$ = ** (highly significant) and $p > 0.001$= *** (extremely significant).

Discussion

It is well known that oxidative injury may be an important part of events involved in the pathogenesis of numerous neurodegenerative diseases including AD. ChR is phenolic compound with antioxidant and neuroprotective properties. Current approaches to incorporate ChR in therapeutics are often hampered by poor targeting and low solubility. Furthermore, free ChR undergoes rapid metabolic transformation resulting in a poor bioavailability. To overcome these limitations and enhance ChR bioactivity, nanoencapsulation has emerged as a promising strategy. Entrapment of active biomolecules in nanoscale carriers can confer them protection against enzymatic degradation, increase their circulation time and guarantee sustained release at the desired site of action [36]. To that end, in the current scientific work, encapsulated antioxidant ChR in Magnetic NPs. Magnetic NPs are considered to be safe and effective carriers for a wide range of applications including local delivery and enhancement of the efficacy of various drugs. Their potential in brain targeting of therapeutic moieties is well established and widely reported in literature [37-39].

Our study demonstrates the neuroprotective efficacy of ChR loaded magnetic PEGylated silica nanospheres against Aβ induced oxidative stress. Our findings suggest that the same therapeutic effect of ChR could be attained at a low concentration used in the current study against amyloid mediated oxidative stress via nanoformulating in MChRPNPs.

Worth to be mentioned is that incubating both MChRPNPs and amyloid at specific concentration in glial and neuronal cell line showed better viability of the cells compared to individual amyloid cell treatment. This scientific observation is in line with previous studies in the literature on flavonoid against oxidative agents in brain tissue related environment [40-41].

However, numerous limitations should be mentioned for the current ongoing work. One of the limitations of this study is the fact that only amyloid induced neurodegeneration was explored. The action of MChRPNPs should be also tested on other AD related processes such as tau pathology to safely assume their potential therapeutic use. More studies are also needed to further describe the mechanisms of ChR action at molecular level. It should also be taken into account that the antioxidant properties of MChRPNPs are affected by physicochemical and biochemical factors such as surface properties, size, as well as their absorption rate. Indeed, it is crucial that the MChRPNPs are able to overcome the blood brain barrier (BBB), thus providing neuroprotection in sensitive areas of the brain linked to neurodegeneration such as the hippocampus. In that respect, their size should be less than 100 nm. Further optimization of the

NPs physicochemical characteristics might maximize their targeting ability and enhance their neuroprotective effect.

Finally, although the current results are encouraging, further in vivo work is needed to investigate the overall mechanisms of action and assess any possible systemic effects.

Given that Aβ toxicity is almost certainly initiated at pre-symptomatic stages of AD, maximum benefits could be obtained from a preventive intervention. The challenge now is to move beyond the epidemiology which has hinted at positive effects of flavonoid rich diets on the development of dementia [42], into clinical applications that directly test the efficiency in individuals that are at risk or those that have already developed mild cognitive impairment.

Conclusion

Chrysin encapsulation in magnetic nanocarriers ensures increased efficacy of this flavonoid against Aβ induced oxidative stress. This study reveals the competence of magnetic pegylated nanoparticles regarding the antioxidant potential and the complete release of their load under conditions of induced oxidative stress in neuronal and glial cell cultures. The collective results set the stage for further work into the development of nanomedicinal materials targeting preventive and/or therapeutic countermeasures to prominent pathogenic mechanisms of Alzheimer's disease.

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Original Article

Callosal Angle and Evans Index predict beta amyloid and tau protein in patients with dementia

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Abstract

Introduction: Aβ and tau protein have been widely investigated for the diagnosis of dementia entities, most commonly Alzheimer's disease (AD). However, their measurement is an interventional, time-consuming procedure, while it requires specialized personnel, unlike the evaluation of radiological markers, such as Evans index (EI) and Callosal angle (CA). This study aims to investigate the correlation between EI, CA, Aβ and total tau in order to outline a basis of diagnostic evaluation for the patient's CSF biomarker profile. **Methods**: Sixty-two (62) patients who presented with dementia symptoms participated in this study. Aβ and total tau levels in their CSF as well as CA and EI values from their MRIs were measured. Multiple regression was employed to predict Aβ and total tau values from EI and CA. From the Durbin-Watson analysis (d1=2.057, d2=1.881) we can assume that there is no first order linear auto-correlation in our multiple linear regression data. **Results**: The variables statistically significantly predicted both Aβ and total tau (F1=8.720, R1=0.484, p1=0.001 and F2=4.110, R2=0.355, p2=0.022). Out of all the variables, it was shown that CA, EI ($p<0.05$) and CA ($p<0.05$) added statistically significantly to the prediction of Aβ and total tau. **Conclusions**: Collectively, these results support a significant correlation between EI and Aβ and CA, Aβ and total tau. This highlights the potential role of radiological markers as rough estimates of biomarker levels in everyday clinical practice, assisting to a robust and inexpensive diagnosis.

Introduction

Dementia poses a widespread threat to global health, as its implications to patients and their families are exceptional in size, costs and impact. Although the exact pathophysiological mechanisms involved in dementia remain to be elucidated, abnormalities in the expression and circulation of proteins like Aβ, tau and phosphorylated tau (P-tau) have been strongly linked to a

number of dementia entities [1-4] Subsequently, CSF levels of the aforementioned proteins have been proven to be indicators of neurodegeneration and disease progression [5. 6] and as such have been used as significant tools in the diagnostic process of dementia, most commonly Alzheimer's disease (AD) [7-10].

Aβ is produced by the cleavage of transmembrane Amyloid Precursor Protein (APP) with the intervention of the enzymes β and γ secretase [2, 11]. In AD, Aβ aggregates in the extracellular space forming neuritic plaques, which constitute the key pathological feature of the disease [5, 12, 13]. Numerous theories have been proposed for the cause of Aβ aggregation, a number of which focus on a pathological clearance of Aβ peptide in the CSF [2, 11, 14, 15]. In addition to AD, low CSF concentrations of Aβ can be found in other dementia types like normal pressure hydrocephalus (iNPH) [4, 16, 17], demonstrating the importance of this biological pathway in neuronal survival and function.

Tau protein seems to also play a significant role in the pathology of most dementia entities, such as AD [6, 12, 18], Parkinson's disease dementia [1] and Frontotemporal Dementia [3]. Tau is a microtubule- associated protein (MAP) with numerous functions, one of which consists of the modulation of the stability of axonal microtubules [6]. In AD, Aβ and tau protein work interrelated, forming an important crosstalk which leads to P-tau [12, 18, 19]. P-Tau increases the capacity of tau to self-assemble resulting in the formation of neurofibrillary tangles (NFTs) [20]. Interestingly, P-tau interferes with neuronal function working both through the formation of NFTs and independently [18, 21]. Thus, CSF levels of tau (and P-tau) are elevated in patients with AD [17], although in other dementias such as iNPH, they appear reduced [4, 16, 17].

In the last decade, the set of overlapping clinical symptoms and radiological signs in dementia has shifted the burden of differential diagnosis to the aforementioned CSF markers [10, 17, 22]. However, their measurement is an interventional and time-consuming procedure, while it requires specialized personnel and equipment. On the other hand, the diagnostic procedure of an MRI, which is necessary for the differential diagnosis of dementia from other neurological diseases, is non-interventional and easily available to the clinician. For this reason, our study tries to investigate the correlation of radiological markers with CSF proteins in order to outline a basis of diagnostic evaluation of the patients' CSF biomarker profile, in a way that is faster, less expensive, non-interventional and easily available for the clinician. It has been proposed that ventricular enlargement may influence the clearance of Aβ [2, 14, 23], whereas tau protein does not seem to abide by the same variables. Tau seems to relate instead to atrophy progression [6, 21]. For this reason, this study used radiological markers which estimate ventriculomegaly and brain atrophy in patients with dementia symptoms.

EI is an indirect radiological marker of the lateral ventricles size [24] which is included in the diagnosis of iNPH, as a major neuroimaging criterion [8, 25]. It is defined as the ratio between the maximal diameter of the frontal horns to the widest diameter of the brain at the same axial slice [26] (figure 1). Based on the most recent guidelines, a value of 0.3 or higher defines ventriculomegaly [27].

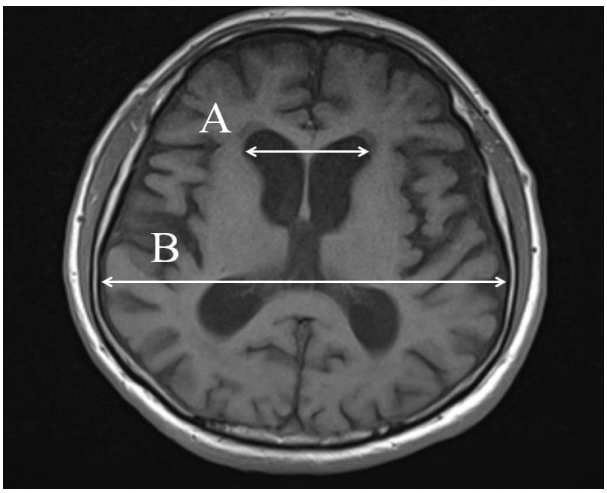

Figure 1: Evans index = $\frac{A}{B}$

CA is a useful radiological marker, used as a diagnostic criterion for iNPH [25, 28] and plays a significant role in the differential diagnosis from other neurodegenerative diseases [29, 30]. It is usually estimated as the angle of the lateral ventricles set at the standard coronal level crossing the posterior commissure (fig. 2). Normal values range between 100-120 degrees and in patients with AD tends to be slightly higher. Values between 50-80 degrees are associated with iNPH diagnosis. Additionally, it has been hypothesized by previous research that CA suggests brain atrophy in patients with neurodegenerative diseases, such as Lewy Body Dementia and AD [26, 31].

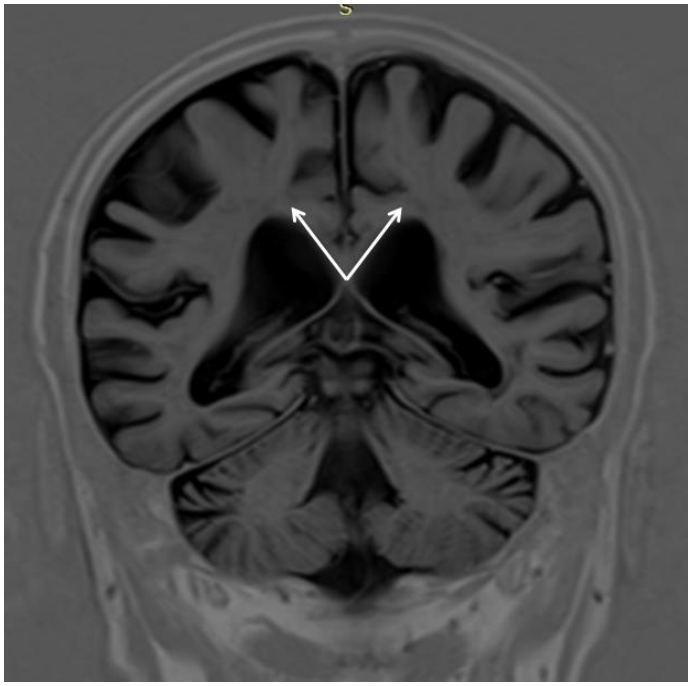

Figure 2: Callosal Angle measured at the level of the posterior commissure.

On this basis, Aβ and total tau CSF protein markers were investigated in this study for possible correlations with Evans index and Callosal angle.

Methods

Patients

Our sample consisted of 62 patients selected from the Greek Association of Alzheimer's Disease and related disorders (Alzheimer Hellas) and AHEPA hospital. The patients were presented with symptoms of dementia and the overall sample consisted of a number of diagnoses, namely 29 AD, 19 iNPH , 2 PSP, 1 FTD, 2 LBD, 2 Parkinson's Disease, 1 mixed type dementia and 6 MCI. All individuals, aged from 52 to 90 years (mean=75.822), underwent MRI brain scans and lumbar punctures to evaluate radiological markers and CSF protein levels respectively.

Radiological Markers

Evans Index and Callosal angle were evaluated via three-dimensional Magnetic Resonance Imaging (MRI). Evans index values were measured from the maximal width of the frontal horns to the internal diameter of the cranium. The callosal angle was identified as the angle of the lateral ventricles set at the standard coronal level crossing the posterior commissure.

CSF protein levels

Patients' CSF was extracted early in the morning through lumbar puncture between L3 and L4 with a G19 and rarely with a G18 needle in a 10ml PP tube. After that the CSF was centrifuged (2000g for 10m) and aliquoted in PP tubes (each tube contains up to 500μl of CSF) for future measurements, and stored in -80oC. Levels of CSF Aβ and whole Tau were measured by a solid-phase enzyme immunoassay (ELISA) using the INNOTEST® β-AMYLOID (1-42), Art. no. 81576 (96T - CE-IVD) kit. All samples and controls were tested in duplicates and each assay was performed according to the instructions and guidelines of the manufacturer.

Statistical analysis

Mean and median values, dispersion, distribution and outliers were assessed using descriptive data, histograms and box plots. The final sample consisted of 60 patients. The relation between Aβ, CA, EI and total tau, CA, EI was tested using scatterplots and multiple regression analysis. The Durbin-Watson statistic was performed in each test and produced values of d1=2.057 and d2=1.881 respectively. Consequently, we can assume that there is no first order linear auto-correlation in our multiple linear regression data. Statistical significance was assumed at $p<0.05$. Analyses were performed using SPSS 24.0 (SPSS, Inc., Chicago, IL, USA).

Results

In the total sample, EI ranged between 0.2302 and 0.411 with the mean value of 0.31448 (SD=0.0412245), CA ranged between 66.5 and 130 with a mean value of 100.008 (SD=18.791), Aβ ranged between 225 and 1400 with a mean value of 631.44 (SD=253.553) and total tau values ranged from 21.7 to 950 with a mean value of 386.57 (SD=230.782). The variables statistically significantly predicted Aβ and total tau in both tests with F1=8.720, R1=0.484, p1=0.001 and F2=4.110, R2=0.355, p2=0.022. Out of all the variables, it was shown that CA, EI ($p<0.05$) and CA ($p<0.05$) added statistically significantly to the prediction of Aβ and total tau, respectively. Specifically, these results are interpreted as: for every 0.01 unit increase in EI a 18.74 unit decrease in Aβ values was predicted (p=0.016) and for 1 degree increase in CA values a 3.876 unit increase in Aβ values was predicted (p=0.023). As for total tau, for every degree increase in CA, a 4.621 unit increase in its values was predicted (p=0.006). Total tau didn't correlate individually with EI.

Discussion

In this study, a sample of 62 eligible patients has been assessed based on their CSF protein markers, CA and EI. Based on our findings, there seems to be a notable correlation between CA and total tau, CA and Aβ and finally between EI and Aβ, although no such correlation was found between EI and total tau.

As both CA and tau have been found to be linked with brain atrophy in the past [6, 21, 26, 31], our findings co-align with existing literature, verifying that CA correlates with CSF total tau.

Another finding of this study was the prediction of Aβ levels in the CSF by CA, meaning that increased values of the angle is connected with higher values of Aβ in the CSF.

A possible hypothesis for this result goes as follows. The decrease of CA is translated as a redirection of the lateral ventricles so that they shift closer to each other [24], due to increased volume of CSF in a non- atrophic brain parenchyma. As a result, the periventricular pressure increases and Aβ clearance decreases, due to reduced periventricular cell metabolism [14, 23]. On the contrast, an angle increase in patients with dementia is linked to a stage of atrophy progression, when the ventricles have decompressed and the periventricular pressure has reduced [26, 31]. As a result, Aβ clearance stays in pathological levels in any case, but relatively increased in higher angles as a result of periventricular decompression.

The correlation between Aβ and EI validates previous hypothesis that ventriculomegaly influences Aβ clearance 31, leading to brain accumulation [14, 23], whereas total tau does not seem to be affected by ventricular enlargement [32]. Instead, CSF total tau is mainly affected by atrophy progression [6] and seems to play a crucial role in differential diagnosis of dementia entities, especially AD and iNPH [26, 30, 31].

The correlation between EI and Aβ holds significance regarding the pathophysiology. Ventriculomegaly seems to play a dominant role in dementia and has been suggested in the past as a common pathway in various dementia entities [14, 23, 33]. It is possible that the increase of

ventricular pressure leads to stress of the periventricular cells, preventing Aβ clearance [14].

However, the results hold particular importance in the clinical practice, as well. Until now, tau protein has been widely used as a marker of brain atrophy. By linking CA with tau protein, we provide further evidence that CA may be in stark connection with brain atrophy.

The aforementioned results provide the clinician with the opportunity of a rough estimation of CSF levels of Aβ and total tau via the measurement of EI and CA in a simple MRI. In this way, we propose a basis for a simpler and quicker way for the clinician to proceed in the differential diagnosis between some common forms of dementia entities, without the need to subject the patient to an interventional procedure such as a lumbar puncture and the expensive measurement of CSF protein markers.

Despite the clinical importance of the aforementioned data, this study has certain limitations. A larger sample would increase the weight of the statistical analysis. In addition, while EI is accepted widely as a rough radiological marker of ventriculomegaly [8, 24], a number of studies have raised concerns on its credibility [34]. Moreover, while a correlation between CA and brain atrophy has been found, CA is not a widely established marker on this matter and more research is needed to provide robust evidence on a strong link between the two.

Future research directions could include statistical analysis of said variables in a larger sample of patients. This would allow thorough evaluation of the results in larger samples consisting of patients with specific diagnoses, in order to seek differences that reflect different pathophysiological pathways. Other prospects would include research on the link between CA and Aβ, which raises multiple questions. On one hand, this correlation seems to have an easy and logical explanation in the case of Aβ reduction in the CSF. On the other hand, it is regarded as a peculiarity in the case of its increase in an already atrophic brain. Thus, future research could investigate the aforementioned relation, proposing an interpretation of this phenomenon.

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The authors declare that they have no conflicts of interest.

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Sir Roy Calne. Water color painting. Liver transplantation (1990).

Original Article

Health, Memory Complaints and Cognitive Performance in Older Adults: Relationship Analysis"

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Abstract

Objective: The aim of the present study was the examination of the relationships among multimorbidity and subjective and objective cognitive performance, as well as the role of some demographic, social and health variables. We also investigated the protective role of social participation and physical activity against the impact of multimorbidity on cognition. Subjective cognitive performance was measured by memory complaints, while the objective one (a part of it) was assessed by verbal fluency. Our sample consisted of 67 participants (58-81 years old) from the Universidad de Experiencia, Zamora, Spain. For the analyses was used the IBM SPSS 20. To examine the relationships between our basic variables we applied partial correlations, while we applied univariate ANOVAs for the rest of our analyses. Neurotism was our control variable. Results revealed that those of the participants who were older and had more diseases presented a more negative self-perceived health and negative perceived health was found to be correlated with worse results in verbal fluency. Multimorbidity itself was correlated negatively with the results in verbal fluency as well. Furthermore, those participants who had a higher educational level, a more intense cultural life and lived just with their partner were found to provide better results. Subjective performance was not found to be related to the objective one and the protective role of the examined variables was found to be different for each dimension. **Conclusion:** It seems that multimorbidity is related to the verbal fluency directly, but it was also found to be related to self-rated health which was also related to the cognitive performance. However, it doesn't seem to be related to the subjective performance.

Introduction

Nowadays, one of the gerontology's main research interests is the understanding of the individual differences regarding the cognitive performance [1]. Another significant topic in the area of elderly is multimorbidity. Since it's widely known that the integral brain function depends on many organic systems which may be altered because of chronic diseases [2] and since by reading different

papers [3, 4], we have seen the existence of common factors (which will be described later) between multimorbidity and cognitive performance we got interested in examining those variables.

Various Psychological Aspects in Aging

Subjective and objective cognitive performance in aging: Memory complaints and verbal fluency

The memory complaints are personal statements about problems/changes in the memory and they are mentioned often by the elders [5] that makes their early detection important considering that a big percent of those who mention them develop MCI or dementia [5-7]. However, it's not always the case and there is an intense polemic about this correlation since according to other findings they are mentioned by younger and healthy population as well [8].

Seeing that memory is influenced not only by cognitive, but also by emotional and motivational paths [6], the modern studies about the origin of memory complaints combine many variables, like biological (apoe4), psychological (chronic stress), clinical (depression), health (multimorbidity) and personality traits (neurotism) [6,9-11].

On the other hand, verbal fluency is an objective indicator of the cognitive performance and it's part of any neuropsychological examination. It's defined as the capacity to recall items of a specific category in a limited period of time [12]. It shows the ability to enter in our mental dictionary, recall grammatical and hearing representations. It is influenced by the acquired knowledge and it is related to the working memory, to the flowing intelligence, the inhibition-variables anyway altered in the physiological aging, but mostly in the beginning of dementia [12,13].

COWAT-FAS is an instrument which measures the verbal fluency and also gives information about the executive functions and in specific, initiation and fluency. Executive functioning is hypothesized to involve the control and the coordination of cognitive operations. The typically uniform positive relations between measures of cognitive abilities and measures of executive functions as well as the similarities between the descriptions of executive functions, some specific cognitive abilities, and the general cognitive factor have led researchers to suggest a confluence of these constructs. In the COWAT-FAS participants are asked to say or write words (both alternatives are valid) which start from the letters F-A-S in a minute for each letter. Many cognitive strategies are required like the inhibition or the ability to change and maintain in mind the orders- factors differently altered in healthy aging, MCI and dementia [13,14].

The multimorbidity in aging and its influence on the cognitive performance

The multimorbidity is generally defined as the co-occurrence of at least two chronic diseases in the same person and it's different from the morbidity which has to do with diseases of the same index [15-18].

It's important to examine the multimorbidity firstly because of its great prevail in the older people (55-98%) [16] and secondly, because of its positive correlation with the cognitive decline. In a survey with 1.763 participants, others [6] found that a series of diseases had a negative impact on the cognitive performance. Besides, we know that diseases like diabetes, stroke and

atherosclerosis are negatively related to the cognitive performance. In a study of 2018, many diseases, and especially the multimorbidity, were positively correlated to greater chances for MCI especially in low income countries [19].

However, multimorbidity influences cognitive performance in an indirect way as well, since it has an impact on a series of variables which, in second place, are related to it. To be more specific, multimorbidity influences the person's function [20] since the majority of times it is related to moving restrictions making it difficult for the person to work out (one of the greatest protective factors against cognitive decline)[21]. It also influences negatively self-perceived health which is related to memory complaints [22] and to health behaviors. Finally, the multimorbidity's impact on the social participation is of great importance since isolation and loneliness are a usual consequence of multimorbidity [3,22]

Self -perceived health in aging

Self- perceived health is a subjective health evaluating factor which can predict the morbidity, the vulnerability and the mortality in aging [17,23]

The increase of diseases in a person is related to negative evaluations about health [24], which have been found to be positively related to memory complaints, especially in case of multimorbidity [22]. Self-perceived health influences someone's health behaviors [23], one of which and of great importance is physical exercise which is a protective factor against cognitive decline and dementia [1,25]. Others [25] mention that the lack of exercise is the cause of seven possibly reversible risk factors for Alzheimer in Europe and America.

The social participation and support

The social participation with its different dimensions (political, professional, educational, social, family, area of information) is a strong characteristic of active and healthy aging [23].

According to others [4], greater social participation in social, physical and educational activities is related to higher cognitive performance and reduced risk for dementia. Likewise, in line with [26,28] and [27], the risk for dementia and cognitive decline is slowed down through the participation in social, educational and leisure activities, something which is explained by the hypothesis of Cognitive Reserve according to which, the environments rich in stimulations reinforce the development of cognitive strategies, the neurogenesis and the synaptic density. Stern [27,28] explains that like this we can understand the brain's capacity to adjust to changes and that cognitive reserve is what stands between the changes in the brain and the appearance of the first symptoms.

However, the social participation and support influence and are influenced by the multimorbidity. That is, it is more probable for the isolated people to suffer from more diseases [29]. According to others [16], big social networks offer a protection against multimorbidity. On the other hand, the number of diseases that a person has may influence the size of its social network and his/her social participation.

Personality traits: Neurotism

It's interesting the fact that in many studies we see that personality traits and especially, neurotism predict the existence of memory complaints and in a bigger grade than objective

cognitive performance [6,10], supporting what it was said before, that is, memory complaints are not always related to pathology.

Neurotism shows one's general predisposition in psychological distress. It's well known that stress, and mostly chronic stress, influences negatively the cognitive performance through the axe of hippocampus [31]. It is also shown that neurotism is directly related to the level of the atrophic nerve cells in brain [30,31]. However, it can also influence the cognitive performance indirectly through its relation to the coping strategies, health behaviors and depressive symptoms [31].

Objectives and Hypotheses

Our main objective is **the** examination of the relationship between multimorbidity, memory complaints and verbal fluency. Because of the observed relationship between our main variables and some demographic, social and health variables, we decided to examine their role as well:

H1- We expect a positive relationship between multimorbidity and memory complaints [6] and a negative relationship between multimorbidity and cognitive performance (verbal fluency) [2]. Because of the controversial predictions, we examine the possible relationship between the memory complaints and the cognitive performance [8].

H2- We expect a negative relationship between multimorbidity and self-perceived health [23].

H3- Older participants [15], with lower educational level [28], who don't have a partner and those who live with less people will have more memory complaints and worse cognitive performance than the rest [4].

H4- Participants with more negative self-perceived health [22], lower level of physical exercise [25], less social contacts [26] and lower level of social participation [4] will have more memory complaints and worse cognitive performance than the rest.

The protective role of the social participation and physical exercise on the impact of multimorbidity on the memory complaints and cognitive performance was also examined.

H5-The elders' social participation of any kind will reduce the impact of multimorbidity on the memory complaints and on the cognitive performance [4, 29].

H6- The physical exercise of any kind will reduce the impact of multimorbidity on the memory complaints and on the cognitive performance [25].

Methods

Participants

The convenience sample was consisted of 67 older adults who were students of the Universidad de Experiencia, Zamora, Spain. It's a university which gives the chance to older people to study in a really low cost in an effort of continuing education in aging.

The 73.1% were women and the 26.9% were men with a mean of 68.8 years old (S.D = 5.44). The 52.5% were married while the 34.3% widowed and the mean number of children were 2.08 (S.D = 1.46). The 37.3% was living with their partner and without their children, while the 34.3% was living alone.

Regarding their educational level, the 34.3% had completed the obligatory education and we could say that generally our sample participated in a high rate in social activities of any kind with exception the participation in associations where the 45.3% has never participated.

Instruments

We created a questionnaire using different instruments in the following line: after the demographic data, there were three questions (number of kids, way of living together, number of people they consider to have a close relationship with(brothers, family in law, grandchildren, nephews/nieces, friends, neighbors) about social network.

The social participation was measured by a total of items which were based on the indicators of social participation (EDE, 2013) and which were used in the ELES study in Spain (Estudio Longitudinal Envejecer en España).

The self-perceived health was assessed through the frequently used question: "Generally, how would you evaluate your health?" which was also used in the ELES study with five possible answers on a Likert scale.

The multimorbidity was measured using a list of 19 mental and physical diseases which was used in the ELES and SHARE (Survey of Health and Aging and Retirement) in 16 European countries in which participants had to choose between YES/NO for each disease [32].

The physical exercise was measured by using four questions about the intense physical activity, the daily walks, the general everyday movement and the number of hours that someone passes without moving from the Yale Physical Activity Scale [33]. We chose those items (from the 35) because they have been found to have the greatest predictive value for the physical and general well-being [34] and secondly, because our objective was to create a questionnaire which would measure many variables.

The memory complaints were measured using the MAC-Q [35] which we translated in Spanish since we saw it is the most frequently used and we couldn't find another valid and short instrument in spanish, (alpha = .85). It is consisted of 6 questions which have five possible answers on a Likert scale and the total score can vary between 7-35. The 25 is considered the cut point above which the participants are considered to have memory complaints. The participants have to answer evaluating their memory compared to their memory in their youth.

For the neurotism were used the 12 questions from the NEO-FFI (alpha = .90).

The objective cognitive performance was assessed using the Controlled Oral Word Association Test which measures the verbal fluency and it offers some information about the executive function as well. The participants had to write words starting from F-A-S in one minute for each letter.

Procedure

The administration of the questionnaires began on May of 2018 at the Universidad de Zamora and last for three weeks. As to facilitate the procedure, we created pairs of questionnaires (COWAT-FAS and the rest) and COWAT was administrated with the presence of the investigator while the other half could be fulfilled by the participant in any time and be returned in the envelope which was given.

Statistical analyses

For the analyses we used the IBM SPSS 20. For the examination of the relationships of our main variables we carried our partial correlation putting neurotism as the control variable. For the rest of the analyses we did univariate ANOVAs controlling again the level of neurotism in every analysis.

Many authors like [6] and [30] have used neurotism as a control variable when they examined the cognitive performance since its impact on it seems important.

We created the followings age groups: 64, 65-69, 70-74, 75+ and the following groups of ways of living together: married, widowed, single/divorced.

Regarding the social network with the total number of people with whom the participants maintained a close relationship we adopted the model of Gierveld & Fokkema (2015) based on European studies: 0-3people (really small network), 4-8(small), 9-14 (normal), 15-28 (big), 29+ (really big).

Because of the similar content, we created a new variable in the category of social participation by grouping the variables: educational and cultural activities (edu/cult activities).

Finally, we grouped the answers “really bad” and “bad” regarding the levels of self-perceived health because of the lack of sufficient responses and we eliminated the answer “not applicable” of the YPAS from all analyses.

Results

MAC-Q’s average score was 22.68 (S.D = 4.14) indicating that the participants didn’t present a higher level of memory complaints (as a mean score) than the population of their age group (25 points). Regarding to the cognitive performance, the average number of words in FAS was 25.40 (S.D = 9.48) with some participants (12) reaching really high scores (35-43 words).

Descriptive data of the subjective cognitive performance (MAC-Q) and verbal fluency (COWAT – FAS)

Variables				
	M	Mín.	Max.	S.D
MAC-Q	22.68	1	29	4.14
FAS	25.40	7	56	9.48

Relations between cognitive functions, self-perceived health and multimorbidity

The **H1** was partially supported since the results showed the anticipated relationship between multimorbidity and cognitive performance (pr = - .26, p = .03), but not with the memory complaints (pr = - .14, p = n.s.).

Complementary univariate ANOVAs revealed that those who had suffered from stroke had a lower score on MAC-Q compared with those who hadn’t, F (1,61) = 11.10, p = .001, η2 = 15%, while people with diabetes had higher scores than those without diabetes, F (1,61) = 4.77, p

= .03, η2 = 7%. Moreover, participants with problems in the urinary system presented lower performance in COWAT than those who didn't have problems, F (1,61) = 8.71, p = .004, η2 = 12%.

There was not any statistically important relationship between the memory complaints and the verbal fluency, while our **H2** was supported since we found a statistically important negative relationship between multimorbidity and self-perceived health, (pr = - .59, p < .001).

Differences on the levels of memory complaints and verbal fluency based on demographic variables

The **H3** was partially supported since there were not found important relationships between demographics and memory complaints.

However, as presented in the **Figure 1**, the expected differences regarding age on COWAT were found (F (3,53) = 7.09, p = < .001, η2 = 28.6%). According to post-hoc, participants over 75 had worse score (M = 16.43) compared to the younger ones.

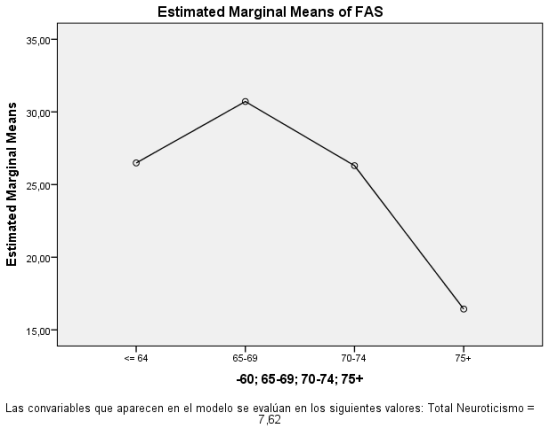

Figure 1. *Scores on COWAT-FAS based on age groups*

According to **Figure 2,** there were important differences regarding the education level and the performance on COWAT (F (4,59) = 5.72, p = .001, η2 = 28 %), where post-hoc revealed that those who had completed the obligatory education had lower score (M = 14.12) than those who had graduated from university (M = 31.30).

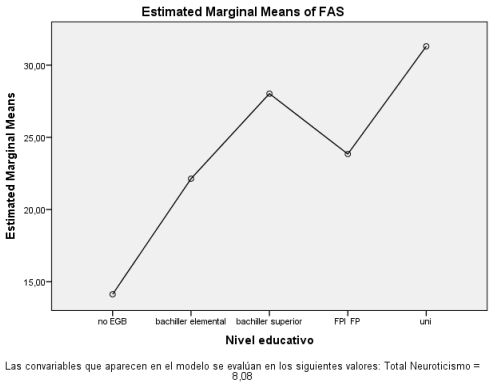

Figure 2. *Scores on COWAT-FAS based on the educational level*

Although not the expected ones, differences between the different ways of living together and the score on COWAT were found (F (3,60) = 3.07, p = .03, η2 = 13.3 %) according to which, those who lived with their partner but without their children (and so, with less people) had higher scores (M = 29.45) than those who lived with their partner and their children (M = 19.83).

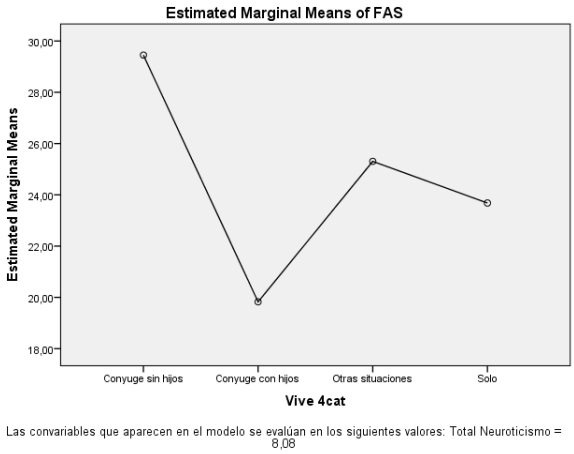

Figure 3. *Scores on COWAT-FAS based on the way of living together*

Differences on memory complaints and verbal fluency based on health variables: multimorbidity, self-perceived health, physical exercise

The **H4** was partially supported since we didn't find the expected differences regarding the memory complaints. Also, there were not significant results about the physical exercise.

Respecting COWAT, the results revealed the expected differences based on the different levels of self-perceived health (F (3,58) = 3.79, p = .01, η2 = 16.4 %). According to post-hoc, those who evaluated their health as really bad/bad presented lower score (M = 7.88) compared to those who evaluated it as good (M = 26.71) or as really good (M = 30.25).

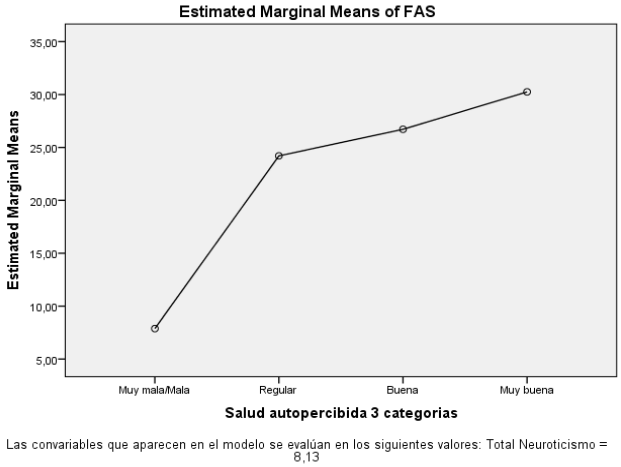

Figure 4. *Scores on COWAT-FAS based on self-perceived health*

Differences on memory complaints and verbal fluency based on social variables: social network and social participation

In contrast to **H4**, didn't come up differences between the grade of memory complaints and the size of the social network or the score on COWAT.

We didn't find the expected results regarding the level of social participation and the score on MAC-Q neither. However, our hypothesis is supported in relation to the edu/cult activities and the score on COWAT (F (2,61) = 3.94, p = .02, η2 = 11.4 %). According to the post-hoc, it is observed that those who didn't participate in this kind of activities had lower score on COWAT (M = 20.33) than those who participated with high frequency[1] ($M = 28.59$).

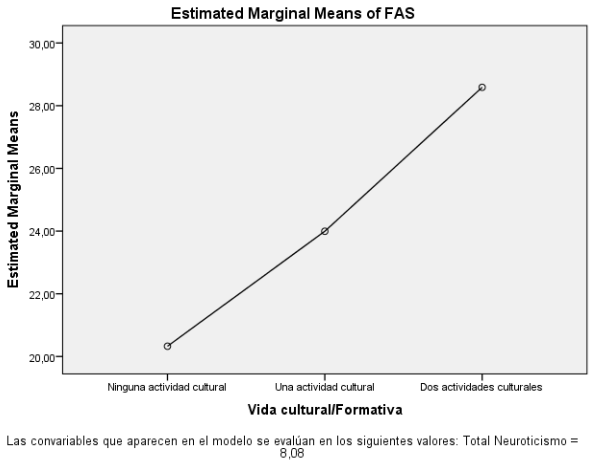

Figure 5. *Scores on COWAT- FAS based on the level of participation in edu/cult activities*

Social Participation

An important interaction was found (F (3,64) = 4.37, p = .008, η2 = 19 %) indicating that the difference in the mean scores on MAC-Q was not the same for all levels of multimorbidity. In contrast to our **H5**, in Figure 6 we observe that from those who didn't have any disease, those who participated in edu/cult activities had more memory complaints (M = 23.57) than those who didn't participate (M = 18.05). The same result came out for those who were suffering from one disease: those who had an intense participation in these activities presented more complaints (M = 23.33) than those who didn't have (M = 15.27). This result will be discussed further later. Although there were no other important results about the other levels of multimorbidity, in the figure can be seen that the participation in those activities reduced the number of the complaints in people who had 3+ diseases.

However, as it's seen in Figure 7, there were not important results regarding the verbal fluency (F (3,64) = 1.69, p = n.s., η2 = 0.8 %). This indicates that there were not significant differences in the scores of COWAT between the participants with multimorbidity who participated in edu/cult activities and those who didn't.

[1] Frequency categories: zero participation-one cultural activity – two cultural activities

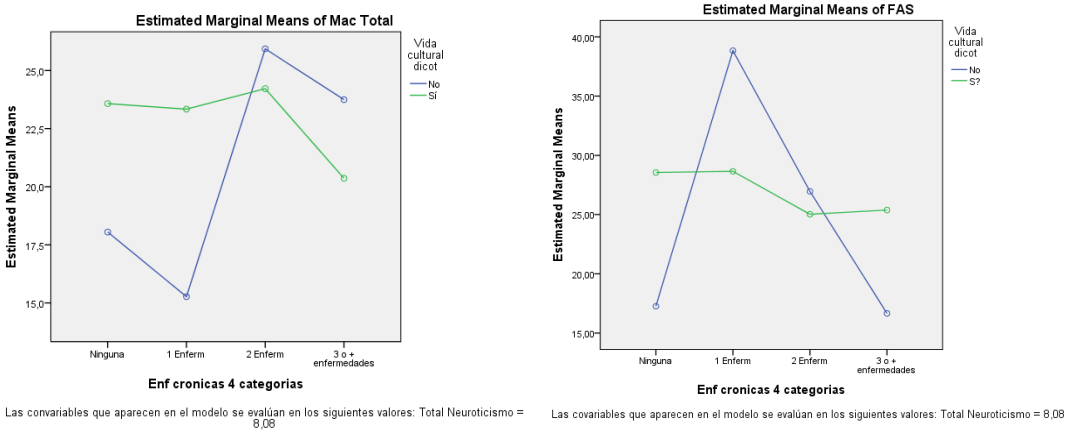

Figure 6 and 7*. Mean scores on MAC-Q and COWAT-FAS based on the interaction between multimorbidity and edu/cult activities.*

Moreover, the analysis revealed important interaction between the tourism and the memory complaints (F (3,53) = 7.98, p = <.001, η2 = 34.7 %) which indicates the existence of differences in the mean scores in the different levels of multimorbidity. There was an unexpected results according to which from those participants who had one chronic disease, those who participated in touristic activities presented more complaints (M = 23.53) than those who didn't (M = 17.70). In contrast to this and in accordance to what we expected, from those with 3+ diseases, those who participated in those activities had less complaints (M = 16.54) than those who didn't (M = 24.78).

However, there was not any important interaction regarding the verbal fluency showing that the those activities were not protective against the impact of multimorbidity on verbal fluency (F (3,53) = 1.57, p = n.s., η2 = 9.5 %).

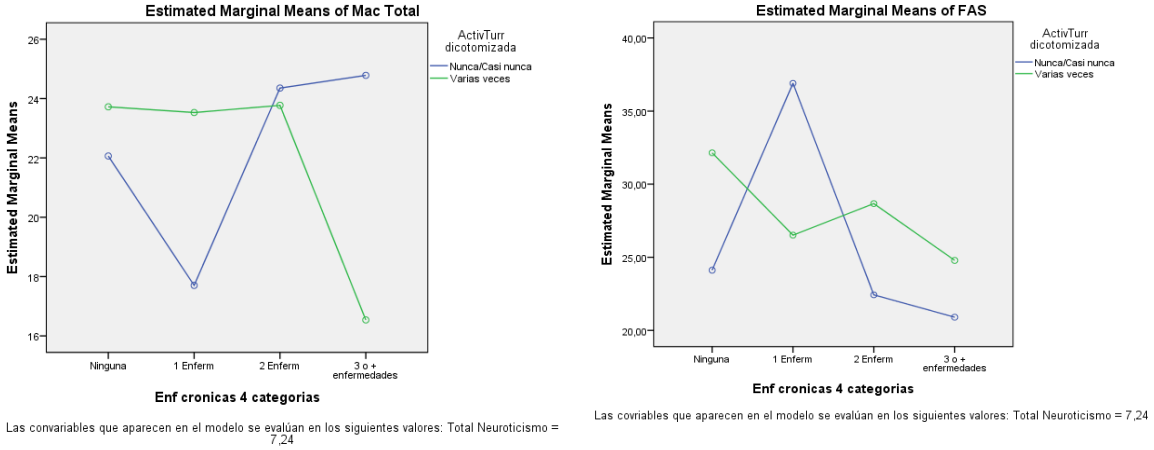

Figure 8 and 9. *Mean scores on MAC-Q and COWAT-FAS based on the interaction between multimorbidity and participation in tourism.*

With respect to the outdoor activities, there was not any significant interaction between the frequency of participation and the reduced number of memory complaints to the people with multimorbidity rejecting our hypothesis (F (3,58) = 1.94, p = n.s., η2 = 10.4 %). However, there were important differences regarding the verbal fluency (F (3,58) = 2.94, p = .04, η2 = 15 %). We see that from those who didn't have any disease, those who participated in outdoor activities had

a higher score on COWAT (M = 31.22) compared to those who didn't (M = 17.06). As we expected, from the elders who had 3+ diseases, those who participated in those activities presented better scores (M = 25.64) than those who didn't (M = 10.43).

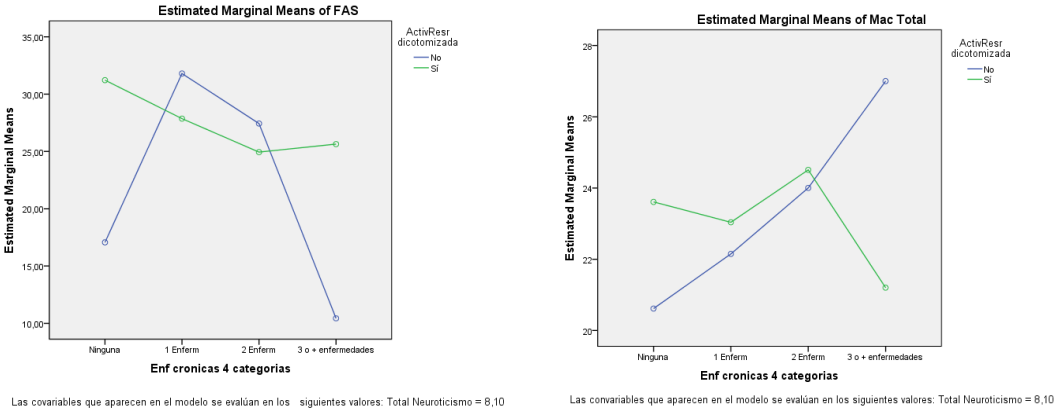

Figure 10 and 11. *Mean scores on MAC-Q and FAS based on the interaction between multimorbidity and participation in outdoor activities*

Physical exercise

Our last hypothesis (H6) which predicted that physical exercise would reduce the impact of multimorbidity on the memory complaints and verbal fluency was rejected for both variables (MAC-Q: intense activity: F (3,54) = 1.73, p = n.s., η2 = 8.8 %, daily walks: F (3,65) = .70, p = n.s., η2 = 3.6 %, daily movement: F (3,64) = .65, p = n.s., η2 = 3.4%. COWAT-FAS: intense activity: F (3,63) = .43, p = n.s., η2 = 2.3 %, daily walks: F (3,65) = 1.90, p = n.s., η2 = 9.2 %, daily movement: F (3,64) = .48, p = n.s., η2 = 2.5%).

Discussion

Having as a basis the health and the cognitive performance/executive function in aging, we examined the relationship between the multimorbidity, the memory complaints and the verbal fluency, testing at the same time the role of some demographic, social and health variables. We also examined the protective role of the social participation and physical exercise against the memory complaints and the cognitive performance in elders with multimorbidity. We used neurotism as a control variable.

According to other studies as well [16], the majority of our sample (56.1%) had multimorbidity reassuring its great prevalence in aging and the need to act. In contrast to our expectations [5], the participants didn't reach the 25 points in MAC-Q, something that indicates that they didn't express more complaints than the average population of their age group. However, we have to keep in mind that our sample was "special" since they kept on studying till this "fragile" phase regarding their cognitive performance.

The number of the chronic diseases was negatively related to the performance on COWAT, something that was already known from other studies [2,8]. What was unexpected was

the fact that it was not related to the grade of the memory complaints. We could say that the participants didn't present memory complaints officially anyway. But, also, like it's known that neurotism can affect the existence of the complaints, there are probably more variables which we haven't tested. For example, [6] found the relationship we expected but in people who perceived themselves as people who forget easily. It's also important to mention that neither all the diseases had an impact nor the same impact on the cognitive performance. To be more specific and as it's presented in other studies as well [6], diabetes favors the memory complaints while the stroke doesn't. This result could enhance the idea of using "clusters" of chronic diseases [36] which function in a homogeneous way.

It's considered important the fact that the memory complaints were not related in any way with the cognitive performance. We have already mentioned the existing polemic about it [11] and like it was mentioned by others [8] it was not the people who were complaining more, those who had the worst real performance. It's about two different variables which measure different dimensions.

Moreover and like it was mentioned by other authors [23,37], those who had more diseases presented more negative self-perceived health. We have to pay attention to this result since self - perceived health influences the general health behaviors [23,24].We also observed what others [23] have mentioned, that is, the elders tend to evaluate positive their health even if they have many diseases.

The fact that we didn't find the expected relation between age and memory complaints is a result found in the work of others [8]. Also, it's possible that the size of our sample didn't permit the existence of a statistically strong relationship. On the other hand, it was expected that the age would influence the performance in COWAT since it's a pretty complicating test which requires strategies that we don't practice daily. What is more, the process speed and the working memory which are some characteristics of this test are altered anyway in physiological aging [13].

Combined to this previous result, we can examine the fact that those who have studied more had a better performance in verbal fluency. According to the hypothesis of the Cognitive Reserve those with a higher educational level maintain more strategies and a richer thinking process compared to those with lower educational level [27,28]. In contrast, we found no important results related to the memory complaints.

Although loneliness and lack of social participation can be risk factors for the cognitive performance [3], we saw that here the way of living together was the dimension who gave us important results, although in an unexpected way. That is, those who lived with their partner but without their children (and so, with less people) presented better performance in verbal fluency than those who lived with their children as well. This could probably be explained by the fact that on the one hand, those participants had an emotional support compared to the widowed and those who lived alone and on the other hand, living with less people may offer less daily stress for taking care of them; it's is well known that chronic stress has a negative impact on cognition [31].

Despite we didn't find significant results regarding self-perceived health and memory complaints, we saw that those with more negative health evaluations presented lower scores on verbal fluency. Self-perceived health is an objective factor which may influence the person's general vulnerability [5] and it's known that cognitive performance is the result of a mix of variables which influence it directly or indirectly [6]. Although we didn't carry out this kind of

analysis, we can suppose the mutual relationships between multimorbidity, self-perceived health and cognition based on the results that those with more diseases, evaluated worse their health and those who evaluated it worse presented worse scores on verbal fluency.

Our hypotheses regarding physical exercise were rejected. However, it's probable that our sample's main "power" till the present time is their education which has favored their mental reserve in a way that overpasses the possible influences of physical exercise.

Combined to this, we see that the intense participation in edu/cult activities was correlated with higher scores in verbal fluency reassuring for once more the hypothesis of the Cognitive Reserve [28]. Notwithstanding, there were no important results regarding the other dimensions of social participation. Others [4] mention that the lack of homogeneous instruments to measure participation and cognitive performance is an important problem. Also, we have to mention that the question about the social network (number of people we maintain a close relationship with) confused most of the participants and probably it's not the best question to be answered individually by older people.

With respect to the protective role of some variables, it was observed that tourism was protective against the impact of multimorbidity on the memory complaints, while the outdoor activities against its impact on verbal fluency.

We have to comment the weird result regarding the edu/cult activities and tourism which not only didn't function in a protective way against memory complains for those with no multimorbidity, but they also worked in the opposite way. About the intense participation in edu/cult activities, we could say that since our sample keeps on being informed about aging, dementia etc. at the university, they are more sensible to the changes they notice in their daily function. About tourism which had worked in a protective way for those with multimorbidity but it didn't for those who had just one disease, we can hypothesize that the intense participation in those activities give more "opportunities" to notice daily cognitive faults and create worries especially if there no more diseases.

Conclusion

To summarize, the majority of the participants had multimorbidity which was correlated to the cognitive performance/executive functioning since it was observed that those who were older and had more diseases had a worse performance. Multimorbidity was correlated with negative self-perceived health and this was correlated to worse performance in verbal fluency. On the other hand, those who lived with their partner and without their children had a higher education level and more intense participation in edu/cult activities presented a better performance.

Memory complaints were neither related to the objective performance nor to the rest variables we tested, indicating that those two are different cognitive dimensions which measure and correlate with different variables.

Outdoor activities were presented as a protective factor against multimorbidity's impact on verbal fluency and tourism against its impact on memory complaints.

To conclude, we couldn't avoid mentioning some limitations of our study like the small and homogeneous sample which didn't allow us to do the analyses we first intended to do. Also,

the lack of a commonly accepted instrument to measure multimorbidity and of a short and valid test for memory complaints in spanish was some limitations of the present study. Also, we have to mention again that we tested just one part of the cognitive performance, that is, the verbal fluency which we considered as a good indicator for our sample in the present case. The results could be different if other dimensions were measured.

We consider useful to examine multimorbidity and its relation with cognitive performance using the proposed "clusters" of diseases measuring more cognitive abilities and executive functions than just verbal fluency. Adherence to treatment is also a factor closely connected to multimorbidity and it could be examined in relation with psychological variables. We suggest the qualitative examination of the perception of the disease, of the loneliness in aging and of the ways the elders use to copy with it.

The authors declare that they have no conflicts of interest.

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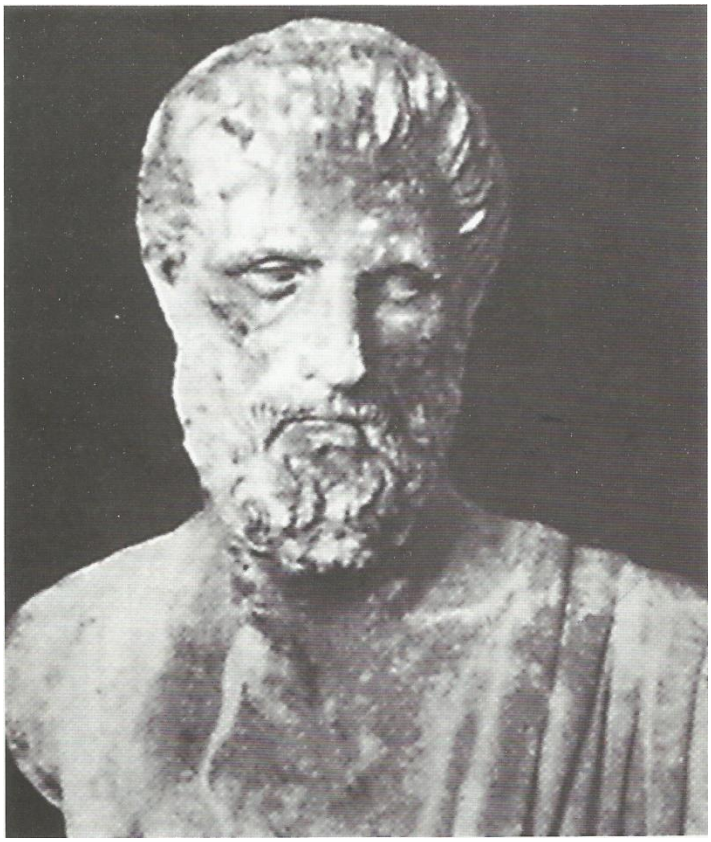

Hippocrates' bust in the Archaeological Museum of the island of Kos where we was born.

Original Article

Cognitive decline in Multiple Sclerosis patients

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Abstract

Cognitive impairment is a common phenomenon in multiple sclerosis (MS), occurring at all stages of the disease, even at the earliest, and can be a major source of disability, social impairment, and impoverished quality of life. Cognitive dysfunction is mainly focused on working memory, conceptual reasoning, verbal fluency, speed of information processing, attention and executive function. Additional clinical factors, including disease course, fatigue and affective disturbance, can impact the degree of MS-related cognitive impairment. We present the results from the two-phases of our prospective study on cognitive decline in MS patients using the data collected from the A' Neurologic clinic at AHEPA hospital, Thessaloniki, Greece. Most of the patients of the present study revealed mild cognitive impairment with mild influence on the everyday function. We found weak correlation between cognitive deficit and the duration of MS, as well as the physical disability status and moderate correlation between cognitive impairment and the type of the disease as well as MRI findings (atrophy and lesion load). Our results also indicate that the currently available battery of neuropsychological tests: California Verbal Learning test (CVLT), Symbol Digit Modalities Test (SDMT), Brief Visuospatial Memory Test and Paced Auditory Serial Addition Test (PASAT) can be used as a reliable tool in the diagnosis of cognitive deficits of MS patients, as related to their degree of disability and to the type of their disease. Evaluation of cognitive functions should be incorporated in the regular assessment and monitoring of MS patients since they seem to be well correlated with the progression of the disease.

Introduction

Multiple Sclerosis (MS) is the most common demyelinating disease of the Central Nervous System (CNS) usually affecting people in their 3rd or 4th decade of life. It is more frequent in women and it can be presented with a variety of CNS-related symptoms given the fact it can

potentially affect almost every neuroanatomical region within the CNS. Cognitive decline secondary to MS has been described in early cases of the disease diagnosed at the end of 19th century. However, many neurologists' considered cognitive decline as extremely rare, given the fact that the cerebral cortex, which has been associated with cognitive processes, did not seem to be affected [1].

The prevalence of cognitive decline has been reported [2, 3] to lie between 13% and 72%, This discrepancy between the studies can be attributed to the utilization of different neuropsychological tests, statistical analyses, individual characteristics and the presence or not of healthy control groups. The prevalence of cognitive impairment of the entire MS patient population is difficult to be estimated, yet, according to exclusively neuropsychological studies , the prevalence of cognitive impairment is ranging between 45% to 59% and it is affecting a larger number of MS patients' in comparison to healthy control groups [3,4].

According to the literature, 20% to 42 % of the MS patients demonstrate a significant deficit in recognizing and recalling verbal and visual stimuli [3]. There is also significant decline in retrieving information from long-term memory either at the onset of the disease or its later stages, whereas short-term memory remains intact [5,6]. Many patients also demonstrate deficits in complex attention and slower efficiency in information processing. Many researchers believe the latter is due to poor working memory [7], which has been linked to a general dysfunction of cognitive processes, while others due to purely motor deficits [8]. Studies have shown a declined ability of problem solving, planning, and prioritization tasks [9, 10]. Such problems affect 13% to 19% of MS patients and they are most likely related to damage to the prefrontal circuits [11,12]. Visual-spatial and speech impairment have been studied to a lesser extent, due to sensory motor and visual deficits which again are highly dependent on the disease related decline and non-cognitive processes [2]. Other studies concluded that severe visual agnosia and aphasia can be linked to MS [4,13], not an unexpected finding since the patients show impaired language performance and, thus, they are more prone to naming and reading tests mistakes than people of the healthy control group [8,14].

Most studies do not demonstrate any significant correlation between cognitive impairment and physical incapability, and the progression and duration of MS [15]. Some studies claim these impairments to be more common in the progressive form of MS rather than its relapsing form [16]. Comi et al. suggest that cognitive impairment may become mostly evident as the disease transits from relapsing/remitting MS to secondary/ progressive MS [17]. MS has been linked to mild to moderate cognitive function decline, while dementia and cerebral cortex function declines (such as eupraxias, speech, memory and gnosias) are rare [18 – 20]. However, cognitive decline can also be identified in patients who suffer from minimal motor dysfunction, or are at the early stages of the disease as the clinically isolated syndrome (CIS). The aforementioned evidence offer an explanation as to why we still have not located the biological etiology of the cognitive decline, and, thus we are still unable to predict the patients who are more likely to manifest such decline and at what stage of the disease [17, 21 – 23].

Our Prospective study on cognitive decline in MS patients conducted at the A' Neurologic clinic, AHEPA Hospital and consisted of two phases. It has been funded by the Greek National Grant Institution, PhD Committee and by the Aristotle Research Committee (ELKE).

Early prospective study: Data, Results & Conclusions

First Prospective Study

The first phase of our prospective study involved 40 patients with definite diagnosis of MS and 20 healthy patients as a control group (Poser et al. diagnostic criteria) [24], during a period of 30 months. From the beginning of the study, the patients were chosen based on their clinically stable picture for at least a year. As a result, patients who required high doses of corticosteroids were excluded from the study. During the study, the MS patients and the control group patients were given neuropsychological tests and clinical examination on a biannual basis. Patients' demographics included: gender, age, marital status, education level, occupation, disease duration since diagnosis, MS type, CNS involvement based on MRI imaging, EDSS scale and treatment. The neuropsychological assessment included patient and family interviews, mini mental state examination (MMSE), short cognitive performance test for assessing cognitive impairment of memory and attention (SKT), depression assessment test (Hamilton and BDI) and depression detection test (GHQ-25).

Patients who demonstrated severe emotional disturbances, either from the beginning or during the study, were also excluded. The MS patients and the control group patients were assessed with neuropsychological tests with the examiners utilizing the same tests and questionnaires each time. All the people involved in the study were informed and agreed to participate anonymously.

Our first study of cognitive decline was focused on the memory and attention aspect. MMSE was proven to be non-specific, given the fact that 91 % of the patients scored above 25 out of 30. The combination of individual interview and SKT were found to be much more useful. More than 57% of the patients showed a difficulty in recalling objects related to their daily activities and social life such as a cup, an umbrella, a chair, a dog etc. Thirty five percent showed a deficit of immediate memory (in recalling the same objects), and only 18 % of patients showed a deficit in recognition of the objects. Twenty percent of the patients demonstrated a difficulty in sorting numbers in descending fashion, a result which was merely attributed to the lack of attention rather than judgment impairment.

The patients' and family interviews suggested easy cognitive fatigue occurring in the 57 % of the patients involved, which was somehow similar to the physical fatigue, especially when multiple data had to be processed simultaneously. This impairment was mainly evident on the patients of higher education (university and above) and patients with increased professional duties, making it difficult for them to be efficient at their work and, thus leading to anxiety and low self esteem. This ''cognitive fatigue'' was related to poor attention span and concentration. Following their MS diagnosis, university students mentioned that they could not achieve high marks easily due to lack of concentration, and their need of frequent breaks in order to achieve their study goals. Regarding prioritization, 43% of the patients demonstrated a decrease in such skills, which subsequently resulted in loss of control, increased stress and panic attacks. The vast majority of the patients admitted that they feel safer when they follow the same order of tasks involved in their daily routine and that they were frightened even with minor sudden changes.

Despite the impairments these difficulties cause in the patients' daily life, they could be

characterized altogether as mild given the fact that they do not lead to reduce overall functioning. Dementia (subcortical/cortical type) was only diagnosed in 2 patients, for whom the disease duration was over 10 years, they had a secondary progressive hemispheric disease with diffuse demyelination in the centrum semiovale areas, cerebellar ventricular dilatiation and high EDSS score showing functional decline [2].

The type of the disease, the duration, and the affected CNS region were mostly related with cognitive impairment. Cognitive impairment can become evident during the first 5 years of the disease and it can be exacerbated with time regardless of either motor dysfunction is present or not. This seems to suggests that the cognitive impairment is solely dependent on the integrity status of the myelin sheaths and axons. The people affected by progressive MS, especially the primary progressive MS, have higher chances of cognitive decline, which is, again, associated with severe demyelination process and neuron axonal damage. People suffering from the hemispheric type of the disease with multiple periventricular lesions show signs of cognitive decline from the first 5 years of the disease [8,25].

Second Prospective Study: Data, Results & Conclusions

During the second phase of our prospective study, Mc Donald's diagnostic criteria 200426 were used. The different patient groups consisted of 6 CIS patients, 22 remitting/relapsing (RRMS) patients, 4 secondary progressive (SPMS) patients and 5 primary progressive (PPMS) patients. The following tools were used: California Verbal Learning Test (CVLT), Symbol Digit Modalities Test (SDMT), Brief Visuospatial Memory Test and Paced Auditory Serial Addition Test (PASAT) [4, 16, 27, 28].

According to our data, there seems to be a correlation between cognitive performance in the CVLT and MS type; poorer performances were linked to progressive disease. (**Figure 1**: MS type patients' performance in California Verbal Learning Test)

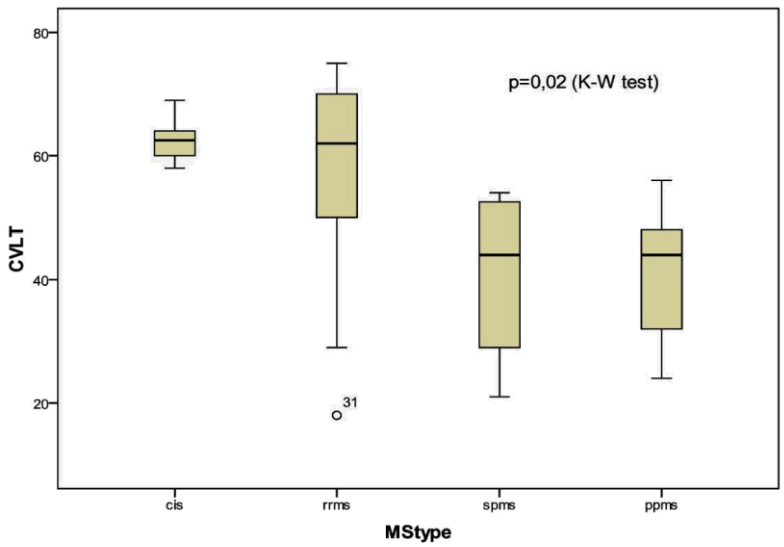

Figure 1: MS type patients' performance in CVLT

CVLT performance also demonstrated to be related with patients' age and EDSS (**Figure 2**: Correlation between CVLT performance and EDSS)

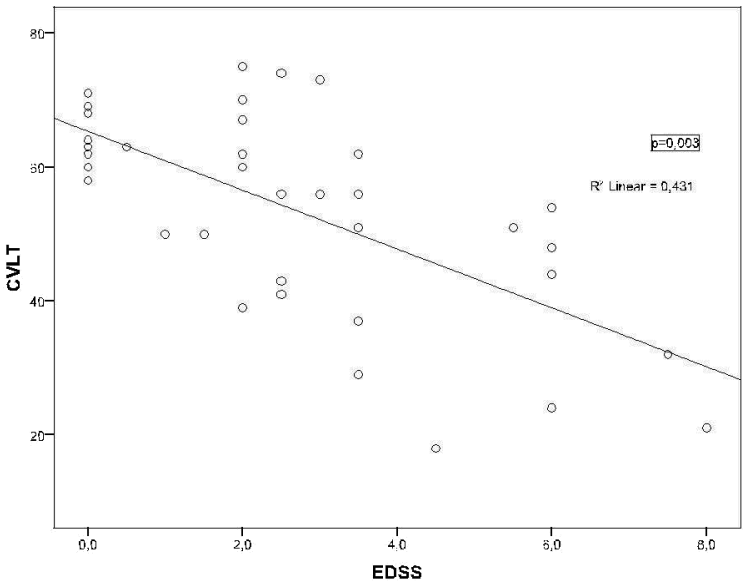

Figure 2: Correlation between CVLT performance and EDSS

Correlation between EDSS and test performance was also demonstrated in Symbol Digit Modalities Test (SMDT) and Paced Auditory Serial Addition Test (PASAT) (**Figure 3**: Correlation between MS patients' performance in SMDT and EDSS (showed in groups) and **Figure 4**: Correlation between MS patients' performance in PASAT and EDSS (showed in groups)

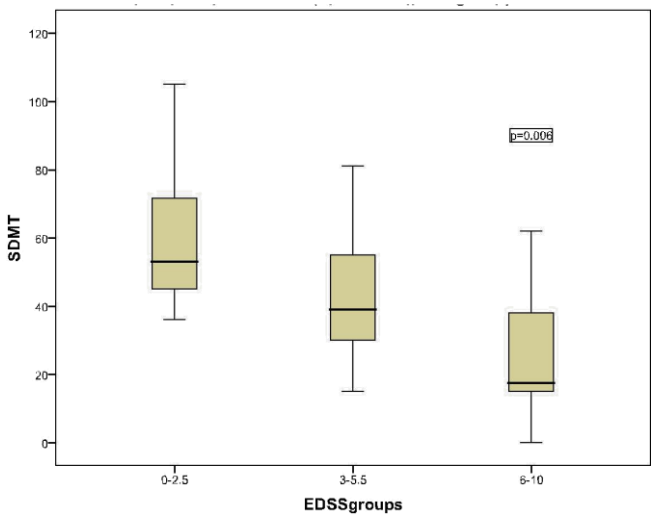

Figure 3: Correlation between MS patients' performance in SMDT and EDSS (showed in groups)

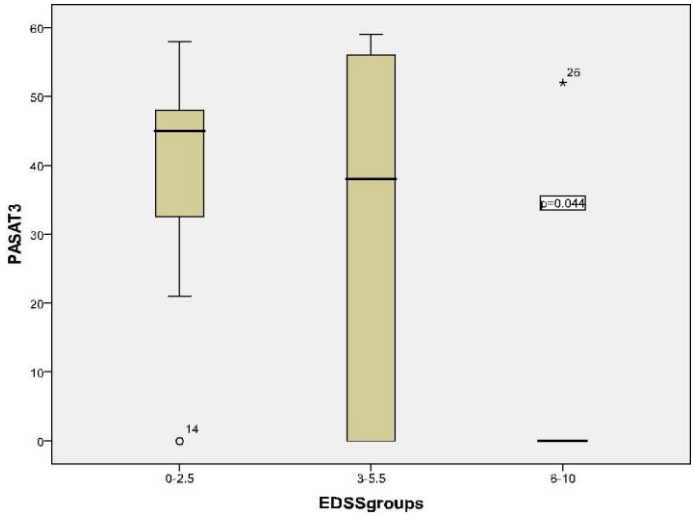

Figure 4: Correlation between MS patients' performance in PASAT and EDSS (showed in groups)

There was no statistical significant link between gender and disease onset.

Despite the fact the sample of patients was somewhat small, the diagnostic and assessment tools used have been known to be sensitive in diagnosing cognitive deficits in MS patients and, thus, we are confident of our results relating with the mobility and the disease type [29].

In conclusion, cognitive function assessment must become an essential part of the routine clinical examination and follow up for MS patients, because and it has been shown to be an important variable of disease prognosis. This can lead to the improvement of the patients' quality of life and functioning. Specific reliable tools must be used for diagnosing cognitive impairment in MS, and clinical confirmation, of different disease parameters must be secured, by employing neuroimaging findings and biological markers.

The authors declare that they have no conflicts of interest.

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Review

Future strategies of management of Alzheimer's Disease. The role of homotaurine

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Keywords: Homotaurine – tramiprosate - 3-aminopropanesulfonate (3APS) - ALZ-801 - pre-clinical and clinical studies - Alzheimer's Disease - Mild Cognitive Impairment.

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Abstract

Dementia and Alzheimer's Disease (AD) particularly will become in future one of the major problems that healthcare systems will have to face with in developed but also in developing countries, because of the progressive aging of the population and the age-associated increase in their incidence. There is a rapid increasing in life expectancy and in elderly percentage. Unfortunately, improvements in lifespan have not been matched by improvements in mental health span. In recent years, there has been a growing interest, supported by a large number of experimental, epidemiological and clinical studies, about the beneficial effects of some natural products in preventing various age-related pathologic conditions, including brain aging and neurodegeneration.

Homotaurine, a small aminosulfonate substance that is present in different species of marine red algae, has been shown, in both in vitro and in vivo studies, to provide a relevant neuroprotective effect by its specific anti-amyloid activity and by its γ-aminobutyric acid type A receptor affinity. The name homotaurine was chosen because of its large homology with taurine (2 aminoethanesulfonate), which is one of the most abundant free amino acids in the brain. The two molecules share a very similar structure, but homotaurine contains one additional carbon. The therapeutic efficacy of homotaurine in AD has been investigated in three phase II, and in three Phase III clinical studies that did not reach their pre-defined primary endpoints. However, post-hoc analyses have shown positive and significant effects on secondary endpoints and subgroups of patients, including a reduction in hippocampal volume loss and lower decline in memory function in the overall cohort, as well as a reduction in global cognitive decline in APOE ε4 allele carriers, suggesting disease-modifying effects. Also in three post marketing (as supplement) studies in patients with Mild Cognitive Impairment (MCI) the results are very promising. In this review, we will present the pre-clinical and clinical evidence supporting the potential role of homotaurine as a promising candidate for both primary and secondary prevention of AD.

Introduction

Current therapies for Alzheimer's disease (AD) are symptomatic with limited impact on the disease itself. Treatment that slows or stops disease progression remains an unmet need. Although the precise events that trigger AD are unknown, there is a large body of scientific evidence suggesting that amyloid peptides, particularly soluble aggregated forms, or amyloid oligomers, cause neuronal damage and cell death leading to AD. The first key driver of AD is amyloid beta in form of soluble oligomers. Pathologically, AD is defined by the presence in the brain of insoluble extracellular amyloid plaques and intracellular neurofibrillary tangles that are composed primarily of tau protein. Amyloid peptides are derived from the amyloid precursor protein (APP), an integral membrane protein, in neurons and astrocytes in the brain. Through the enzymatic cleavage of APP, amyloid monomers are produced normally at low levels and cleared from the brain via cerebrospinal fluid. One view of AD is that APP is cleaved at an accelerated rate, producing increased amounts of soluble amyloid monomers. These monomers then aggregate to form larger soluble amyloid oligomers, which are neurotoxic and, over time, lead to loss of neuronal synapses, nerve cell dysfunction and, ultimately, nerve cell death. The consequences of this progressive cascade include the formation of amyloid plaques, loss of brain volume, particularly in the hippocampus, and a progressive decline in cognition and the ability to function. AD might be prevented or effectively treated by decreasing production of Aβ and tau, preventing their aggregation or misfolding, neutralizing or removing their toxic aggregate or misfolded forms, or by combinations of these modalities.

Recent research and clinical trials support the importance of targeting amyloid oligomers early in disease progression, including the following findings: 1. Amyloid oligomer formation begins in AD patients years before clinical signs of the disease appear. 2. Accumulation of amyloid oligomers in the brain correlates with AD progression. 3. Patients with APOE ε4 have higher levels of amyloid oligomers compared to non-carriers, which predisposes them to increased risk and early onset of AD, and 4. Results from clinical trials of aducanumab and BAN2401, both injectable monoclonal antibodies that target amyloid oligomers, showed reduced amyloid plaques in the brain and slowing of cognitive decline in mild AD patients [1, 2].

As a potential disease-modifying treatment for AD, Alzhemed (tramiprosate) is a compound that binds to soluble amyloid-beta peptide (Aβ) and inhibits the formation of neurotoxic aggregates that lead to amyloid plaque deposition in the brain. It is discovered that the brain has an endogenous molecule, 3-APS, that has potent anti-amyloid oligomer activity. 3-APS is also the primary metabolite of tramiprosate, the active agent of ALZ-801, and, it was found that its levels in the brain increased with administration of tramiprosate in clinical trials. Endogenous nature of major metabolite of tramiprosate may help explain safety, excellent brain penetration & potential efficacy of ALZ-801. As a potential disease-modifying treatment for AD, Alzhemed (tramiprosate) is a compound that binds to soluble Aβ and inhibits the formation of neurotoxic aggregates that lead to amyloid plaque deposition in the brain. ALZ-801 is a novel, oral anti-amyloid drug candidate that is an optimized prodrug of tramiprosate, which has shown promising results in analyses of clinical data and therapeutic mechanism of action. This includes the discovery of its novel molecular mechanism of action blocking the formation of toxic amyloid

oligomers [3] associated with the development and progression of AD [4].

Although promising as an AD treatment, tramiprosate exhibited two limiting deficiencies: high inter-subject pharmacokinetic (PK) variability likely due to extensive gastrointestinal metabolism, and mild-to-moderate incidence of nausea and vomiting. To address these, it was developed an optimized prodrug, ALZ-801, which retains the favorable efficacy attributes of tramiprosate while improving oral PK variability and gastrointestinal tolerability.

The AD therapy ALZ-801 works by preventing protein components from clumping together to trigger the amyloid plaque accumulation in the brain that causes the disease. Disease-modifying drugs for AD and other neurodegenerative diseases could evolve from the findings. Researchers knew that tramiprosate, the active ingredient in Alzheon's ALZ-801, inhibited the Aβ protein.

ALZ-801 has the potential to be differentiated from other emerging therapies targeting AD pathology due to 1. Its' novel mechanism of action, 2. oral mode of administration, and 3.potential efficacy in a genetically-targeted population. If ALZ-801 is approved, then it has the potential to be among the first drugs to intervene in an underlying mechanism of AD. The oral absorption, conversion to tramiprosate, distribution and elimination characteristics of ALZ-801 prodrug are shown on **figure 1**.

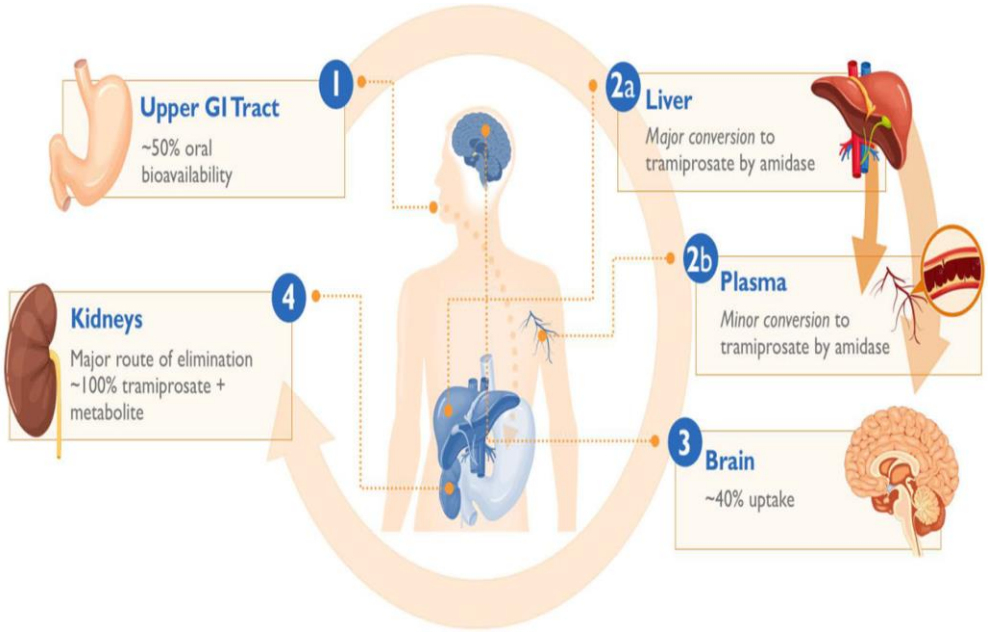


Figure 1. (After permission Hey et al 2018)

Mechanisms of action

In Vitro Studies

Homotaurine (HT) has been demonstrated to have neuroprotective effects in rats systemically administered with kainic acid [5] or following ischemic stroke [6]. The neuroprotective effect of HT has been shown to be GABAa dependent in some assays or GABAa independent in othert assays.

Against Amyloid beta and activation of GABA A receptors

In vitro, tramiprosate provides neuroprotection against Aβ-induced neurotoxicity in neuronal and **mouse organotypic hippocampal cultures**, and reverses Aβ-induced long-term potentiation (LTP) inhibition in rat hippocampus, in part, through activation of β-aminobutyric acid A (GABA-A) receptors [7]. HT inhibits the activation of caspase pathway induced by amyloid beta and by other apoptotic agents in primary rat neurons and OHC. This effect can be mimicked by GABAa agonist and blocked by GABAa antagonists.

This mechanism of action of tramiprosate, previously studied extensively, has been further elaborated in a recent work using molecular approaches, including ion mobility spectrometry-mass spectrometry (IMS-MS), nuclear magnetic resonance (NMR), and molecular dynamics [3].

HT has been shown to provide also neuroprotection against Aβ-induced neurotoxicity in **rodent neuronal and organotypic hippocampus cultures** and to reverse Aβ-induced inhibition of long-term potentiation (LTP) in **slices of rat hippocampus**, at least in part through its binding to GABA type A receptors. **Figure 2.**

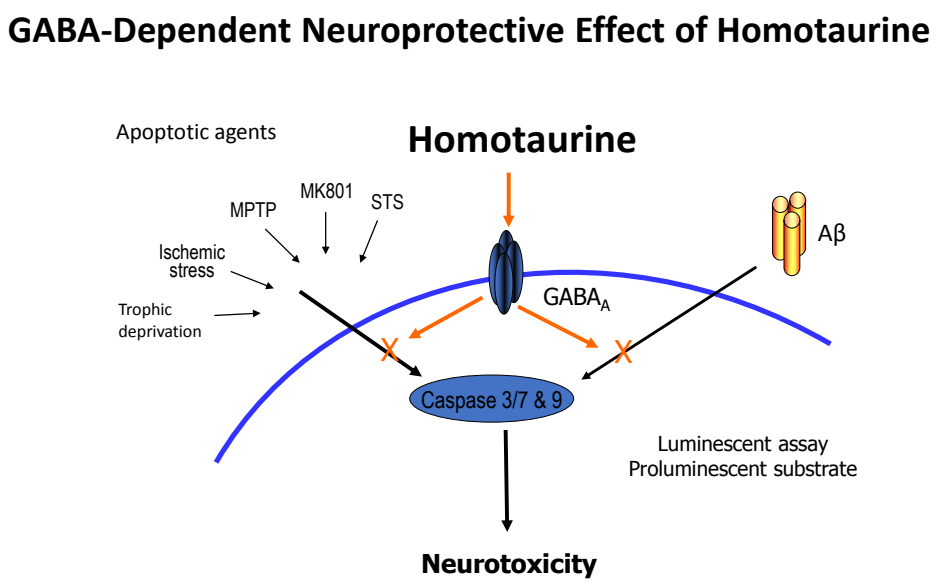
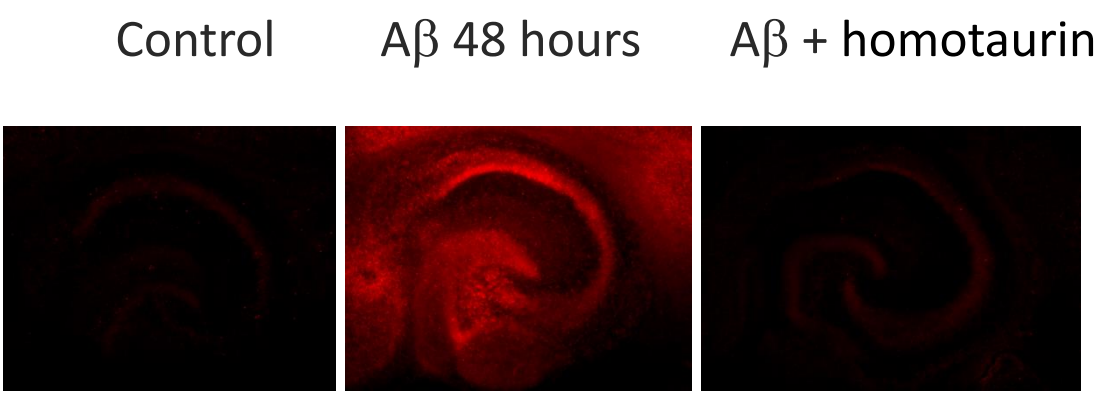


Figure 2: Gervais F., 2007 (After permission)

HT blocks the activation of phospho Extracellular Signal Regulated Kinase (erk) system and fragmentation of DNA induced by amyloid beta (in primary neurons and Shyshy cells (devoid of GABAa R). This effect is not blocked by GABAa antagonist.

When high concentration of AB proteins are incubated for 24 h, large amounts of amyloid fibrils are formed and can be seen by EM. When the same amount of Aβ amyloid proteins are incubated in the presence of HT, amyloid fibrils formation is inhibited Demonstrating the anti-fibrillogenic effect of HT [8].

HT has also been shown to protect brain cells against toxicity amyloid fibres in various assays. In an experiment neurons from hippocampus were incubated with the marker of apoptosis, Propidium Iodide (PI), which has characteristic to penetrate only in dead cells highlighting them with red coloration. The first group represents healthy brain cells after 48 h incubation with PI and the cells are alive (control). The second group showed dead neurons that were incubated for 48 hrs with toxic amyloid. And the third group showed alive neurons incubated with toxic Aβ but in presence of HT. **Figure 3.**



Propidium iodide staining

(shows dead cells: red coloration)

Figure 3: Homotaurine Protects Against Aβ-Induced Neurotoxicity in Mouse Hippocampal Cells

Against tau aggregation

HT has been also shown to favor polymerization of tau **protein in cellular models** similarly to sulphated GAG (sGAG), such as chondroitin sulphate or eparan [9]. Nevertheless, the authors of the paper demonstrated that this attitude to promote tau aggregation was not toxic for the cells, because homotaurine does not affect the binding of tau to microtubules, and more, it significantly decreased tau-actin complexes, that represent a major toxic aspect for neurons. Recent study indicates that 3-APS favors tau aggregation, in tau transfected non-neuronal cells, and in neuronal cells. But it was also found that 3-APS does not affect the binding of tau to microtubules but may prevent the formation of tau-actin aggregates [10].

We would like to emphasize the importance of testing on both types of pathology (amyloid and tau) the potential drugs to be used

In Vivo Studies

In vivo Tramiprosate (3-amino-1-propanesulfonic acid; 3APS; Alzhemedtrade mark) was found to maintain Aβ in a non-fibrillar form, to decrease Aβ (42)-induced cell death in neuronal cell cultures, and to inhibit amyloid deposition. Tramiprosate crosses the **murine** blood-brain barrier (BBB) to exert its activity. Treatment of **TgCRND8** mice with Tramiprosate resulted in significant reduction (approximately 30%) in the brain amyloid plaque load and a significant decrease in the cerebral levels of soluble and insoluble Aβ (40) and Aβ (42) (approximately 20-30%). Tramiprosate produced dose-dependent reductions of Aβ in the brain of transgenic mice (hAPP-TgCRND8). A dose-dependent reduction (up to 60%) of plasma Aβ levels was also observed, suggesting that Tramiprosate influences the central pool of Aβ, changing either its efflux or its metabolism in the brain [11].

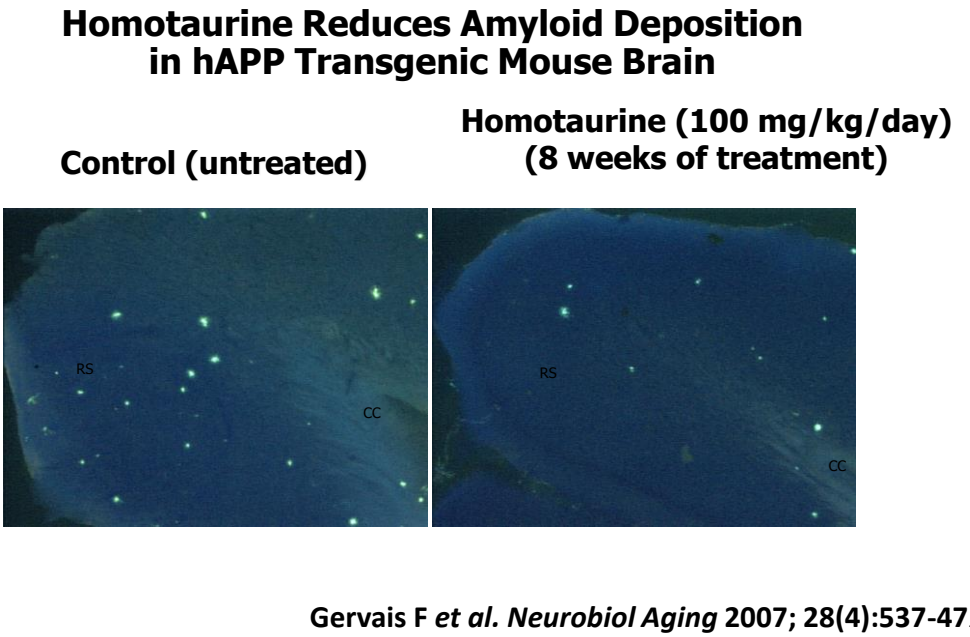


Figure 4. (After permission)

Due to its GABAergic effects and ability to inhibit neuronal dopaminergic firing, calcium acetylhomotaurinate – a homotaurine derivative also named acamprosate – has been successfully used to reduce ethanol self-administration and relapse to alcohol drinking **in both animals and humans**. Considering GABA relevance in the physiology of cognition, the potential central nervous system activity of homotaurine has also been explored in learning and memory processes **in animal models** [12-14].

In a Preclinical Toxicology/ Pharmacokinetics study it was safe & well tolerated upon chronic exposure (39 wks). Only diarrhea & vomiting was recorded **in a dog study** (≥ 500 mg/kg). The hepatocellular hypertrophy and hyperplasia of gastric mucosa (rat, 2000 mg/kg/day) was reversible. There were no adverse effects on reproduction (< 300-1000 mg/kg) and there was no effect on liver enzyme activity (CYP 450). There was only one major metabolite (2-carboxyethane sulfonic acid) and it was not mutagenic [7].

In vivo HT can pass the blood brain barrier and HT can decrease brain soluble and insoluble levels $A\beta_{42}$ and $A\beta_{40}$ so we can confirm that HT can reduce aggregation and, at the same time, promote the physiological clearance.

Clinical studies in AD and CAA

The safety, tolerability, pharmacokinetic/ pharmacodynamic effect and the clinical efficacy of tramiprosate have been evaluated in Phase I studies and randomized, double blind, placebo-controlled Phase II and III clinical trials: a total of 3464 subjects (288 healthy subjects, 24 patients with cerebral amyloid angiopathy (CAA), 2863 with AD and 288 with MCI) were exposed to tramiprosate.

Phase 1 studies

There are totally 10 clinical Phase I studies with 288 healthy subjects (74 elderly) exposed to homotaurine for 7-10 days. The endpoints were safety and pharmacokinetics. It was safe & well tolerated in young & elderly. The most frequent AEs were nausea, vomiting, dizziness. The GI adverse events were dose-related (≥ 300 mg SD). No serious adverse events and no effect on cardiac conduction (QTc) were reported. Also there were no effects on lab, vital signs, and neurological tests [14].

The most recent phase I bridging program was to evaluate the safety, tolerability and pharmacokinetics (PK) of ALZ-801 in healthy volunteers. ALZ-801 is an orally available, valine-conjugated prodrug of tramiprosate with substantially improved PK properties and gastrointestinal tolerability compared with the parent compound. Oral ALZ-801 represents an advanced and markedly improved clinical candidate for the treatment of AD [15].

Phase II studies

In a randomized, double-blind, placebo-controlled Phase II study in which 58 patients with mild-to-moderate AD were randomly assigned to receive placebo or 3APS 50, 100, or 150 mg BID for 3 months the safety, tolerability, and pharmacokinetic/ pharmacodynamic effect of 3APS was assessed. At the end of the double-blind phase, 42 of these subjects entered an open-label phase in which they received 3APS 150 mg BID for 17 months. Assessments included plasma and CSF 3APS concentrations, CSF levels of Aβ-40 and Aβ-42, and total tau, as well as cognitive (Alzheimer's Disease Assessment Scale-cognitive subscale, and Mini-Mental State Examination) and clinical (Clinical Dementia Rating scale-Sum of Boxes) measures. 3APS had no significant impact on vital signs or laboratory test values. The most frequent side effects were nausea, vomiting, and diarrhea, which were intermittent and mild to moderate in severity. Seven 3APS-treated subjects discontinued because of side effects (all causalities) over the course of the study, and there were no 3APS-related serious adverse events. 3APS crossed the blood-brain barrier, and dose-dependently reduced CSF Aβ-42 levels after 3 months of treatment. There were no significant score differences in tests between groups over the 3-month double-blind period. Long-term administration of 3APS is safe, tolerated and reduces CSF Aβ-42 levels in patients with mild-to-moderate Alzheimer disease [16].

A 5-center Phase II double-blind trial was performed to evaluate the safety, tolerability, and pharmacokinetics of tramiprosate in 24 subjects with **lobar intracerebral hemorrhage**, affected by probable or possible cerebral amyloid angiopathy. This 12-week study, performed with 3 different oral doses (50, 100, or 150 mg b.i.d.), confirmed the safety of tramiprosate. Enrolled

patients were had mean age 70.8+/-5.4, (range 61 to 78) and had more advanced baseline disease (measured by number of previous hemorrhages) than consecutive subjects in a CAA natural history cohort. No concerning safety issues were encountered with treatment. Nausea and vomiting were the most common adverse events and were more frequent at high doses. Nine subjects had new symptomatic or asymptomatic hemorrhages during treatment; all occurred in subjects with advanced baseline disease, with no apparent effect of drug dosing assignment [17].

Phase III studies

A. Patients were randomized to receive Placebo BID (n = 109), tramiprosate 100 mg BID (n = 103), or tramiprosate 150 mg BID (n = 100) for 78 weeks. A total of 508 patients underwent volumetric MRI scanning. Of these, 312 provided scan pairs for assessing hippocampus volume changes and were included in the analyses. Exploratory analysis of the volumetric MRI subgroup suggests that tramiprosate slows hippocampal atrophy, and reveals some evidence of a beneficial effect on cognition. Slope analyses of ADAS-cog score changes showed significant differences in favor of the 150 mg BID group, and when both active groups were combined, in comparison to the placebo group. No between-group differences with respect to changes to each visit in the CDR-SB were observed with either modeling approach. Although there was a similar dose-response relationship observed in the hippocampus volume and ADAS-cog final model analyses, the overall changes in psychometric scores and hippocampus volume were not significantly correlated [18].

B. A total of 1,052 mild to moderate AD patients were enrolled and were given either placebo or homotaurine for 18 months and 790 (75.1%) completed the 78-week trial. Homotaurine/placebo administered as add-on to AChE inhibitors ± memantine Cognitive function was tested using the standard, validated ADAS-cog test every 3 months. Brain Volume (hippocampus) was measured at baseline and after 18 months of treatment in a subset of patients. Patient discontinuation and reasons for withdrawal were similar across groups. Planned analyses did not reveal statistically significant between group differences. Lack of adequate statistical validity of the planned analysis models led to the development of revised predictive models. These adjusted models showed a trend toward a treatment effect for ADAS-cog (P = 0.098) and indicated significantly less HV loss for tramiprosate 100 mg (P = 0.035) and 150 mg (P = 0.009) compared to placebo. The incidence of adverse events was similar across treatment groups. The primary planned analyses did not show a significant treatment effect, but were confounded by unexplained variance. Post-hoc analyses showed a significant treatment-related reduction in Hippocampus Volume loss. However, there was only a trend towards slowing of decline on the ADAS-cog and no slowing of decline on the CDR SB. These results must be interpreted in consideration of the limitations of clinical and disease-modification outcome measures and their relationship, the heterogeneity of the disease and the impact of confounding demographic and clinical variables [19].

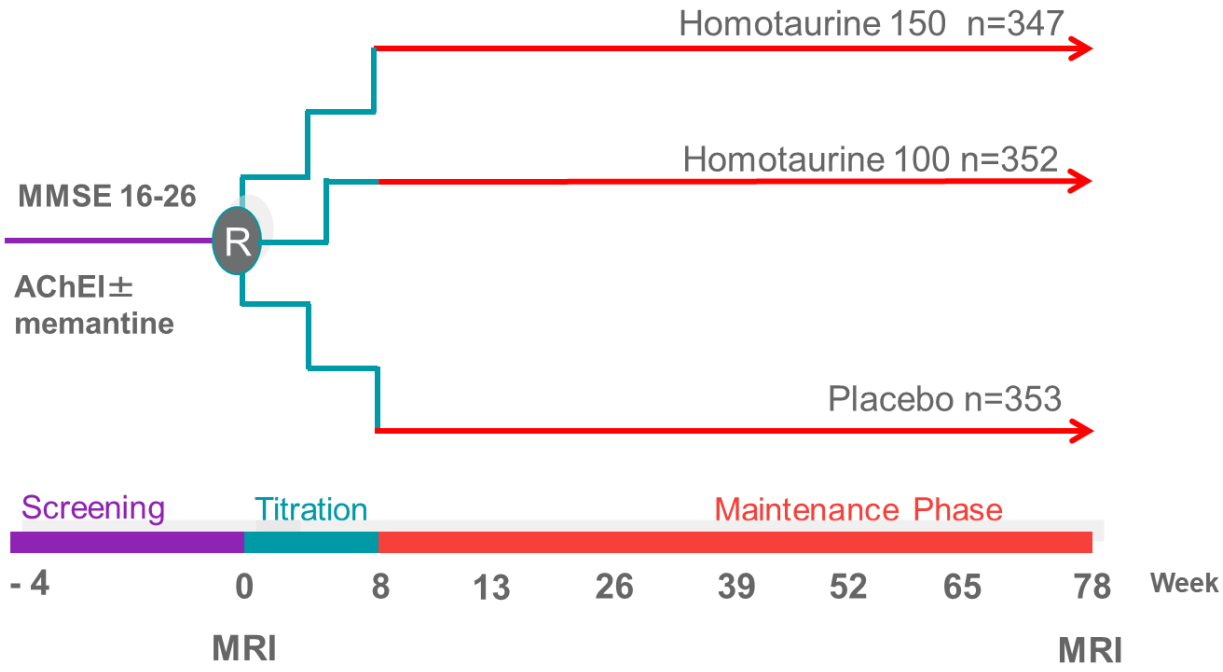


Figure 5: Design of Phase III study after permission J Prev Alzheimers Dis. 2016;3(4):219-28

C. The clinical data for ALZ-801 and its active agent, tramiprosate, suggest long-term clinical efficacy in AD patients with the APOE4 genotype, along with a favorable safety profile [20]. The initial Phase III program for ALZ-801 focused on patients with the homozygous APOE4/4 genotype at the Mild stage of AD, with the potential for future expansion to additional AD populations.

D. A double-blind for 3 months and then open label for 36 months

The safety, tolerability, and pharmacodynamic effects of Alzhemed were assessed in a double-blind study in which 58 individuals with mild-to-moderate AD (MMSE 13-25) were randomized to receive placebo or Alzhemed 50, 100 or 150 mg BID for 3 months. At the end of the double-blind phase, 42 of these subjects entered a 36-month open-label (OL) phase in which they received Alzhemed 150 mg BID. Assessments included plasma and cerebrospinal fluid (CSF) Alzhemed concentrations, CSF levels of Aβ, as well as cognitive (Alzheimer's Disease Assessment Scale-cognitive subscale, Mini-Mental State Examination) and clinical performance (Clinical Dementia Rating scale, Sum-of-Boxes) measures. Alzhemed was safe and well tolerated, crossed the blood-brain barrier, and dose-dependently reduced CSF Aβ 42 levels after 3 months of treatment (**Figure 6**, After permission).

Mild AD subjects (MMSE 19-25 at entry) displayed greater reduction of CSF Aβ 42 levels than moderate AD participants (MMSE 13-18 at entry). As soluble peptides are in constant equilibrium between the ISF and the CSF, altering the levels of Aβ oligomers in the CSF would also alter the levels of such proteins in the brain parenchyma.There was no effect of Alzhemed on the cognitive or clinical measures after 3 months of treatment. The OL follow-up suggested a stabilization of cognitive function especially in mild AD subjects over the 36-month study period.

Alzhemed thus appears to be well tolerated with long-term exposure and reduces CSF Aβ 42 levels in mild-to-moderate AD subjects [16]. (**Figure 6**)

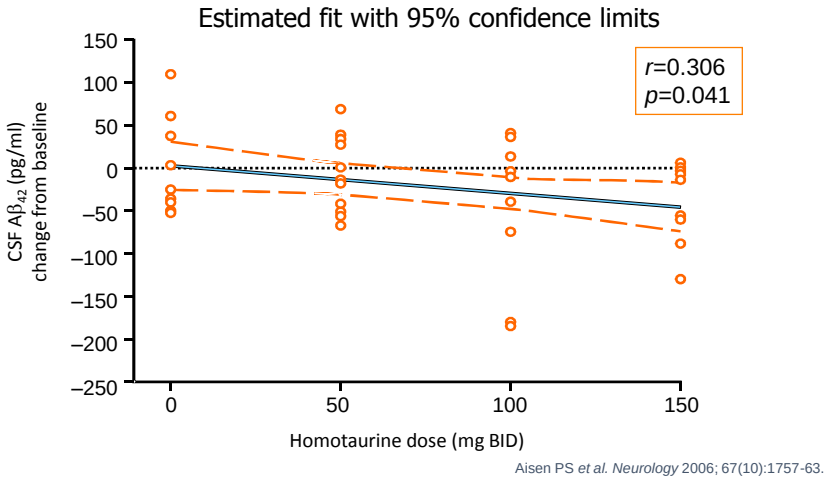


Figure 6: Phase II: Dose-Dependent Decrease of $A\beta_{42}$ CSF Levels in AD Patients . (CSF Aβ 42 (pg/ml) changes from baseline after permission) Aisen PS, et al. Neurology 2006; 67: 1757-63.

The conclusions of phase III studies are 1.Strong trend of reduced cognitive decline with homotaurine vs. placebo, 2. Statistically significant reduction of cognitive decline with homotaurine vs. placebo in APOE4+, 3. Homotaurine reduced significantly brain volume loss (hippocampus) and 4. It is safe and well tolerated.

Clinical studies in Mild Cognitive Impairment (MCI)

Disappointing clinical trials over the last several years have led to a growing consensus on the need to intervene earlier in the disease process, before the onset of any clinical symptoms. However, drug development at this stage is challenging given the difficulty of assessing a therapeutic benefit in subjects who are, by definition, almost clinically healthy. The US FDA [21] and EMA [22] recently issued new drafts guidance for trials in early AD, which revised the taxonomy of AD by recognizing four stages of the disease, including an expanded view of the pre-dementia stage. These guidelines further advance regulatory support for clinical trials in earlier stages of AD, because several studies have now reported that cognitively normal older individuals with low cerebrospinal fluid Aβ1-42 or high positron emission tomography amyloid binding demonstrate disruption of functional networks [23] and decreased brain volume, consistent with

the patterns seen in AD [24].

It was showed that AD patients have impaired LTP-like cortical plasticity, as measured by standard theta burst stimulation protocols applied over the primary motor cortex (M1). Furthermore, AD patients have a weakened short latency afferent inhibition (SLAI), a neurophysiological measure of central cholinergic transmission, which changes reflect the cholinergic dysfunction occurring in the pathology. The aim of the first study was to investigate whether homotaurine administration could modulate in vivo measured mechanisms of synaptic plasticity, namely LTP and LTD, and also SLAI in a group of MCI patients. It was observed that homotaurine administration did not induce relevant changes of both LTP and LTD recordings, while induced changes of SLAI in this group of patients. The authors suggest that homotaurine effects are dependent on changes of cortical GABA transmission suggesting a potential role for this compound in ameliorating the cholinergic transmission by modulating the inhibitory cortical activity [25].

A recent study aimed to evaluate the effects of homotaurine supplementation on cytokine serum levels and memory performances in MCI patients. Neuropsychological, clinical and cytokine assessment was performed at baseline and after 1 year of homotaurine supplementation in 20 patients categorized as carriers (n = 9) or no carriers (n = 11) of the ε4 allele of the apolipoprotein E (APOE) gene, the strongest genetic risk factor for AD. Following homotaurine supplementation, patients carrying the APOE ε4 allele showed a significant decrease in IL-18 (both in its total and IL-18BP unbound forms), in turn associated with improved short-term episodic memory performance as measured by the effect of the Rey 15-word list learning test immediate recall. Thus, homotaurine supplementation in individuals with aMCI may have a positive consequence on episodic memory loss due, at least in part, to homotaurine anti-inflammatory effects. This study strongly suggests that future research should focus on exploring the mechanisms by which homotaurine controls brain inflammation during AD progression [26].

A recent study investigated potential neuroprotective effect of homotaurine on the hippocampus structure and episodic memory performances in amnestic MCI (aMCI). Neuropsychological, clinical, and neuroimaging assessment in 11 treated and 22 untreated patients were performed at baseline and after 1 year. Magnetic resonance data were analyzed using voxel-based morphometry to explore significant differences (Family Wise Error corrected) between the two groups over time. Patients treated with homotaurine showed decreased volume loss in the left and right hippocampal tail, left and right fusiform gyrus, and right inferior temporal cortex which was associated with improved short-term episodic memory performance as measured by the effect of the Rey 15-word list learning test immediate recall.

Conclusively, homotaurine supplementation in individuals with MCI did not induce relevant changes of both LTP and LTD recordings, while induced changes of SLAI in MCI patients, patients with MCI carrying the APOE ε4 allele showed a significant decrease in IL-18 (both in its total and IL-18BP unbound forms), in turn associated with improved short-term episodic memory performance as measured by the effect of the Rey 15-word list learning test immediate recall and had a positive effect on hippocampus atrophy. Future studies should further clarify the mechanisms of its effects on brain morphometry.

FDA grants Fast Track designation to Alzheon lead clinical investigational drug, ALZ-801, for Alzheimer's disease. FDA-Accepted Study Design in APOE4/4 AD Patients. It is well known

that 3-SPA is the primary metabolite of ALZ-801, a prodrug of tramiprosate that is in clinical development for the treatment of Alzheimer's disease. 3-SPA penetrates the brain and, in a tramiprosate phase III North American (NA) trial, achieved brain concentrations associated with prevention of Aβ42 oligomer formation and clinical outcome benefit in patients with Alzheimer's disease carrying the ε4 allele of the apolipoprotein E gene. We hope that this drug, ALZ-801, will be the first modifying drug for AD patients

The authors declare that they have no conflicts of interest.

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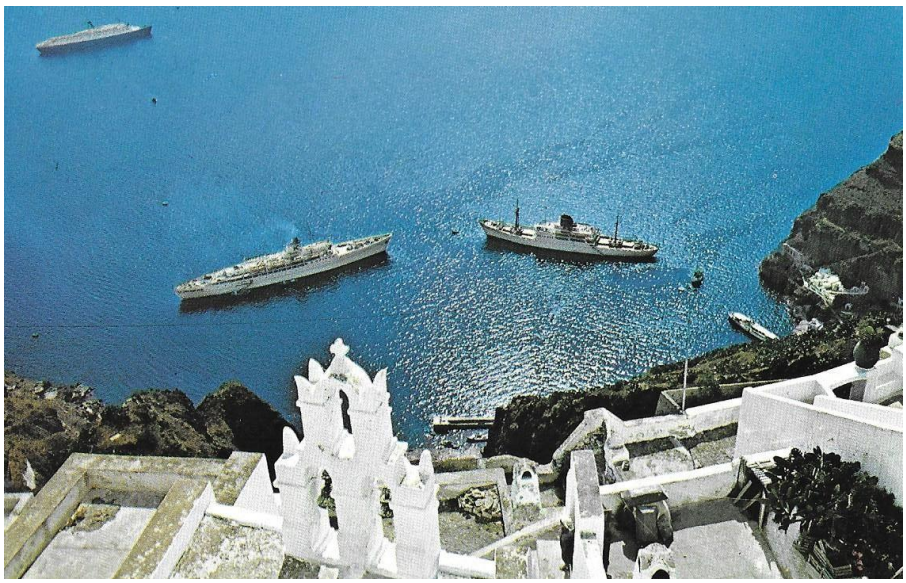

Santorini

Review

Hereditary causes of ischemic cerebral small vessel disease

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Keywords: Cerebral Small Vessel Disease - CADASIL - CARASIL - COL4 - Vascular Cognitive Impairment

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Abstract

Hereditary types of ischemic cerebral subcortical small vessel disease (SSVD) are rare, usually autosomal dominant, diseases, due to an abnormality in vessel wall synthesis. They may present with various combinations of migraine with aura, ischemic events (transient ischemic attacks, lacunar strokes) and progressively worsening ischemic lesion load in brain imaging. Eventually, vascular cognitive impairment (usually of the frontal-subcortical type) develops, frequently accompanied by behavioral-psychiatric symptoms and bilateral pyramidal and pseudobulbar signs leading to severe disability and premature death. In some patients, microbleeds and hemorrhagic strokes may be present. Despite their rarity, these disorders offer a statistically homogeneous population (usually without additional pathology such as Alzheimer's disease), suitable for the study of vascular cognitive impairment. The few studies on the relative frequency of these disorders indicate that the most frequent (or rather the least rare), accounting for more than half of patients, is CADASIL, due to mutations of the NOTCH3 gene, followed by COL4A1/A2-related disease, autosomal dominant forms of HTRA1-related disease and leucoencephalopathies with calcifications and cysts. Mutations of TREX1, GLA, FOXC1 and CARASIL are less frequent. Despite the genetic nature of these disorders, their phenotype, severity and rate of progression may be adversely affected by classical cardiovascular risk factors such as hypertension, diabetes, dyslipidemia and smoking and control of these risk factors is strongly advised for all patients.

Introduction

Vascular cognitive impairment (VCI), is the second most common cause of cognitive impairment in the senium [1]. It may be due to multiple large vessel infarcts or strategically located infarcts; however, subcortical small vessel disease (SSVD) is probably the most frequent cause [2]. It is

usually a sporadic disorder in patients over 60–65 years of age, due to classical cardiovascular risk factors such as diabetes, hypertension, dyslipidemia and smoking [3]. Up to 2/3 of such patients may additionally encapsulate Alzheimer's disease pathology [4], thus they suffer from mixed disease rather than pure VCI [5].

Inherited cerebral small vessel diseases [6] comprise a group of rare monogenic disorders leading to cerebrovascular disease and stroke [7]. Despite their rarity, these diseases may offer homogeneous patient samples, usually with no additional pathology, more suitable for studying pure VCI and understanding the relationship between lacunar stroke, SSVD and VCI [8].

Cadasil

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoancepahlopathy (CADASIL) is due to mutations of the NOTCH3 gene at chromosmome 19q12 [9]. These mutations cause alterations in the vessel wall of arterioles, with deposition of granular osmiophilic material, resulting in brain tissue ischemia [10].

Typically, disease onset occurs either with migraine with aura at about the age of 30, or with early ischemic events (transient ischemic attacks or lacunar stroke) at 41–50 years [11]. When migraine is present, it is almost always the first manifestation of the disease, followed by ischemic stroke 17 years later. Neuroimaging features include multiple and progressively confluent ischemic lesions in the white matter and basal ganglia with characteristic involvement of the anterior temporal white matter and external capsule [12]. By the age of 40, almost all patients have abnormal imaging findings. As the disease progresses, the ischemic lesion load increases and, psychiatric-behavioral manifestations, cognitive decline and bilateral pyramidal and pseudobulbar signs become evident, leading to dementia, and significant motor disability. Episodes of encephalopathy and seizures may also occur [11,13]. Premature death usually occurs at or before the age of 65–70 [13].

A clinical scale, called the "CADASIL-scale" has been proposed as a useful screening tool, in order to identify patients with a high probability to carry NOTCH3 mutations and, thus, being more suitable for genetic testing, which is required for definite diagnosis [14]. However, a significant phenotypic variation may occur among different families carrying the same mutation and even among patients of the same family, leading to unusual presenting features [15-17], deviations from the above described patterns [16–18] and diagnostic difficulties [19].

The diagnostic probability of CADASIL increases in the absence of cardiovascular risk factors. However, the presence of hypertension, diabetes, dyslipidemia and thrombophilia has been reported in many patients and does not exclude the diagnosis of CADASIL [20]. In fact, the presence of such factors may affect the clinical features in some patients [21]. Hypertension increases the risk for stroke [20,22,23] and disability due to dementia [24]. Smoking also increases the risk of stroke [20, 25] and dementia [25]. Hypertension and diabetes with increased HbA1c may increase the risk for microbleeds [23,26]. Controlling these risk factors, especially hypertension and smoking, may delay lacunar stroke, disease progress and functional disability [20,25] and, such a disease modifying, preventive approach, is currently strongly recommended [27]. Indeed, preventive measures such as physical activity and early control of dyslipidemia, resulted in less severe imaging findings and delay of first stroke in a family with CADASIL [28].

There are families with autosomal dominant inheritance and clinical/imaging features similar or identical to CADASIL, in which NOTCH3 mutations are not detected [14]. Such patients are called “CADASIL-like”.

COL4A1/A2-related disorders

COL4A1/A2-related disorders are due to mutations of the genes encoding for chain alpha-1 or chain alpha-2 of collagen type IV [29]. Both genes are located in close proximity at chromosome 13q34. The disorders are autosomal dominant and result in multiple abnormalities of the basement membrane [30]. Since collagen type IV is widespread in many tissues, COL4A1 or COL4A2 mutations may present with either one of multiple, sometimes combined or overlapping phenotypes, including infantile hemiparesis and porencephaly, ocular anterior segment dysgenesis, migraine and cerebral small vessel disease [31,32]. A specific phenotype characterized by hereditary angiopathy with nephropathy, aneurysms and cramps (HANAC) has been described [33]. Recently, pontine autosomal dominant microangiopathy with leukoencephalopathy (PADMAL) has been recognized as another phenotype due to mutations upregulating COL4A1 expression [34].

The cerebral small vessel disease caused by COL4A1/A2 mutations is characterized by multiple/confluent white matter lesions and lacunar strokes, but the characteristic lesions in the white matter of the temporal pole present in CADASIL are practically always absent [31,32]. Microbleeds are not infrequently seen and hemorrhagic strokes [35] or parenchymal hematomas sometimes provoked by minor trauma may cause an additional hazard in these patients, necessitating avoidance of most athletic activities and of anticoagulant or even antiplatelet agents [36].

Recently, it has been suggested that some common, non-pathogenic variants of the COL4A1/A2 genes, may be associated with increased risk for sporadic cerebral small vessel disease [37].

A family with an autosomal dominant disorder and with clinical and imaging features suggestive of HANAC, but with no COL4A1/A2 mutations has been reported indicating that, similar to “CADASIL-like”, there are “COL4A1/A2-like” patients [38].

HTRA1-related disorders

These disorders are due to mutations of the HTRA1 gene, located at chromosome 10q26.13 [6]. The gene encodes for the high temperature requirement A serine peptidase 1 (HTRA1), and mutations result in inability to suppress transforming growth factor beta TGF-β activity in the wall of small cerebral arteries, but also in skin and bone, resulting in dysregulation of TGF-β signaling [10]. There are two disorders with SSVD related to HTRA1 mutations: the rare autosomal recessive CARASIL and a less rare, autosomal dominant CADASIL-like disorder [39].

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL or Maeda syndrome), is a rare SSVD, usually observed in patients of Asian (Japanese and Chinese) origin, but it has been described in Western populations [10]. Patients suffer from recurrent ischemic events (mainly lacunar infarcts), leading progressively to bilateral pyramidal signs and VCI (including behavioral psychiatric symptoms) usually by the 4th decade of life. A significant percentage may develop VCI, without clinically

obvious strokes. In addition, most patients experience premature diffuse alopecia and degenerative disk disease with herniation and spondylosis deformans in the lumbar (often leading to back pain) and cervical regions [7,10]. Migraine is usually absent. In MRI, white matter lesions occur in deep white matter, basal ganglia, periventricular areas and brain stem [7,10,39]. Involvement of external capsule and temporal poles (characteristic in CADASIL) is usually absent, but may be observed rarely [40].

Recently it has been shown that autosomal dominant HTRA1 mutations, not only exist, but they are more frequent than CARASIL [41]. One such patient has been described in Greece [42]. Patients usually present with a less severe phenotype, with later onset of the disease, later development of VCI and absence of non-neurological manifestations [43]. However, more severely affected patients with alopecia and spondylosis have been described [44].

TREX1-related disorders

Three prime repair exonuclease 1 (TREX1) is a 3'-5' exonuclease encoded by the TREX1 gene at chromosome 3p21 and mutations of this gene may lead to either one of 4 disorders: Aicardi-Goutières syndrome, familial chilblain lupus, (susceptibility to) systemic lupus erythematosus and the autosomal dominant Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL) [45]. The latter is characterized by various combinations of migraine, retinopathy with gradual visual loss, brain ischemic lesions, renal and gastrointestinal, or even bone and hepatic involvement and may present as one of four overlapping phenotypes: cerebroretinal vasculopathy (CRV), hereditary vascular retinopathy (HVR), hereditary endotheliopathy with retinopathy, nephropathy and stroke (HERNS), or hereditary systemic angiopathy (HSA) [7,39,45]. Patients develop migraine, transient ischemic attacks and/or strokes and neuroimaging reveals SSVD, but additionally, tumor-like, mass lesions with contrast enhancement, surrounded by edema [39,45,46]. Cognitive decline is accompanied by psychiatric-behavioral symptoms. Visual impairment typically precedes the vascular brain symptoms and death in CRV and HERNS occurs within 10 years from disease onset [39, 46].

Other genetic causes of SSVD

Mutations of FOXC1, ABCC6 (pseudoxanthoma elasticum) and CBS (homocystinuria) may be causes of SSVD [45]. Cerebral amyloid angiopathy (CAA) due to many causes (including amyloid beta-related CAA), may also be associated with ischemic SSVD, in addition to the most characteristic hemorrhagic lesions (lobar hemorrhages, microbleeds, cortical superficial siderosis) [39,45,47].

Microangiopathy with calcifications and cysts is easily recognized due to the characteristic imaging features; however it is not one single entity. It may be due to leucoencephalopathy with cerebral calsifications and cysts due to SNORD118 mutations (Labrun syndrome) [48], or cerebroretinal microangiopathy with calcification and cysts due to CTC1 mutations (Coats plus syndrome) [49].

Relative frequency of the various causes of ischemic SSVD

Due to the rarity of these disorders, their epidemiology is not well studied. In a recent study it has been shown that CADASIL is the most frequent (least rare) disorder, accounting for 58% of cases, followed by COL4 mutations, accounting for at least 13% [50]. Heterozygous HTRA1 mutations may account for 3.5%-5% of patients [41,43] and leucoencephalopathies with calcifications and cysts for 4% [50]. Next generation or whole exome sequencing may provide a tool for easier diagnosis of such patients which, in turn, may help in better understanding of these disorders [45].

The authors declare that they have no conflicts of interest.

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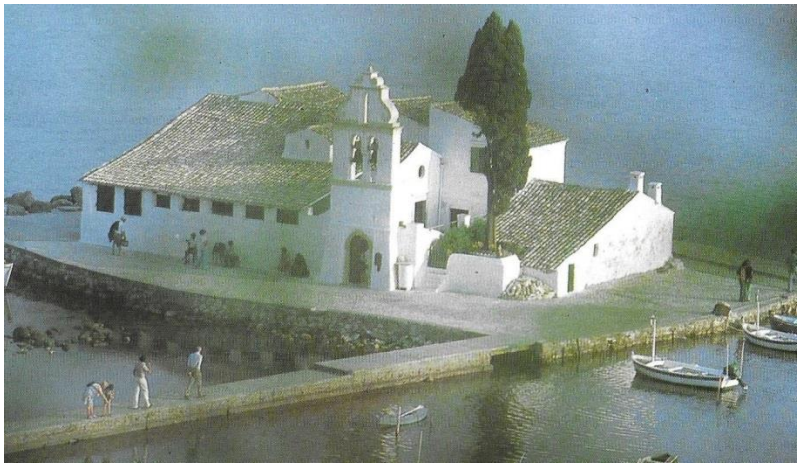

Paleokastritsa, Corfu

Review

Cognitive functions and social cognition in multiple sclerosis: An overview

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Abstract

Cognitive decline has been widely reported in patients with multiple sclerosis (MS) despite its clinical heterogeneity, at all stages and in all subtypes of the disease. Deficits are most commonly present in attention, processing speed, working memory, verbal fluency and executive function. However, MS patients also show decreased performance in tasks related to social cognition, i.e. mental operations thatdrive interpersonal skills such as social perception, empathy and theory of mind. Social cognitive deficits are an underestimated but important aspect of impairment in MS, reflecting how people process, store, and apply information in social interactions. Deficits in these domains have been associated with reduced social and psychological quality of life, even after controlling for severity and duration of the disease, age, and neurocognitive performance. Social cognition impairment is not entirely dependent on and parallel to general cognitive dysfunction, given that some patients experience disorganization of their social life before a significant or detectable cognitive impairment. The decrease in performance of social cognition tasks may reflect changes in brain activity and brain structure, either general or regional. Both subtle diffuse pathology and acute local lesions have at least partially independent effects on aspects of social cognition. The observed white matter damage contributes to a mechanism of disruption in the network of brain connections involved in social cognition. Undoubtedly, there is a wide variability in the relationship of social cognitionand neuroanatomical findings, not only due to the brain's complex connectivity, but also to the lack of a unique operative definition of these cognitive domains. Furthermore, it is difficult to compare study results, given the variability of clinical presentations in all stages of the disease.More research would contribute in understanding social cognition deficits better and in determining whether and what kind of training could be beneficial.

Introduction

Multiple Sclerosis (MS) is a chronic inflammatory autoimmune degenerative disease of the central nervous system (CNS). It is the most common non-traumatic neurological disorder among young adults leading to disability. The etiology of MS involves white matter pathology, cortical atrophy, cortical lesions, and microstructural abnormalities in deep gray matter that affect structural and functional connectivity between various brain regions [1, 2]. Cognitive impairments in MS result from this diffuse disruption in brain networks [3, 4]. Besides cognitiveimpairments, sensorimotor [5, 6], visual [7, 8], bladder, cerebellar [9] and emotional symptoms [10, 11] are also present, leading to functional disability [12, 13] and reduced quality of life [14].

The disease course can be very heterogeneous through which following types have been described: Relapsing MS, Active (with relapses and/or new lesions on MRI), Not Active (no relapses or MRI activity), Progressive MS (Secondary Progressive MS and Primary Progressive MS), Active with Progression (relapses/MRI activity and clinical deterioration not due to relapses), Active but without Progression (relapses but no clinical deterioration), Not Active but with Progression, Not Active and without Progression (stable disease) [15]. The use of subtypes' terms is primarily for descriptive purposes and for setting reasonable expectations for the treatment. Using the word "Active" to describe clinical relapses and/or MRI activity and the word "Progression" to describe clinical deterioration, highlights changes of the clinical framework. The ongoing inflammatory or neurodegenerative processes affect disease activity and clinical progression in either relapsing or progressive cases [16].

In this paper, we provide a brief overview of cognition in MS, with an emphasis on social cognition and its associated neuroanatomical substrate. We also discuss the impact of social cognitive deficits on the functional capacity of MS individuals.

Cognitive functions and social cognition

Cognitive functions in MS

As with the disease course, the cognitive impairments in MS are also heterogeneous depending on MS type [17-22] and cognitive function [1, 23, 24]. There is also a temporal gradient in cognitive impairment, as it is palpable in the early stages but more severe in progressive types [25, 26]. Cognitive symptoms are usually hidden by more visible deficits, like the former mentioned sensorimotor and cerebellar symptoms. Patients may not be fully aware of them, or may underestimate them, compared to emotional complains, fatigue or pain [27]. This is why clinicians should not rely on self-reported cognitive impairment but rather on real cognitive test performance [28]. Indicated sensitive neuropsychological tests and batteries measure all cognitive domains compromised in MS [20, 29]. Evaluations should be performed annually as their outcome may affect prognosis and therapeutic decisions [15, 30].

Nearly two-thirds of patients with MS present cognitive impairmentat some point during their life [31]. Primarily it concerns attention [32], processing speed [33-36], working memory [37, 38], episodic memory [26, 39-44], verbal fluency [20, 45, 46] and executive functions [47-50].

Patients with reduced information processing speed require more time to perform mental tasks. Deficits in attentionparticularly divided attention, lead to difficulty in multitasking and keeping one's train of thought. Patients with problems in acquiring, retaining and retrieving new information are often disorganized and forgetful. Changes in executive functions lead to difficulties prioritizing, preserving motivation, engaging in goal-directed action, and controlling behavior.

Cognitive reserve is crucial in determining the cognitive phenotype of MS [1, 51]. The theory of reserve posits that genetic factors (maximal lifetime brain growth), neurodevelopmental factors (nutrition, and physical health) and environmental factors (quality of parenting, adequate education, leisure activities and intellectual enrichment) contribute to resistance against disease-related cognitive decline [52]. Cognitive reserve may stall the expression of cognitive deficits in MS, even when there is a significant reduction of brain volume [53, 54]. Normally, persons with more severe disease are at greater risk for cognitive impairment, indicating that there is a negative relationship between cognitive status and disease burden. However, given the cognitive-pathologic dissociation favored by cognitive reserve, many MS patients hold out against significant disease burden without cognitive impairment [32, 55, 56].

Social cognition

Social cognition is one of the less measured aspects of cognition. The fact that it is not a uniform theoretical construct makes it difficult to assess and to compare across studies. It refers to a set of neurocognitive processes underlying the individuals' ability to perceive, interpret, remember, and apply information about themselves and the social world. Social cognitive processes include social perception, social understanding and social decision-making. The first refers to perception of emotion through facial expression or prosody [57, 58]. The second refers to affective empathy, i.e. perception and attribution of a mental or affective state to othersand carries the sense of feeling or interpreting the feelings of others [59].The third involves theory of mind (ToM) that refers to complex metacognitive understandings of our own minds as well as the minds of others, which enables planning behaviors while taking also into consideration others' behaviors [60, 61].

Recently, researchers showed increased interest in the biological basis of social cognition, from genes to brain processes. Biological factors and environmental variables interact to produce individual differences and pathology. There is an attempt to explain the more complex phenomena of social cognition by basic cognitive processes, such as visual perception, memory and attention but the answer is much more complex and interdisciplinary. Failures of social cognition may lead to abnormal social behavior, as seen in neurodevelopmental (autism), psychiatric (schizophrenia) and neurological disorders (Fronto temporal dementia). Autistic persons show lack of the intuitive ability to attribute thoughts and feelings to others leading to incapacity to social interaction and communication impairment [62, 63]. In certain types of schizophrenia, a similar deficit appears in intuitive empathy [64, 65]. In the behavioral variant of frontotemporal dementia, social skills are severely compromised [66, 67].

Social cognition and neuroanatomical substrate in MS

Social cognition has not been systematically assessed as part of common clinical evaluations but it should be since social cognitive dysfunction in MS is actually affected in all stages of the disease and all types of clinical course types. The specific neural basis of the social cognition

impairment in MS is still unclear. Brain atrophy, including measures of global and regional grey matter volume, cortical thickness or structures such as the corpus callosum have been linked to cognitive performance [68, 69]. Recent studies have begun to explore this clinical radiological relationship in social cognition, including involvement of the amygdala, associative frontal/temporal/parietal areas [70] and diffuse patterns of white matter damage [71].

Emotion recognition from facial cues is the most commonly studied social cognitive ability. In the human brain, a network of limbic structures that includes the amygdala, insula, orbitofrontal cortex and subcortical structures is activated in response to emotional features in faces [60, 61]. Multiple sclerosis individuals face difficulties in recognizing facial emotions. Not only are they less accurate at recognizing basic emotions in comparison to normal controls but they have longer reaction times [72, 73]. The impairment lies rather on emotion processing, then on facial identity discrimination [74]. Impaired recognition of facial emotions by patients with MS seems to be associated with both cognitive and affective aspects of the disease, mainly depression [75]. According to the frontal framework of social cognition, three dimensions have been identified [76]: a medial-lateral dimension processing internal/emotional to external/cognitive, a ventral-dorsal dimension representing the stimulus driven to a reflective dimension, and an anterior to posterior dimension to lessen complexity. In patients with MS, an impairment in information transmission from temporal visual processing areas to frontal regulation areas may explain facial emotion recognition. Temporal white matter lesions might cause an impaired interconnection of temporal facial processing and ventro-lateral prefrontal emotional facial recognition [74, 77].

Another key social cognitive ability is theory of mind (ToM), the ability to deduce other peoples' thoughts based on verbal and non-verbal cues. Similarly, empathy is the ability to deduce other peoples' emotions based on verbal and non-verbal cues. The neuronal processes of ToM and empathy involve different brain networks which partially overlap [78], like the medial prefrontal cortex, the temporoparietal junction and the temporal poles. ToM stimuli lead to increased activation of the lateral orbitofrontal cortex, the middle frontal gyrus, the cuneus and the superior temporal gyrus, and other more distant brain regions depending on the task used [57, 79]. Empathy is associated with enhanced activations of the paracingulate, the anterior and posterior cingulate and the amygdale [80, 81]. Subtle difficulties in empathic abilities, reading others' complex mental states and understanding pragmatics can contribute to interpersonal problems observed in MS [82, 83]. Evidence further suggests that MS patients might also subjectively report difficulties in recognizing their own emotions and empathy [84, 85]. Deficits in ToM, empathy and emotional prosody have been reported in the early stages of RRMS even in patients who have no substantial neuropsychological deficits [86, 87]. White and gray matter pathology in MS affects multiple brain regions and disrupts a number of social cognition neural networks. White matter pathways, which have a key role in coordinating the information flow between different regions of gray matter, are particularly vulnerable in MS [88]. Patients with lesions located in the ventromedial prefrontal cortex demonstrate selective deficits in ToM. Lesions in the inferior frontal gyrus compromise empathy and emotion recognition [89].

Discussion and Conclusion

There is a wide variability in the relationship of social cognition and neuroanatomical findings, not only due to the brain's complex connectivity, but also to the lack of a unique operative definition of these cognitive domains. The social cognitive deficits may reflect changes in brain activity and brain structure, either general or regional. Both subtle diffuse pathology and acute local lesions have at least partially independent effects on aspects of social cognition. Furthermore, it is difficult to compare study results, given the variability of clinical presentations in all stages of the disease. More research would contribute to the understanding of the longitudinal course of social cognition deficits and their relationship with MS neuropsychological and neuropathological characteristics and treatment strategies.

Although standard clinical neuropsychological evaluation does not include measures of social cognition, they should be part of comprehensive batteries. Clinician need to be aware of these difficulties alongside the more established aspects of cognition that may be negatively affected.Early identification of social cognition deficits enables early-intervention cognitive rehabilitation that focuses, besides attention and memory, on emotion perception, empathy and ToM, using either restorative or compensatory activities. More importantly, clinicians need to address the "real world" implications of these deficits and to develop effective transdiagnostic interventions for MS patients.The nature, magnitude and specificity of social cognitive impairments each play an important role in therapeutic decision-making.

Social cognitive deficits appear to be a core cognitive phenotype of many developmental, neurological and psychiatric disorders. Only through transdisciplinary research, can we learn more about social cognition. Because of the nature of the deficits, research needs to be easily accessible and applicable to everyday life.We have to go beyondthegenetic, neuronal, cognitive and social level and include the educational and political one. Outcome measures should combine neuroimaging and neuropsychological testing results with qualitative reports and interviews, and observational methods in real-life contexts.

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Review

Comparison of two electronic screening tests (culturally neutral & culturally customized) for major and minor neurocognitive disorders.

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Abstract

Introduction: While attempting to test the population for Mild and Major Neurocognitive Disorders (M. - M. N.D.) there are tests in conventional and electronic form which are applied. However, a large proportion of elderly avoids being tested as the process itself causes stress and fatigue. The structure of the tests is often irrelevant to the experiences and life of the elderly as it focuses on the standard arraying of the diagnostic criteria. **Objectives**: A) the introduction of a culturally oriented screening test based on folk tradition. B) exploiting the advantages of technology in an electronic version of this test, in order: To be administered by non-specialist and / or people of the elderly's environment. To be given to people with sensory impairments of vision or hearing - speech. Methodology: 1. Automation of the delivery and evaluation process in order to avoid the bias effect due to differences between examiners. Use of multimedia and innovative interfaces. 2. Application of the electronic version to a sample of 300 people of both sexes and 60+ years old. The evaluation of the culturally customized test will be done on the following axes: Metric Capacity in detection of Major and Mild ND. For this case, the reference point are normalized screening tests. Evaluation of usability and acceptance by examinees, examiners, caregivers and health professionals using normalized tools and interviews. 3. Comparative study with an electronic state-of-the-art screening test (TAB CAT) for M-MND in a sample of 120 individuals aiming in comparing the tests for ease of use and acceptance by the test subjects.All participants will be from 60 years old and above, having the Greek language as native language, without any limitations on the educational level. Participants will be divided into 3 groups according to their

diagnosis (normal, Mild ND, Major ND)Expected Benefits: The potential effectiveness of the innovative screening method for Mild - Major ND is expected to increase the number of elderlies who treated for neurocognitive impairment while reducing examination costs.

Introduction

Increased life expectancy combined with declining birth rates has led to a strong aging trend. Over the next five years it is estimated that people aged over 65 years old will overcome the number of people under the age of 5 years old for the first time [1]. The aging rate in Italy, Bulgaria, the Czech Republic, Greece and Japan is expected to reach the point that for each young person two elderly people will be matched [2]. ADI [3] has a proportional image as between 2015 and 2050 it is estimated that the number of elderly will rise in the high income countries by 56%, in countries over the average income by 138%, in countries below average by 185% and at the low income countries by 239%. Accordingly, the proportion of people suffering from dementia will increase by 116% in the high income countries, by 227% in the above the average, by 223% at below the average and by 264% in the low income countries.

At the same time, the incidence of diseases associated with cognitive functions disorders increases exponentially [4]. Every 3 seconds a person is diagnosed with dementia. In 2018 [5] it was estimated that 50 million people worldwide suffered from dementia and this number is expected to be tripled, reaching 152 million people in 2050. The very high cost of dementia that reaches 1 trillion dollars in 2018 is estimated to be 2 trillion dollars in 2030 and that will stress national health systems and families bearing the primary care of patients. In Greece, the frequency of dementia is estimated at 9.59% in people over the age of 70 [6] and 3.6-9.2% over the age of 65 years [7-9].

Although the general public believes that dementia is intertwined with aging, in reality, despite the fact that the risk of developing the disease is increasing over the years, it is not a legitimate consequence of aging [10]. The attempt to distinguish aging and dementia and the encoding of similarities and differences has brought to the foreground the Mild Cognitive Impairment (MCI) [4,11]. Originally characterized as the precursor stage of Alzheimer's Disease (AD) [12,13]. The term MCI includes a set of mild syndromes characterized by a lower, in comparison to dementia, but detectable reduction of one or more mental functions compared to the expected performance for the age and educational level of the subject examined in the neuropsychological evaluation. In the revised version of the DSM-V**Σφάλμα! Δεν έχει οριστεί σελιδοδείκτης.** taxonomy, it is referenced as a separate neurocognitive disorder, with specific diagnostic criteria. It requires indications of moderate mental decline compared to the previous level in one or more areas (attention, capacity, perception, learning and memory, language, social cognition). Labels should be based on reports by the person himself, a person with training in the field of mental disorders or the clinic, that there is impairment of cognitive function. Cognitive deficits must not prevent day-to-day functionality.

The multidimensional nature of the MCI has recently established the term neuropsychological profile [14], as a clinical description of the clinical picture and the deficits of cognitive functions. Shacks-Ericson & Blazer [15] underline that our knowledge of Mild

Neurocognitive Disorder (Mild ND) as described in the DSM-V**Σφάλμα! Δεν έχει οριστεί σελιδοδείκτης.** is derived from the MCI research as previously defined in the international literature. Similarly to DSM-V**Σφάλμα! Δεν έχει οριστεί σελιδοδείκτης.**, the term dementia is replaced by Major Neurocognitive Disorders (Major ND). It includes disorders whose central and main characteristic are clinically significant deficits in cognitive functions. Disorders are acquired (and are not part of the developmental pathway - the last stage of which is aging), and point of reference is the initial level of functionality.

The transition from normal aging to Mild and Major N.D. is insidious, gradual and often lacks attention. Participating in screening tests of population, although is not appropriate for all the elderly, is important for people at risk [16] and is the first step towards further, thorough testing of clinical characteristics, application of neuropsychological tests, laboratory and neuroimaging control which is necessary. The application of screening tests can detect medical conditions that cause memory and cognitive impairment and are attributable to potential reversible factors such as anemia, vitamin B12 deficiency, folic acid deficiency, depression, drug toxicity, etc. As cognitive decline appears to begin earlier than the diagnosis of Mild -Major ND, the definition of a reference point as long as the individual still retains its functionality within the normal range is of great importance in the case where some of these individuals will suffer from dementia in the future.

Although in recent years there has been sufficient information about Mild and Major ND there is an average period of 2 years [17] (in the Greek population 6-16 months for 52% of cases [18]) from the appearance of the first symptoms to the diagnosis of the disease. In the U.S.A. and the U.K. it is reported that 50-66% of the elderly diagnosed with dementia at the primary health care do not have such a record in their personal file record [16,19].

The systematic excess of the above difficulties has been an objective for several years. The entrance into the digital era and the use of high-speed broadband networks has provided a powerful boost for the development of computerized tests. These are computer-assisted or tablet-based tests that are used either to screen the population for cognitive impairment or to analyze the cognitive functions of the subject. Over time and by conducting studies, their pros and cons began to appear in the scientific literature [20,21]. Electronic Tests (e-tests) make possible the systematic evaluation of the clinical condition and the longitudinal monitoring [22].

Advantages of e- tests:

- Can be used by a wealth of health care professionals at low cost, short-term training.
- The responses are recorded exactly and data is automatically stored.
- Repeated examinations can be compared.
- Tests can be tailored to the candidate's level to cover a wide range of cognitive functions.
- Data is stored directly in the device's storage space, while being able to transfer it directly to a cloud infrastructure and a research database.
- They allow the simultaneous transmission of data to telemedicine programs.

Restrictions:

- Lack of regulatory data and psychometric standards.
- The low familiarity of the elderly with computers.
- Professionals are not familiar with this type of tests.
- Equipment / software costs.
- Insufficient integration into healthcare systems / services.

The review of the literature and the clinical experience from the daily use of equipment used in brain stimulation – empowerment programs, points out that the elderly indicate a positive attitude towards new technologies. This finding is in contradiction with current views (of the general public and professionals also) that present the elderly detached from technology and being cautious or hostile to it [23-25], as in the past few years the number of portable electronic devices (tablets, smartphones) has grown to the general public, including the elderly.

The perception that dementia is a natural consequence of aging, the inability and / or reluctance to recognize and accept the severity of initial mental and behavioral changes [26], and the accompanying fear of diagnosis and stigma raise barriers in searching for medical help, with significant implications for everyday life and clinical course of the -un-diagnosed elderly. Information obtained from professionals – who often lack of special education - that come into regular contact with the elderly, is not sufficient. The primary screening of the Major ND population may even double the number of elderly patients receiving a diagnosis [17] as the clinical judgment by itself lacks in comparison to the combination with receiving a primary screening test [27].

There are a large number of Major ND screening tests that are culturally neutral, reliable and commonly accepted widely, which are used in both day-to-day clinical practice and epidemiological studies. Nonetheless, Major ND is sub-detected.

Some elderly people are reluctant to contact mental health professionals for the evaluation of their mental functions. The test structure itself, whose lesions directly correspond to diagnostic criteria, increases the stigma esteem and daunts the elderly, especially in the elderly with a low educational level and a strong feeling of stigma. Adaptation and validation of existing screening tests in the examining population, although it significantly diminishes the differences due to cultural factors, leaves untouched the whole profile of the screening test, which, mainly for people with low education level, and therefore unfamiliar with this way of thinking, can cause inhibitions that are accumulated in the fears of a possible diagnosis of neurocognitive disorder and act as a deterrent or, in some cases, leads them to pay less of their potential effort [28]. According to literature research it appears that until now the attempts to create a screening test with a high metric capacity and a "friendly" profile to the user are steadily moving around the axis of cultural neutrality. In this comparative study, two screening tests are provided electronically (culturally adapted and culturally neutral) in order to demonstrate the strength of their metric capacity and to measure the degree of acceptance by the examinees and the examiners. The ultimate objective is to check the hypothesis of increasing the proportion of the elderly scanned for Mild -Major ND in our country so that, in the event of success, the project will be the basis for the creation of corresponding texts for elderly people living in different cultural contexts.

Mathews's [29] highlights the need for a critical view of the principle of the globalization of

neuropsychology as it was born and developed in the context of the western culture in combination with Ardila's [30] corresponding for the controversial cultural neutrality of the tests used to evaluate mental functions as many of them use strategies that may not be familiar or even they violate socially accepted norms of the populations to which they are currently applied are now current more than ever, as the expected rapid increase in dementia cases in developed countries where there is a high percentage of people with low levels of education will soon bring us in front of this reality.

Method

A) Tests

1. The brain health assessment test (B.H.A.- TAB-CAT) [31] is provided electronically via tablets and has an Ios operating system. The BHA test was developed by the University of California San Francisco and consists of four sub-scales, which were designed to evaluate the respective cognitive functions (a) memory, executive function and speed, visuospatial ability and speech. A scale of functionality has been incorporated into the B.H.A., which is self- reported by a person well aware of the condition of the person under consideration. Changes in functionality and the appearance of cognitive disorders have been noted over the past 5 years. The B.H.A. has 84% sensitivity to MCI detection and 100% for dementia detection with 85% specificity [31].

The device should have a screen of 9.7 inches and above, and an Ios operating system. The administration of the test lasts about 10' and the grading is automated. The Tab-CAT was selected for comparison with the HAST, as it is a good example of a culturally neutral computerized screening test. It belongs to the latest generation of computerized screening tests using tablet and focuses on the early detection of cognitive disorders while being characterized by a short duration of administration [21].

2. The Hagia Sophia Test (HAST)

The HAST [32] includes the text of a popular legend that relates to the building of the Cathedral Church of Hagia Sophia in Constantinople (now Istanbul Turkey) by the Byzantine Emperor Justinian's at 537 A.C. . This particular text has come to us today from the oral transported folk tradition as well as other corresponding texts in folklore. In the 128 words that compose the myth, the incidence of grammatical and syntactic phenomena, the similes and metaphorical speech and the implied information are resulting from context or are part of the prior knowledge. Thus, these words create an intimacy due to cultural stimuli but also a demanding environment of varying degree of difficulty in its representation by the participants. Levi-Strauss C. [33] states that the texts of myths are not read only in the usual way from left to right, but at the same time horizontally and vertically and from the front to the back. To narrate the myth, we must read it horizontally. To understand it, we need to read the vertical columns. The myth combines the knowledge of the real world, the negation of this knowledge, and the existence of counterfactuals in a dialectical context that sets into test lexical-semantic factors that can influence the process of comprehension [34]. Normally (without cognitive disorders), the presence of a false dialectical framework can eliminate the effect of an event that negates real world knowledge but it is

appropriate into the false frame (for example, the bee gets the holy bread with her mouth and flies out of the window). Understanding the semantic links of metaphorical expressions requires a verbal controlled process as the metaphorical speech has a special status in semantic memory [35]. Unexpected - novel events- in narrative alternations - and recall, respectively - of the myth cause the attention and are more efficiently memorized by triggering increased neuronal activity related to perception, attention, learning and memory [36]. The project, due to its originality, confronted us, as we were counting, with plenty of difficulties. To ensure the stability of the case, we relied on part of the tests provided in a prospective study conducted since 2009. The preliminary findings from the pilot administration of HAST in conventional form [32], provide us a boost for the second phase - creating an electronic application for e-HAST through tablet.

B) Developing the online version of the test

The design of a standalone application for tablets with size up to 10.1 " for Android environment. The application allows the implementation of the test and its usage by a (specially trained or non-professional) practitioner or a person of the elderly's environment.

1) Architecture

Google's Android environment. Capability of automatic execution of the application any time a user wishes. Automated storage of (a) the demographic data memory, the respondent's answers, the audio file of the grant. b) online connection to a server for transferring file image for secure storage as well as connection with Alzheimer's Federation's online database for clinical and research purposes by certified users.

2) Content and Presentation

Includes:

A) Demographics download screen.

It is completed by the user (professional or a person of the subject's environment).

B) Narration of the test.

Narration with simultaneous presentation of representative images of the myth and subtitles (for people with severe hearing problems).

Narration of myth (for people with severe vision problems)

C) Intermediate stage.

At the end of the narration, the user will be introduced into the visual perception assessment application screen, using L. Ghent's [37] complex realistic images designed to investigate the possible weakness of visual perception, nominal aphasia, and critical ability in children. The use of these images gives the opportunity to investigate the possible influence of the visual perception deficits in the e-HAST and TABCAT performance.

D) Completion of the test. Transfer in to the screen where the subject has to complete the test entries.

E) Export and exploitation of results. Presentation of the test's result to the user and appropriate feedback.

3) Answers

The person giving the test, records on paper the subject's answers and then types them in the device. At the same time, an audio file is created to complete the test for speech impairment assessment by the clinic.

4) Evaluation

The data of the preliminary allocation of the HAST in its contractual form (n = 950) brought to light a significant number of related words which the examined ones used in the requested items to be completed. The calibration of the test was based on a 4 degrees Lickert scale (a) correct, b) approximately correct c) error, d) no answer. Episodic and semantic memory disorders have a negative effect on the understanding and production of speech. Specific areas of the brain such as the Wernicke and Brocca regions are associated with speech disorders and influence the performance on language tests. Depending on the type of dementia we might have phonological paraphrases, semantic or phonological errors [38], simple phrases, reduced speech flow and thematic coherence, decreased phonological awareness, vocal paraphrases, etc. The perspective of exploiting the richness of the words that the subjects used interchangeably to supplement the HAST created the hypothesis of improving the metric properties of the test. In order to measure the 'specific weight' of each of the words used by the respondents to answer, the vocal and phonological, morphosyntactic and semantic characteristics were calculated. The problem of evaluating the performance of the subject is solved by exploiting the computing power of the electronic device on the basis of a relevant algorithm.

Results

User is informed about her/his performance and receives a recommendation for a possible repetition of the test, after 6/12 months or for an immediate test by a specialized in memory doctor's office (depending on the test's performance) with the information of such of these offices that are close. The mental health professional may have access to analytical data relating to the areas of the cognitive functions examined by the test (graph showing parallel reference values in the normal population), completion time and consolidation and compliance with the test's completion instructions. At the same time, the recorded sound record allows the investigation of any aphasic disorders and/or mistakes in phonology - syntax, the structure of speech, fiction etc.
Each user's results, will be uploaded to a server (if a broadband connection is available). There will be stored together with the personal demographic data anonymously, verifying the personal data's security and protection. The goal is to build a large database which will allow data extraction for research exploitation.

Methodology

- Implementation of the e-HAST, pilot application, improvement and construction of the final version
- Completing the evaluation of the conventional (paper and pencil administration) version

- Defining user groups a) in healthy elderly individuals, b) with Mild Cognitive Impairment and c) Demented.
- Administration of HAST in a sample population of 300 people
- Parallel administration of the Tab-CAT to a sample of 120 people for usability testing
- Statistical analysis of the data, processing the data and conclusions

Design

The present study will consist of a Cross Sectional design

Participants

The participants in the study will be men and women over 60 years in the prefectures of Attica and Thessaloniki. The sample of the study consists of three different groups of elderly. Each group will consist of 100 persons. Each participant will be 60 years old and above, regardless of their level of education, and their mother tongue will be Greek. The participation in the study will be voluntary and the participant will sign an informed consent statement.

The first group will consist of healthy participants (control group) living in the community who do not exhibit symptoms of moderate to severe depression (GDS performance from 11-15), and do not have a history of psychiatric problems, a history of addictions, or a cognitive decline. The second group will consist of participants diagnosed with Mild ND based on DSM-V. The type of syndrome will have been diagnosed after a neurological and neuropsychological assessment. They will retain their functionality and, according to defined criteria, will not be able to be diagnosed with dementia. The third group will include elderly people with a diagnosis of Major ND. The syndrome will be diagnosed following neurological and neuropsychological assessment and based on the criteria of DSM-V.

All participants will be examined with a neuropsychological array that will evaluate all cognitive functions as well as the existence of functional and neuropsychiatric problems as well as symptoms of anxiety and depression. At the same time a clinical interview will be conducted by a neurologist or psychiatrist. The final diagnosis (and the corresponding distribution in one of the three groups) will be given by the team of scientists of the Hellenic Association of Alzheimer's Disease and Related Disorders after an evaluation of the neuropsychological array results and the above mentioned examinations. Researchers who provide e-tests will not know the diagnosis of each participant in order to preserve their neutrality.

Tests and usability evaluation

All exercises, as well as additional material (instructions, solved examples, texts and multimedia material) will be given in the mother tongue of the participants (Greek). The implementation of the two screening tests will take place at the day centers of the Hellenic Society for Alzheimer's Disease and Related Disorders and in the Municipality of Ilion, Attica, in the framework of the first cognitive control of MND. In the subgroup of participants receiving the Tab CAT test (40

individuals from each group for a total of 120 individuals) and the satisfaction and usability assessment questionnaires,

The examination will take place at the same session where the e-HAST will be awarded in order to evaluate the two tests under similar circumstances. To avoid the effects of fatigue, there will be a 15-minute break between the two tests and also before the assessment of the satisfaction and usability questionnaires.

Data collection and analysis

In this context, we will collect data from user's responses, test completion statistics (total test time, average time per response, total score) as well as questionnaires for users attitude towards the platform and application. Overall data with the reference test data, demographics and clinical diagnosis will be coded anonymously on an electronic basis. The data analysis will be realized by parametric and non-parametric statistical methods, depending on the sample's normality test of the sub-group of each analysis, aiming at the extraction of qualitative and quantitative conclusions.

Limitations

- In order to save our program resources, even though the application will be designed for the three operating systems, it will be tested only in an Android environment, which is the most widespread in our country
- The requirement of elementary computer knowledge (mainly input-output devices) by the person who will provide the e-HAST
- The level of education (restrictions in case of functional illiteracy)
- The sensory inability of the examined person seeing and hearing combined.
- The e-HAST will be provided by non-experts. The expert will be present as an observer to ensure that there is no significant deviation from the administration protocol.

Innovation

The text of Hagia Sophia's legend is a part of folklore. Applied for the detection of Mild.- Major ND because of the wealth of linguistic elements that compose it, the psychometric properties, and its ability to stimulate emotion and retrieve cultural memories by examining more familiar and accessible to the elderly. The investigation of cognitive impairments with the particular test based exclusively on the use of speech (oral and written). The fact that the answers to the test entries (in their computerized version), they are not ordinal or categorical, but allow -as words flowing speech- calibration of the test based on series of factors such as morphosyntactic, semantic and context relativeness. This type of scoring may not be made by the examiner as it requires valuable time. The electronic application gives us the advantage of accuracy and speed in the results

Meanwhile, as we introduce our words with the keyboard, we have the opportunity to enrich the vocabulary (in the phase of monitoring and upgrading the software). The saved vocabulary allows the examiner to interfere with the assessment of the qualitative characteristics

of the test (e.g. in cases of aphasic disorders). An additionally reason that HAST is an innovative screening test is that provide us the ability to non professionals and people in the familiar environment of the elderly to administer the test. So, we examined the hypothesis of increasing the number of elderly people screened for cognitive impairment. HAST it is not just an attractive screening test but also a test that can be given to the daily (and intimate) environment of the elderly from lay people, which will reducing effectively the stress caused by the examination and enabling the screening of cognitive functions to elderly who are unable to visit memory clinics or other specialized structures. Also, the possibility of administering in 2 forms, limits problems due to sensory impairment or visual or hearing impairment, but not to the coexistence of these two conditions.

Our effort is novel because it introduces a screening test like this, having the aforementioned features and exploiting the advantage of speech. In addition, the possibility of experimentally using this innovative test (e-HAST) along with the Tab-CAT allows us to compare it with the state of the art of existing computerized screening tests. As well as, drawing conclusions for better use of existing and innovative e-tests in preventive medicine programs focused on the mental health of elderly Greeks. The potential success of this project will trigger the opportunity to be created similar tests in other populations. And, a possible failure will prevent similar steps by saving resources [39].

Future implications

1. The creation of a screening test administered by non-specialized professionals and individuals of family environment.

a) Installing the diagnostic criteria in a structured grammatical and syntactic speech.
b) Exploitation of texts of folk tradition rich in grammatical and syntactic phenomena in cognitive empowerment – stimulation programs in elderly with cognitive disorders.

2. Introduction of combined utilization of human and technological intelligent analysis of psychometric properties and cultural stimuli of e-HAST.

a) Introduction of an exhaustively analytical system for the calibration of the screening tests, utilizing the computational power of the dispenser in regard to the response to the entries of the examined elderly.
b) Possible increase of sensitivity and specificity.

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Brief Review

Ageing and Down syndrome: Neurocognitive characteristics and pharmacological treatment

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Abstract

Individuals with Down Syndrome (DS) are commonly characterized by unique neurocognitive and neurobehavioural profiles that emerge within specific stages in the developmental continuum. A plethora of studies have confirmed DS's relationship to premature aging and subsequent cognitive decline. Due to having three copies of the amyloid precursor protein (APP) gene which results in amyloid-beta plaque deposition, the cognitive decline often resembles the decline observed in Alzheimer's disease. More specifically, as individuals with DS mature in age (>40) they experience a dramatic increase in difficulties in several cognitive domains, such as language, visuo-spatial abilities, executive functions, working memory, etc. Especially, frontal functions are reported to show an inverse correlation with age. In contrast to the pronounced and well-described neuropsychological deficits, psychiatric symptoms presented by this patient category are not uniform. Mental health disturbances commonly include general anxiety, obsessive-compulsive or oppositional/aggressive behaviors, depression and sleep disorders, as well as self-injury and behavior belonging to autistic spectrum disorders. Therefore, the purpose of the present review is twofold. Our first goal is to depict the cognitive and behavioural phenotype of adults with DS and our second goal is to review the current treatment options available for the behavioral and psychological symptoms, with an emphasis put on the quality of evidence available through meta-analyses and appraising critically the anecdotal treatment often applied. We also present a review on the psychotropic medication, especially acetylcholinesterase inhibitors, that can potentially slow the progression of cognitive decline of those patients. Finally, novel therapeutic strategies, psychological interventions and future diagnostic and therapeutic challenges are discussed.

Introduction

Down syndrome (DS) is the genetic manifestation of a chromosomal defect, which is the well-known trisomy 21. Its estimated prevalence is 13.65 per 10.000 live births [13] or according to other sources 1 in 800 live births with a global incidence of more 200.000 cases per year [46]. DS is the leading genetic cause of intellectual disability accounting for 25%-30% of people with mental retardation [42]. The degree of intellectual disability varies widely from borderline intelligence to severe mental retardation, with the 80% of individuals displaying moderate mental disability [52]. Despite considerable variability, individuals with DS have been described as having phenotypically distinct and consistent behavioral patterns in language and cognition across their entire lifespan [15,39].

Neuropathological changes in DS closely resemble those identified in both sporadic and early onset Alzheimer's Disease (AD) in the general population, and tend to accumulate as individuals with DS advance in age. More specifically, by the age of 40 nearly all individuals with DS have detectable neuropathological findings related to AD [38]. These pathological alterations include extracellular appregates consisting primarily of fragments of the amyloid precursor protein (APP) [35]. Neurocognitive decline as a consequence of brain changes is commonly observed in adults with DS, which increases rapidly after the age of 40 [16]. Health morbidities also increase with age for individuals with DS at risk of dementia [40] and need to be considered in both diagnosis and treatment of older individuals presenting cognitive decline [51]. Although neuropathological signs of dementia are observable in adults with DS, however not all of them undergo an obvious cognitive decline. For example, Tyrrell and colleagues [56] reported that the average prevalence of DS with AD is about 13.3% at the age of 54.7 years.

Taken all the above into consideration, people with DS exhibit unique neuropsychological and behavior profiles during specific periods of their development. Nevertheless, adulthood in DS is related with more dramatic changes as a result of the emerging neuropathological alterations. Thus, the aim of the present review is twofold. Firstly, we describe current findings on the neurocognitive and behavioural functioning in adults with DS, in order to depict the neurobehavioural profile of DS. The second aim was to review the current perspectives on pharmacological treatment that addresses behavioural and psychological disturbances.

General cognitive functioning in adults with DS

As regards research on cognitive functioning, a plethora of studies focused on the deficits that present children with DS (i.e., in language and working memory). However, literature on the cognitive abilities of adults, and especially older adults, with DS remains relatively limited. More specifically, the adult brain of individuals with DS displays several structural abnormalities; decreased hippocampal volume and circuit abnormalities are believed to be the main cause of cognitive decline met in DS [43,59]. Moreover, behavioural and mental health issues are not rare in DS, since attention and memory deficits, hyperactivity, depression and psychosis are very common among adults with DS [2,51]. Finally, it is claimed that approximately 6-10% of people with DS have a co-morbidautism spectrum disorder [28,41].

General cognitive decline is observed among people with DS as they advance in age.

Approximately, by the age of 60 years, 75% of people with DS meet the criteria for AD [51]. Nevertheless, linguistic skills in individuals with DS are preserved at a relatively good level despite the age-related changes demonstrated in other cognitive functions in DS [14]. Thus, for example, receptive vocabulary and reading skills remain stable [10]. However, there is some evidence that not all language functions are preserved to the same degree. For instance, Rondal and Comblain [53] claimed that syntactic abilities and expressive vocabulary are affected by the progressive age increase. Moreover, studies have shown that communication skills decrease as people with DS get older. Prashner and Chung [48] noticed that about 20% of people with DS around the age of 50 and 70 years old present impairments in their speech ability. Nelson, Orme, Osann and Lott [45] also claimed the existence of deficits in expressive vocabulary and in pragmatics among adults with DS.

Cognitive decline in adults with DS starts at a younger age compared to healthy adults and adults with intellectual disabilities of unknown etiology [61]. The mean age of cognitive decline in this population is located in their mid-fifties, although there is some evidence suggesting that cognitive decline may start ever earlier in their mid-forties [54]. In a longitudinal research, Coppus, Evenhuis, Verberne, Visser, van Gool et al. [17] studied 506 people with DS and revealed that in adults over 49 years old the percentage of cognitive decline was estimated at 8.9%, and increased with age reaching a percentage of 17.7% in adults between 50-54 years and 31.1% for ages between 55-59 years. Finally, in the last age group (over 60 years) the percentage climbed to 25.6%. Although the latter percentage was not anticipated in their study, researchers suggested this finding reflects the negative impact increased health issues which older people with dementia face. Moreover, Zigman, Schupf, Devenny, Miezejeski, Ryan et al [62] found that adults with DS over 50 were more likely to present symptoms of dementia than their counterparts with intellectual disabilities of unknown etiology by an analogy of 8.56 to 1.68.

The association between DS and dementia has been a main area of research interest for over a century [21,26,58]. As mentioned earlier, neuropathological changes are very common in adults with DS, especially between the ages of 35 and 40 [25]. The relationship between brain abnormalities in DS and dementia is mediated by many factors. For example, the gene area of the 21st chromosome is associated with the coding of APP (amyloid precursor protein), a large interferon that transmits glucoprotein. This glucoprotein of APP is supposed to lead to dementia as it produces APP fractals when it breaks [49]. Since people with DS have trisomy of these genes, it is almost certain that increased production of APP is associated with fast development of neuropathological problems in this population [9]. Furthermore, more genes that belong in the 21st chromosome are more likely to play an important role in the development of neuropathological issues [29,45,47,57]. Many researchers believed that it was almost certain that adults with DS would present dementia at some later stage of their life [61]. Yet, in adults that ultimately present AD it is still not easy to detect symptoms before the age of 50 years, making researchers to believe that there is a latent precursor period of time that extent for 25 to 30 years.

Neuropsychological and behavioural functioning in adults with DS

Longitudinal studies through middle adulthood have confirmed the ongoing cognitive decline with increasing age, which is also detectable with the use of standardized tests for intellectual or neuropsychological functioning [23]. Even in the absence of clinical sings of dementia, which, as

discussed earlier, are mostly associated with AD, neurodegenerative alterations (e.g., volume loss in frontal, temporal, parietal lobes and reduced network connectivity among brain regions) take place leading to neuropsychological deficits in several cognitive domains [1,6]. One significant area of difficulties is the linguistic domain, where the increase of age puts additional demands to the diminished linguistic abilities (phonological processing, morphosyntactic abilities, articulation) resulting in lack of speech fluency with hesitation and pauses, difficulties in speech organization and word discrimination [7]. Other neuropsychological domains in DS affected by age-related changes involve the executive function (both lower EF, such as attention, inhibition, and higher EF such as working memory, planning/organization, self-monitoring, flexibility), visuo-spatial abilities, learning, long-term memory and behavioural functioning [23], which will be discussed below.

Concerning the executive functioning, adults with DS demonstrate a more consistent pattern of impairments in comparison to that which was observed in previous developmental stages (i.e., during childhood where some EF aspect have been assumed to be preserved) [32]. Lower level executive dysfunction is related with impairments in many attentional subfunctions (sustained and selective attention for both visual and auditory stimuli), which during adulthood affect the ability of prioritizing and staying engaged with a certain task which affects their everyday living. Difficulties in inhibitory control and processing speed also continue through adulthood. With respect to higher EF, verbal/auditory working memory has been consistently reported to be less developed than visuo-spatial short-term memory in persons with DS. The latter has served as an explanation for the continuous difficulties they experience with language acquisition, characterized as atypical, in comparison to mental age-matched individuals. This pattern seems to persist across their entire lifespan [23]. However, the advantage of the relatively preserved visuo-spatial short-term memory has shown to be compromised when the visuo-spatial task load increases. Additional EF impairments in adults with DS include difficulties with planning a solution for a problem or applying a newly introduced strategy, which is reflected by a delay in execution actions. Flexibility, which refers to the switching among different mental tasks, as well as the simultaneous processing are also points of weaknesses in DS [23]. Moreover, adults with DS display poor self-monitoring in verbal comprehension tasks. They fail to identify the point in a conversation where they misunderstood or missed some information and they do not display the ability to ask for clarification. Besides that, they are also vulnerable to disruption form irrelevant information when performing a task, as they experience difficulties in controlling or filtering interfering verbal material [30]. Although visuo-spatial abilities (e.g., visual processing, spatial orientation, visuo-construction) have been considered to be preserved in relation to verbal abilities, current research suggests the existence of a trend within the visuo-spatial domain which is considered to account for some of the observed difficulties [11]. More specifically, individuals with DS tend to approach presented stimuli in a more global way, thus, failing to perceive and integrate details embodied within the information. The ability of learning new skills is relatively preserved in DS. However, they display an advantage in learning through observation (by observing someone performing an action) rather than instrumental learning (in which they are forced to make the appropriate environmental changes to meet their goal). Personality characteristics such as responsiveness to positive reinforcement and high social motivation contribute to learning through social observation [50]. Finally, deficits in long-term memory are

also observed in adults with DS both in verbal and nonverbal memory tasks. Memory performance is affected by impairments in encoding, retrieval and memory consolidation. The etiological underpinning of memory dysfunction in adults is related to the neurodegenerative processes affecting temporal lobes and hippocampal function [31].

Regarding the behavioural domain, people with DS are often described as friendly, cheerful and socially motivated, even though sometimes exhibiting stubbornness and poor task persistence. When compared with other types of intellectual disability, that are secondary to genetic disorders, individuals with DS present less maladaptive behaviours, but increased rates of depression. The level of social motivation is reduced significantly in older adults with DS. They smile less and more briefly than they used to [36]. Other psychiatric problems such as hyperactivity, obsessive-compulsive disorder, sleep disturbances, auditory hallucinations, uncooperativeness and self-injuries emerging with aging have been associated with the development of dementia, especially the early-onset Alzheimer type [20]. A 75% of individuals with DS that is above the age 60 suffer from dementia, with the diagnosis being established around 55 years. In spite of the neuropathological changes, that are commonly considered indicative of AD, some persons with DS still do not meet criteria for dementia. Early neuropsychological signs, even prior to 2 years to a dementia diagnosis, include selective attention impairments, executive dysfunction and episodic memory deficits [4]. Finally, neuropsychological profiles of persons with AD with DS and those from the general population have shown a high level of similarity even after being controlled for gender and functional impairment [18]. Besides the cognitive deterioration, it is very important that older adults with DS, whether they meet a diagnosis for dementia or display prodromal psychiatric and neuropsychological signs, support and pharmacological treatment is of outmost importance for the DS.

Pharmacological treatment in DS

There is a very limited evidence base concerning psychopharmacological treatments of intellectual disabilities in general, including DS. The clinical polymorphism of the disorder, the limited use and applicability of diagnostic criteria otherwise used for the general population, the presence of somatic and/or psychiatric comorbidities excluding patients from research studies pose challenges in the on-label prescription of psychotropic medication [55]. In these terms, all available guidelines are mostly based on consensus and favor non-pharmacological measures, especially assessment of the environment and any related changes or psychological interventions. Medication is thus considered a second-line treatment, applied where there is a plausible indication of a possible improvement of the target symptom through its use.

In general, medication is prescribed in DS for mental illnesses, such as depression, anxiety, sleep and psychotic disorders, for the management of behavioral symptoms (both in long-term functioning and short-term aggressive behavior), as well as antidementia drugs, both for the cognitive and the behavioral/psychological symptoms of dementia [3]. Before the prescription of medication, a thorough evaluation of the medical history and current health status, current medication, previous pharmacological interventions as well as a functional analysis of behavior (e.g., sleep diary, evaluation of aggressive episodes) has to take place [8]. Moreover, the capacity of consent to the instauration of medication has to be assessed, in addition to

determining the treatment plan with the patient and/or his environment. Since most of medication prescribed is off-label, this last point and the thorough documentation of possible side-effects are crucial. Although there is inconclusive evidence of patients with DS being more sensitive to psychotropic medication due to altered pharmacokinetics [24], the frequent concurrent treatment with anti-epileptics and the presence of somatic disorders and oppositional behavior poses a challenge to the prescription and administration of the medication [55]. In these terms, the principle of "starting low and going slow" is applicable, using lower dosages than in the general population and avoiding polypharmacy. After a longer stable period (minimum of 6 months), a progressive withdrawal of the medication should be attempted [8].

Affective disorders in patients with DS can be not only expressed through the "normal" emotional symptoms, but also through regression, social withdrawal, avoidance behavior, irritability and self-injury, making the diagnosis of a depression a complex task. Selective serotonins reuptake inhibitors (SSRIs) have a better tolerated profile, especially when anti-epileptics are simultaneously used [27]. However, the evidence based is limited and the response rate is rather low. An increase in aggressive and disorganized behavior has been also observed through the use of antidepressants, more commonly with tricyclic agents (TCA) [8]. The combination treatment strategy of antidepressants and antipsychotics has a very limited evidence base and therefore should be implemented only on an individual basis. Mood stabilizers and lithium have been used for the treatment of aggression, either self-injury or violent behavior toward others, with an effect size superior to placebo in the case of lithium in RCTs [27]. These positive effects have been shown with sodium valproate or carbamazepine only on open studies, despite the common use of these pharmacological agents.

Antipsychotics are not only used for psychotic disorders, but for a number of problem behaviors such as aggression, stereotypical behaviors and self-injury [3]. Although risperidone is the only medication licensed for the use in aggressive behavior for a short period of time for patients with dementia and autism spectrum disorders [55], treatments such as haloperidol, aripiprazole, zuclopenthixol and olanzapine have been studied, with positive results. However, the evidence base is poor, the adverse effects are non-negligible, as well as the risk of cardiovascular events is elevated. Moreover, a recent study showed that the careful tapering of antipsychotics can lead to an improvement to behavioral problems [27]. A careful assessment targeting specific symptoms with side-effect monitoring is therefore required.

Benzodiazepines have important adverse effects and may actually worsen the behavioral symptoms, especially when used in contexts other than the rapid tranquillization [8]. Other than the risk of dependence and withdrawal symptoms, there is also a significant cognitive decline in older patients, where this treatment category should be avoided.

Melatonin seems to be a well-tolerated and efficient treatment choice in sleep disorders, although most of the studies have been undertaken in pediatric populations [27].

According to therapeutic guidelines [19], despite the difficulty of evaluating and therefore staging dementia through neuropsychological testing, patients with DS should benefit from antidementia drugs. Acetylcholinesterase inhibitors, especially donepezil, have been shown to improve cognitive and global functioning, as well as slow down cognitive deterioration in long-term follow-up studies, with a small advantage over placebo [5]. Donepezil in particular seems to be well-tolerated, with the evidence base being still small. Memantine (a NMDA antagonist),

despite the efficiency in older people with dementia both in terms of cognitive decline and behavioral and psychological symptoms (BPS), did not show any significant effect in patients with DS in a RCT [5]. However, this study did not differentiate between patients with and without dementia. Interestingly, SSRIs have been reported to slow cognitive decline in a retrospective chart study [24]. In terms of the management of BPS, there have been no specific studies in patients with DS and dementia; according to the expert consensus, the same treatment plans as in patients without Down syndrome should be attempted after thorough psychological/behavioral evaluation [19].

The development of a transgenic mouse model of DS in recent years (Ts65Dn) has opened up novel research options, especially for patients with dementia [22]. The studied pharmacological agents promote neurogenesis, improve synaptic plasticity and cognitive performance in the model. However, there are limitations in the transfer of the applicability to humans: for example, despite the marked improvement in cognitive performance and reduction of neurodegeneration through administration of vitamin E in the mouse model, no significant effect was noted in the subsequent RCT in humans [5]. Through the early diagnosis by means of neuroimaging and other markers [44], the interventions against neurodegeneration can be implemented at an earlier stage, before the development of clinical symptoms of dementia [12].

Psychotropic treatment for patients with DS is a demanding area of psychiatric management for patients, clinicians and care-givers. The symptoms presented by this patient category are not uniform, the therapeutic options are limited and combined with adverse effects. In this case, more studies are needed, in order to build up a robust evidence base, enabling thus the informed prescription of individual therapeutic plans.

Conclusion

The concurrent literature on neurocognitive and behavioural functioning in adults with DS reveals a unique pattern of deficits in multiple cognitive (especially in episodic memory, executive functioning, and expressive language) and behavioural domains, which has been demonstrated to reflect the ongoing development of brain neuropathology that takes place as individuals with DS mature in age. Relative strengths are observed in nonverbal abilities and social motivation. Weaknesses are observed especially when task load in neuropsychological testing increases independently of the modality through which testing material was presented. The results of the studies mentioned in this review on the neurobehavioural and cognitive profile of adults with DS support the idea of a distinct pattern of performance as compared to intelligence-matched healthy individuals and patients suffering from other genetic syndromes (e.g., William's syndrome or Fragile X syndrome). Regarding the pharmacotherapy of DS, the diagnosis and treatment of psychiatric disorders is challenging, given the communicational limitations in DS and the frequent co-existence of physical disorders. Additionally, the relatively small body of evidence-based literature and the neglect of the assessment of care environments lead to over-prescription, with little assessment of the real benefit provided for the patients. A major concern for this patient group is thus polypharmacy, especially concerning antipsychotic medication for possible aggressive behavior. Taken all the above into consideration, we underline the importance of

structured evaluation of DS patients and the interaction with their environment, in order to optimize the therapeutic options, both pharmacological and psychological.

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Brief Review

Speech and language intervention for language impairment in patients in the FTD-ALS spectrum

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Abstract

Amyotrophic lateral sclerosis (ALS) is the most common neurodegenerative disease that belongs to the group of motor neuron diseases. Motor deficits like reduce in tongue strength, may coexist with cognitive deficits compatible with frontotemporal dementia (FTD), also known as frontotemporal lobar degeneration (FTLD). FTD is a neurodegenerative syndrome with two main clinical variants: behavioral (bvFTD) and language or Primary Progressive Aphasia (PPA). ALS and FTD have significant clinical and neuropathological overlapping so that for some researchers they are "the ends of the same disease spectrum". A key intervention in this patient population is the speech language therapy (SLT), a specific form of cognitive intervention, which evaluates communication skills and designs a personalized intervention plan to improve communication abilities. It has been used in patients with aphasia of different etiologies and has been shown to be effective. There is limited research in SLT interventions in patients in FTD-ALS spectrum, and the initial findings indicate success to some extent. Due to progressive neurodegeneration in FTD-ALS spectrum, the main goal of the intervention is not the complete rehabilitation of linguistic deficits but the reduction and, if possible, the delay of language decline in order to improve patient's communication and the quality of his/her life. In this paper, we critically review the reported approaches of speech language therapy (SLT) for monitoring language impairments and the impact of interventions in patients with FTD-ALS spectrum. Initial findings are supporting more systematic treatment of speech and language impairment in patients in the FTD-ALS spectrum

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common neurodegenerative disease that belongs to the group of motor neuron diseases. The disease onset age is usually 55 to 65 years. In ALS

both upper and lower motor neurons are equally affected, disrupting voluntary mobility, respiratory function, speech and swallowing. The variable mixture of upper motor neuron (UMN) and lower motor neuron (LMN) signs contribute to the clinical heterogeneity of ALS [1]. Like most neurodegenerative diseases, it begins focally and spreads. ALS is classified as 'spinal' if the initial symptoms affects the limbs or 'bulbar' if speaking, swallowing or coughing are affected. UMNs impairment leads primarily to spasticity whereas paresis of LMNs, leads to flaccidity. Despite of the subtype, ALS progresses and eventually affects all skeletal muscles [2]. Speech and swallowing problems are the initial symptoms in 20% of cases which present with a bulbar onset [3]. Depending on the location of primary pathology, patients may develop different symptoms like tongue atrophy which leads to speech and swallowing disorders (bulbar ALS, affecting brainstem motor neurons that innervate muscles of tongue movement, chewing, swallowing and articulation) [1,3,4]. The prevalence is approximately 4–6 cases per 100,000 people [3]. While onset is commonly in mid-adulthood, ALS may begin earlier or later in life [4]. Paralysis of respiratory muscles results in respiratory dysfunction which is the cause of death. Although some forms demonstrate protracted survival, death occurs 3-5 years after diagnosis [4]. Studies have shown that cognitive and behavioral deficits are also present in ALS.

Frontotemporal dementia (FTD), also known as frontotemporal lobar degeneration (FTLD), is a heterogeneous disorder with distinct clinical phenotypes [5,6]. FTD is a neurodegenerative syndrome characterized by progressive loss of nerve cells in the frontal and temporal lobes [7,8]. In a meta-analysis of 73 studies with early dementia cases under the age of 65, FTD was the second most common type of dementia in the majority of studies with a prevalence ranging from 3% to 26% [9]. FTD has a reported prevalence of 10-15 per 100,000 population in individuals aged 45-65 years, and is a common reported cause of younger onset dementia, although with large variability across studies [10].

FTD has three main subtypes [7-9,11-13]: i) behavioral variant (bvFTD), with predominant behavioral and personality changes and ii)progressive non-fluent aphasia(PNFA), which mainly affects speech production and iii) semantic dementia(SD), which is connected with impaired word comprehension and semantic memory[8]. PNFA and SD can be included in the more general term "primary progressive aphasia" (PPA) [14]. Another subtype of PPA has recently been described, called logopenic progressive aphasia (LPA) or the logopenic/phonological variant of PPA, which predominately associates with Alzheimer's dementia (AD), characterized by impaired lexical retrieval and sentence repetition [15,16].

The association between FTD and ALS, has been recognised since the first half of the 20th century [17, 18]. FTD and ALS are two neurodegenerative conditions which share pathological, genetic and clinical features [4,14,17,19-23]. In the syndrome of FTD with motor neurone disease, behavioural or language dysfunction may evolve in tandem with ALS [24] and recent reports suggest that the two disorders often develop together in the same patient [17]. Although around 10–15% of patients with FTD develop ALS (FTD-ALS) there is an even higher prevalence of 'subclinical' evidence of ALS, with electromyographic evidence of ALS or subtle ALS-like clinical signs, such as fasciculations, in 60% of FTD patients (FTD-ALS). Conversely, while 10–20% of ALS patients meet diagnostic criteria for FTD, at least 50% of patients presenting with ALS develop cognitive or behavioral impairment, termed ALSci and ALSbi [9,10,16]. A concurrent onset of bvFTD and PPA may predict later development of ALS [11]. In addition,

findings from a research [17], confirm ALS in many patients with FTD. This overlap of cognitive, behavioral and motor symptoms and early disease onset can be very challenging in making the correct dementia diagnosis [14]. The overlap between ALS and FTD, worsens the prognosis with mean survival of 2 to 3 years from the onset of symptoms [6]. The management of symptoms in the FTD-ALS spectrum, requires a multidisciplinary team of experts to coordinate. This patient population may also develop motor disorders in speech such as dysarthria and apraxia, as well as swallowing disorders. In the present paper we focus on the basic cognitive and language deficits of patients in FTD-ALS. The speech and language therapist's state of the art approach to the cognitive and linguistic difficulties will be discussed, presenting the evidence supporting the described methods of evaluation and intervention.

Language-cognitive impairments in FTD-ALS spectrum

The clinical significance of language deficits in ALS has been questioned due to the difficulties in articulation resulting from dysarthria, respiratory failure and motor dysfunction [25]. The deficits can be identified in all subdomains of language: syntactic comprehension, naming, semantics, morphology, grammar and pragmatics and associate with atrophy in motor and posterior frontal cortex. In particular, language disorders observed in this patient population included reduced speech production, difficulty in naming objects, echolalia, producing stereotyped expressions and using semantic paraphrases [18]. Given that, there is increased possibility during the disease progress, symptoms of PPA to be present. In other words, deficits in naming objects, have been reported in some studies of patients with ALS [20] and this indicates that there is a malfunction in the basic word-finding process. Additionally, individuals with ALS exhibit behavioral changes similar to those found in behavioral variant of FTD. Hence, it has been argued that there is an overlap between bvFTD and ALS.

Types and subtypes of FTLD

Communication disorders in patients with bvFTD are related to the blunted expression and comprehension of emotional information like prosody, affecting pragmatics of communication [10]. As the disease progresses, individuals with bvFTD may begin to develop expressive language impairments along with deficits in the initiation of verbal or gestural communication [12]. However, there are not many reports of language deficits in this type of FTD.

The term PPA refers to a progressive neurodegenerative disorder [13]. For these individuals, communication disorders lie in the weakening of language system. Difficulties with language include word-finding problems, production of paraphasias, effortful speech, grammatical and/or comprehension deficits. These are the principal causes of impaired daily living activities, like performing routine job responsibilities that require verbal communication. Moreover, early symptoms of PPA may be the presence of cluttering or stuttering and difficulties in using more specific vocabulary related to semantic category [24]. Main language impairments and the location of brain atrophy of subtypes of PPA, are present in Table 1.

The identification of the type of PPA, is performed after careful evaluation of the clinical signs coupled with neuroimaging findings [7,9,16]. Accurate diagnosis is particularly important for prognosis of the disease and crucial to its monitoring. But, the differential diagnosis is a most difficult procedure [15], especially when characteristics of logopenic progressive aphasia (LPA),

are present. LPA is histopathologically associated with Alzheimer's disease [12, 16], so it is not always included in FTD spectrum. Recently, LPA was included as another subtype (logopenic or phonological) of PPA [16], indicating that there is a link between LPA and FTLD. Diagnosis of PPA subtype, in final stages, is a clinical challenge because there is an overlap between clinical features of subtypes and ALS [24].

Specifically, SD and PNFA represent models of language dysfunction in neurodegenerative diseases of frontal and temporal lobes [13] and sometimes are referred to together as PPA [7], a broader spectrum language disorder syndrome.

SD is the most well-known clinical syndrome of PPA [15,26] and associates with anterior temporal lobe atrophy [27]. When the left temporal lobe is involved, as in left svPPA, symptoms are mainly language-based with a slow loss of semantic knowledge [5]. More specifically, there are expressive and receptive language difficulties, with individuals experiencing problems in word finding process during a conversation with others [26]. The most prominent early feature in SD is the reduction of expressive vocabulary, commonly described by patients as a "loss of memory for words" [15]. The spontaneous speech is empty and meaningless. SD is more likely to lead patients to surface dyslexia/dysgraphia [12], where the words are written as pronounced. This also means reduced writing ability with irregular or informal spelling. When the right temporal lobe is involved (right svPPA), behavioral symptoms are evident. As time progresses both temporal lobes become involved and symptoms begin to overlap [5].

PNFA is evident in 25% of patients with FTD [7,14] and associates with inferior frontal atrophy [27]. The cases of PNFA indicate a wide range spectrum of language disorders, which include anomia, phonological errors, oversimplification of grammatical structures and/or grammatical errors during speech production [11] . Phonological errors can be: substitutions, deletions, simplifications, and additions of a phoneme [15]. Problems in understanding sentences with complex syntactic constructions can occur [14]. PNFA can present with articulatory errors, effortful speech, loss of prosody, as a result of disorganization of motor planning or speech execution, that is, a sensorimotor speech disorder rather than, or alongside, an aphasia [13]. Many studies have documented "fluency" problems in PPA variants, like PNFA. A typical problem is long pauses during speech [28]. Agrammatism is another feature of PNFA typically presented with the use of short phrases, so called "telegraphic speech", because of the omission of short connecting words, dropping the verb ending, saying the words in the wrong order [7,13,14]. Mild grammatical errors are present in writing [5]. The most characteristic feature of PNFA, is apraxia of speech (AOS), defined as impaired motor speech planning resulting in articulation deficits and orofacial movements in the effort to produce the correct sounds [14] with individuals making phonological errors in speech [15,28]. Some patients can initially display AOS but eventually aphasia will be evident [12]. With disease progression, speech becomes increasingly effortful with patients often eventually becoming mute [12,15,24].

LPA is a recently described subtype [16] of PPA. Neuroimaging indicates that atrophy is centred around the angular gyrus [27]. The rate of speech in LPA is slow with difficulties in the word finding process which lead to long pauses during speech production [15]. Anomia and disturbances in repetition of words and sentences are present. Also, there are phonological errors which are evident even in writing [16]. Other deficits in attention, memory or visual-spatial ability may be evident too, and as the disease progresses, these deficits can cover language deficits

[15]. It is difficult to recognise if the underlying pathology of these patients is Alzheimer's disease (AD) or another pathology [16]. Patients with severe LPA (approximately 70% of patients with AD) are hardly separated from patients with PNFA (predominately patients with FTD) [11,16].

According to the above mentioned research findings we can hypothesized that ALS, FTD with PNFA and bvFTD represent a clinical and pathophysiological continuum in regards to the extent of motor versus cognitive disorder [28,29].

Cognitive-language impairments in FTD-ALS spectrum

Cognitive, language and behavioural changes which occur in ALS, in combination with its correlation with FTD, led to the concept that these two disorders are part of a spectrum [22,29]. Cognitive/behavioural deficits are reported to occur in 50-75% of ALS cases and may develop in parallel with motor deficits although either can occur initially in the same patient [21], while approximately 15-25% of patients meet criteria for FTD [27]. In addition, the majority of the researchers have argued that there is a continuum between FTD and ALS while genetic findings [22,31] show a link between the two disorders, which supports the concept of spectrum [11].

Symptoms of motor neuron dysfunctions are likely to appear in patients with FTD, although only a few studies have systematically investigated motor neuron dysfunctions in FTD subtypes and estimated their incidence, distribution, severity and functional significance [2,31].

The reduction of language ability, is an important feature of FTD-ALS spectrum [29]. In particular, studies have been carried out where there was deficit in naming ability, syntax [21], and grammar in patients with FTD-ALS, indicating a continuum between them [25]. Most cases of PPA-ALS have PNFA symptoms [7], and more rarely ALS symptoms appear in patients with SD [15] or LPA [16].

As FTD progresses, it looks like patients develop ALS more frequently than other upper or lower motor neuron syndrome or neurodegenerative disease [5,14]. The variants of FTD, are equally likely to be associated with the clinical features of the ALS and vice versa [6,14]. Furthermore, it seems that patients with bulbar-onset ALS may be at increased risk of developing dementia compared to spinal-onset patients [6,23] and the incidence of FTD in patients with bulbar-onset ALS has been reported as high as 48% [17]. This association has practical importance. ALS coexisting with FTD significantly impacts the survival of these patients [17]. It is considered that the less advantageous prognosis concerns the case of overlapping between FTD and ALS, leading to death within 3-5 years of onset of symptoms [7], [24]. In contrast, the SD and PNFA have longer survival time from symptom onset about 11 to 12 years and 9 years, respectively [7].

Speech and language approach in FTD-ALS spectrum

For ALS patients with FTD, the impairments will progress over time, leading to greater impairment in life functioning. For this reason, interventions are planned regarding the patient needs depending upon the stage of the disease process and the severity of the impairments [32]. Interventions generally fall into two categories, compensatory or restorative. The aim of all interventions is the patient's participation in daily life activities with the lowest level of assistance as possible within the least restrictive environment [32].

PPA is a very disabling disorder. At present, there is no available treatment [33]. An

increasing area of interest in the field has been the development of behavioral therapies aimed to ameliorate language deficits [13,33]. To date, there are very few studies of low-level evidence investigating behavioural intervention for the speech and language impairments in PPA. All have examined appropriately applied treatments previously designed to target specific impairments observed in stroke-related aphasia, such as word-finding difficulty or sentence construction and discourse problems. These preliminary studies are providing promising directions for larger scale trials, especially in the period of the disease when language symptoms predominate and cognition is relatively intact [13].

SLT has been shown to be effective in patients with aphasia of various etiologies, where it has been extensively used. It aims to maximize the subject's communicative abilities. A recent meta-analysis identified 30 controlled trials of SLT, performed over 40 years, showing beneficial effects in a variety of language measures, like aphasia severity [33]. Functional neuroimaging studies have shown neural reorganization following SLT, confirming clinical results. The main goal of SLT intervention, is to develop an individualized plan for the patient with FTD (depending on his/her social environment and the stage of the disease), maximize his/her communication skills, but not to regain lost language ability [32]. If therapy is maintained throughout the course of disease and realistic goals are set, that are patient-centered and include caregivers, SLP services will be useful for individuals with FTD.

The development of a telephone-based screening battery to detect frontotemporal changes in ALS, helps patients with severe physical disabilities. Telephone-based tests, are cognitive tests for neurodegenerative diseases, which have shown to successfully relinquish some of these associated burdens and costs and may increase generalizability of results by reducing selectivity and increasing sample sizes. Unfortunately, the majority of these screening tools assess age-related, Alzheimer's-type memory impairment and are not completely practicable to the assessment of FTD for ALS patients [34].

The use of a structured, semi-quantitative instrument that allows the clinician to rate the relative severity of impairment in each language domain based on language assessment and patient and partner interview, may provide a more complete clinical picture, rather than solely using information from test performance, in PPA research programs and clinical care [35]. The clinician rates impairment on a five-point scale from "normal" (0), to "questionable/very mild" (0.5), "mild" (1.0), "moderate" (2.0), or "severe impairment" (3.0). The Progressive Aphasia Severity Scale (PASS) profile shows the rate of change of individual language skills relative to each other, which may be useful for monitoring of impairments.

Analysis of speech and pausing is useful in describing the performance of individuals with various neurodegenerative diseases. Typically, it is obtained during speech/ language/ cognitive assessments. Speaking measures such as articulatory rate have been used in ALS for tracking the progression of bulbar signs whereas pausing measures have been suggested as possible diagnostic markers of cognitive changes in dementia, including subtypes of PPA [28]. A recent study examined the usefulness of performing a simple reading test with an algorithmic method of assessing speech versus pause behaviors across the FTD-ALS spectrum. The articulatory rate indicated motor speech abnormalities in the FTD-PNFA group and bulbar ALS and was found to be a predictive measure of motor speech deficit. Pause measures, were also impaired for FTD-PNFA group. This finding was consistent with reports of the motor speech disorder of AOS, which

is currently a core diagnostic feature of FTD-PNFA. However, recent studies, suggested that decline in articulatory rate might be associated with the presence of cognitive impairment [28]. Other screening tools for assessment of language subdomains in PPA [22], are present in Table 2. Finally, it is clear that motor speech assessment should be performed in patients with FTD to identify speech motor abnormalities.

A recent study [36] examined the capacity of the Greek version of two self-report instruments to detect Subjective Cognitive Impairment (SCI) in older adults. However, the assessment of subjective estimations is a useful indicator of early cognitive deficits in AD.

There is currently no cure for PPA, however, initial research suggests SLT may enhance quality of life. Despite positive research findings, individuals with aphasia because of dementia are under-referred for SLT services. This may be in part because of a lack of evidencePP-based data and formal training of therapists for providing SLT to individuals with dementia [37]. The SLT intervention focuses on three primary areas: i) impairment-based approaches based on a home exercise program ii) activity and/or participation strategies to accommodate communication in daily life and iii) disease education, counseling, and caregiver training [37]. All of these components are substantial for providing clinical care for patients with PPA.

A recent study [33] suggests that PPA patients subjected to SLT when compared to a control group that had not been subjected to SLT, showed, a less severe reduction of language, namely concerning naming abilities. This study also suggests that the implementation of language-based intervention in PPA might attenuate the progression of some language impairments, and should prompt further studies using randomized, controlled, rater-blind procedures to ascertain the effectiveness of SLT in PPA.

Currently, two Alternative and Augmentative Communication systems, the eyetracking (ET) and brain–computer interface (BCI), have been tentatively used with the aim of management of neuropsychological tests, in ALS patients [38].

A positive effect of language rehabilitation on picture naming has been previously reported. Most results were obtained in single-subject experimental research and case reports. Louis et al. [33] found that after intensive training on phonological skills in three PPA patients, over a 42-days training period, some language functions (fluency, written comprehension, repetition, reading, and reduction of phonemic paraphasias) either remained stable or improved, in spite of global decline of language abilities.

A recent study reported on an intervention aiming at improving the patients' ability to communicate by verbal means with others in everyday life, through a stimulation approach [33]. Improvement in comprehension and expression of both spoken and written language was targeted through different exercises. The intervention included picture naming, description of picture actions, complex auditory-verbal comprehension, reading and writing, facilitation of expression of feelings and opinions, and enhancement of conversational skills. This study of 10 FTD patients (2 PNFA, 2 LPA, 6 SD) showed that patients subjected to SLT for 12 months had significantly less language decline (concerning naming abilities) compared with a control group that did not undergo SLT.

In another study patients used the personalized Communication Bridge Web application [37]. This study indicates that, Internet-based SLT provides an attainable method for improving access to care for individuals with mild and/or moderate aphasia symptoms, who have prior

familiarity with a computer, and an engaged caregiver. This study used person-centered, impairment directed and activity- and/or participation-based interventions and disease education. SLT intervention may contribute to prolonging the independence of individual and decrease caregiver burden. For patients with AD, information and communication technologies (ICT) have been developed through web-based applications [39].

Much attention has focused on whether people with SD can relearn lost vocabulary with some positive results in experimental settings [26]. Recent work has focused on word retraining in SD, through learning word-picture pairings, over multiple sessions. Encouragingly, there are positive results for retraining in SD. Moreover, these patients are able to demonstrate this knowledge when tested using different visual stimuli, indicating generalization in some extent. This technique is relatively new, however it suggests that preserved cognitive skills may offer some improvement at a functional level for SD patients [15]. More recent studies have adapted a similar technique in LPA, and the reported results are positive. Interestingly, studies which have combined behavioral interventions with functional MRI have demonstrated increased functional activity in brain regions which are not atrophic indicating that such interventions work by recruiting non-affected brain regions to compensate for impaired function.

Few studies explicitly targeted everyday skills in SD [26]. Studying conversation provides an opportunity to explore the changing needs of people with dementia in conversation, and the way in which others can adapt to these changes. Interventions that are empirically grounded in the experiences of people with dementia and their family members, and also have a clear theoretical framework and that can be tailored to individual needs, are valuable [40].

There are two published studies examining everyday conversation. One study described how a man with SD, had a repeated practice of acting out scenes within conversation, changes in vocal characteristics, pitch and loudness, facial expression and body posture. This enabled him to participate in conversation and the listener to gain at least some meaning from him, despite his language difficulties. The second study, presented a new or expanded communicative behavior, often spontaneously acquired and systematically employed, to overcome a communication barrier in order to satisfy both transactional and interactional goals of communication [40].

In individuals with PNFA speech therapy interventions may also be of assistance. For example, one case study [15] demonstrated that reading out loud reduced the number of errors during speech production in an individual with PNFA whose primary symptom was apraxia of speech. Encouragingly, there was maintenance one year later and word and sentence repetition appeared also to improve, suggesting a degree of generalization. Group studies, however, are scant.

A patient with LPA was reported to be submitted to an intensive 2-weeks semantically based intervention, which was successful at producing lasting and generalizable effects of improved lexical retrieval over a 6-months period [32]. Another study followed 2 individuals with progressive language impairment and a stroke aphasia patient in a daily 90-min semantically based intensive treatment and showed to improve lexical retrieval at two weeks [33]. Results were positive for all patients, who showed improved lexical retrieval on a generative naming task for specific categories trained during intervention. The same authors, reported also positive results for an LPA patient who performed follow-up assessment at 3 weeks, 4 and 6 months after intervention. The improvement that this patient also has, in naming on the training task,

generalized towards an improvement in standardized measures of confrontation naming.

There are reports that suggest SLT can benefit a patient with FTD and ALS, especially if intervention is maintained during the progression of disease and realistic goals are set. All evidence-based SLT interventions, discussed above, are presented in Table 3. Also, the participation of carergivers is also beneficial. Most importantly a patient in the FTD-ALS spectrum, particularly in the final stages, is at high risk of aspiration and choking when receiving food. The SLP intervening for speech training should educate caregivers to be alert in order to help patients through compensatory techniques who are equally trained by SLPs. It is considered that the participation of caregivers, in this situation, can greatly benefit the patients [15].

Conclusion

Neurodegenerative diseases are behind most dementias [8]. Patients in FTD-ALS spectrum lose the control of themselves as a result of cognitive, language, behavioural and motor impairments, that they display. Self-service is impossible for patients with FTD-ALS. They are completely dependent on their carergivers, while communication with them is becoming more and more difficult and this impacts their quality of life.

The main goal of interventions for patients in FTD-ALS spectrum in every multidisciplinary team, is the improvement of quality of life and the maintenance of safety. Also, some interventions may reduce carer burden and improve carer quality of life [15]. Especially, SLT, can contribute to evaluation and intervention in language deficits which are evident in a FTD-ALS patient. However, the generalization of research results for SLT interventions can be difficult, even impossible for good reasons. Systematic reviews confirm the positive outcomes of speech and language interventions, but most researchers find it difficult to generalise conclusions due to the small numbers of studied patients [41].

The neurodegenerative nature of the disease, heterogeneity of clinical symptoms, and variable rate of disease progression are many of the challenges associated with planning interventions in PPA. Hence, the majority of intervention studies have been case reports or small cohorts providing evidence of concept that a particular therapeutic approach may be useful [32]. Furthermore, there is a misconception that SLT services are not appropriate for patients with neurodegenerative syndromes because of their progressive course and the heterogeneity of language and other cognitive symptoms among individuals. Thus, evidence-based research on the effectiveness of SLT intervention in dementia has been limited to small cohorts and case studies [37]. To complicate things even more, dementia is difficult to study in patients with ALS due to motor problems which interfere with cognitive testing while ALS is difficult to assess in FTD due to limited cooperation of patients for motor studies [17].

Unfortunately, there are no known disease-modifying interventions and very limited medical interventions that have been found to assist in the management of the symptoms. Neurorehabilitation specialists and community-based activities offer individuals with bvFTD/PPA interventions and services to ameliorate life functioning and life satisfaction. Participation in educational conferences and support groups provides an opportunity both for caregivers and patients to meet others facing similar family and life situations so that they can share their

thoughts, learn from each other and feel less isolated [32].

We can conclude that there is mostly a theoretical approach from speech and language therapy which has been applied in some patients, with significant benefits for both patients and caregivers, however, due to the limited number of studies and samples, generalization can be difficult. Future research should focus on evaluating the efficacy of different types of speech and language intervention in large samples of FTD-ALS, so that generalization of results can be achieved. Full rehabilitation is not considered a realistic goal due to the nature of the disease. Even the reduction in the rate of linguistic and cognitive weakening, is considered a success. In some cases of FTD-ALS, a speech and language therapist can intervene by educating both the patient and the caregivers on the use of alternative augmentative communication devices (AAC).

PPA subtype	Language deficits	Brain atrophy
SD	Finding the right word for objects Naming the objects Word comprehension A lack of vocabulary Surface loss of literacy skills	Predominant left hemisphere cortical loss; grey matter loss predominantly in inferior temporal and fusiform gyri, temporal pole, parahippocampal cortex, entorhinal cortex; losses extend to anterior cingulate, orbitofrontal, inferior frontal, and insular cortices
PNFA	Slow and hesitant speech Apraxia of speech Grammatical errors Worsened understanding of complex sentences Finding the right word for objects Loss of literacy skills such as reading and writing	Predominant left hemisphere cortical loss; pars opercularis atrophy in the inferior frontal gyrus extending to prefrontal and temporal cortices, caudate and putamen atrophy bilaterally
LPA	Word-finding pauses lead to abnormalities of fluency that can be intermittent (disappearing when patient is allowed small talk and circumlocutions) Object naming deficits may or may not be present Relatively preserved syntax and single word comprehension	Atrophy of left temporoparietal and posterior cingulate

Table 1. Classification of PPA subtypes

Verbal expressive language	Boston Diagnostic Aphasia Examination (BDAE) (Goodglass &Kaplan 1983), Mental Deterioration Battery (Caltagirone et al. 1979)
Verbal fluency	FAS test (Benton 1967), Controlled Oral Word Association Test (Lezak 2004),Verbal Fluency Index (VFI) (Abrahams et al. 1995), Thurstone Written Word Fluency (Thurstone 1938)
Confrontation Naming	Boston Naming Test (Kaplan et al. 1983), Graded Naming Test (McKenna & Warrington 1983), National Adult Reading Test (Nelson & Willison 1991)
Semantic Processing	Pyramids and Palm-trees Test (Howard & Patterson 1992), Hodges' Semantic Battery (Hodges et al. 1991), Psycholinguistic Assessments of Language Processing in Aphasia (Kay et al. 1996), Category Specific Names Test (McKenna 1998), Peabody Picture Vocabulary (Dunn & Dunn 1997), British Picture Vocabulary Scale II (Dunn et al. 1997), Sydney Battery (Savage et al. 2013)
Auditory Comprehension	Token Test (De Renzi &Vignolo 1962), Test of Reception of Grammar(TROG) (Bishop 1982), Bilingual Aphasia Test (Paradis &Canzanella 1990)
Action Verb Processing VS Object Noun Processing	Kissing and Dancing Test (Bak& Hodges 2003), Italian Battery for the Assessment of Aphasic Disorders (Miceli et al. 1994)
Reading & Writing	Graded Difficulty Spelling Test (Baxter & Warrington 1994), Spot the Word Test (Baddeley et al. 1993)

Table 2. Screening Tools for the assessment of language subdomains in FTD-ALS

Intervention	Goal	Exercises
individualized multimodality stimulation approach (Farrajota et al, 2012)	comprehension and expression	picture naming, description of picture actions, complex auditory-verbal comprehension, reading and writing, facilitation of expression of feelings and opinions, enhancement of conversational skills
behavioral intervention (Henry et al, 2013) [42]	reduction of speech production errors	reading
person-centered telepractice web-based (Rogalski et al, 2016)	Maximize impact on the participant's quality of life by teaching strategies that can be implemented in everyday life situations	lexical retrieval for targeted words compensatory strategies and care-partner training on appropriate cueing techniques
cognitive intervention (Kumfor et al. 2016)	Word retraining	word-picture pairings
everyday conversation(person-centered approach) (Kindell et al. 2013, Simmons-Mackie & Damico 1997) [43, 44]	communication difficulties in everyday life	natural Conversation Conversation about past experiences
behavioural intervention (Intensive semantically based) (Beeson et al. 2011)[45]	Lexical retrieval	generative naming tasks by semantic category (demanding task)

Table 3. Evidence-based SLT interventions

The authors declare that they have no conflicts of interest.

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Short Communication

Robust regional cerebral blood flow perfusion deficits in relapsing-remitting multiple sclerosis patients with executive function impairment

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Abstract

Objectives: Cognitive impairment is present in up to 65% of Relapsing Remitting Multiple sclerosis (RRMS) patients and can be extremely debilitating. Although deficits in episodic memory and processing speed occur more frequently than executive deficits, executive dysfunction tends to have a significant impact on MS patients' ability to generate strategies, think divergently, solve and estimate problems, and reason in abstract terms with substantial negative impacts on activities of daily living. In the present study we investigated perfusion detection rate and pattern, as well as the association between perfusion rates and cognitive dysfunction in cognitively impaired RRMS patients. **Methods**: We present findings from 17 cognitively impaired RRMS patients who were evaluated with a comprehensive neuropsychological battery and additionally evaluated by brain perfusion radiopharmaceutical technetium-99m hexamethyl-propylene amine oxime (Tc-99m HMPAO). **Results**: RRMS patients had hypoperfusion in several predefined Brodmann areas and lobes of the brain, relatively to demographically matched healthy controls according to an established normative database NeuroGam™. However, we noted blood flow reduction, mainly in the frontal lobes and other related

prefrontal areas, involving both hemispheres, but with asymmetric left hemisphere predominance. Moreover, associations between measures of response inhibition, set shifting (executive functions) and severity of hypoperfusion in the left frontal lobes were also established. **Conclusions**: Cerebral hypoperfusion is an integral feature of MS pathology. Executive dysfunction is associated with robust cerebral perfusion deficits in the frontal and prefrontal cortex of cognitively impaired RRMS patients.

Introduction

It is now commonly accepted that 50 -70 % of individuals with Multiple Sclerosis (MS) will experience cognitive deficits over the course of the disease [1] with a direct impact on daily competence and health related quality of life [2]. Cognitive dysfunction of a mild to moderate severity can occur early in the disease course, even in the absence of major physical disability [3]. The heterogeneous nature of MS generally precludes manifestation of a typical cognitive impairment pattern, and cognitive dysfunction is characterized by a varying combination of domain-specific deficits rather than a uniform general cognitive decline [4]. Although deficits in episodic memory and processing speed occur more frequently than executive deficits, executive dysfunction tends to have a significant impact on MS patients' ability to generate strategies, think divergently, solve and estimate problems, and reason in abstract terms with substantial negative impacts on activities of daily living [5].

Cerebral perfusion which is usually quantified as cerebral blood flow (CBF) represents the blood volume passing through a given volume of brain parenchyma per time unit [6]. The utilization of brain perfusion SPECT in MS patients is scarce and the literature investigating the association between cognitive functions and perfusion deficits is indefinite requiring further investigation. In one of the first relative studies to be published [7], the authors noted predominantly frontal and greater left than right temporo-parietal perfusion deficits in their MS patients compared to healthy controls. Another early study demonstrated that MS patients with a progressive disease course had significantly reduced cerebral blood flow in the frontal gray matter, compared to RRMS patients and healthy controls [8]. A more recent study that recruited secondary progressive MS patients [9] reported that SPECT was able to highly detect decreased cerebral perfusion in their sample. Although as noted previously, very few perfusion studies have been published so far in patients with multiple sclerosis, findings of very early focal perfusion changes during the inflammatory phase of the disease [10], and of generally reduced grey matter perfusion in MS patients compared to healthy controls [11] might indeed influence our understanding of the underlying disease processes, and could be important for future therapeutic considerations.

Results from the previously mentioned studies suggest that cerebral hypoperfusion is an integral part of MS pathology, irrespective of the clinical subtype. In this study we investigated perfusion detection rate and pattern, as well as the association between perfusion rates and cognitive dysfunction in cognitively impaired RRMS patients. We hypothesized that due to the heterogeneous demyelinating and neurodegenerative nature of the MS disease process, variable hypoperfusion deficit rates will be evident in each of the four cerebral lobes and predefined Brodmann areas that will be evaluated.

Furthermore, moderate to large correlations between performance on neuropsychological measures and rCBF perfusion rates in the respective cerebral regions associated with the cognitive task were expected.

In the present study, we present findings from the pretreatment phase of a cognitively impaired relapsing remitting multiple sclerosis (RRMS) patient cohort that was allocated to receive computer-assisted functional cognitive rehabilitation.

Methods

Participants

Seventeen RRMS patients from a larger cohort of RRMS individuals that were eligible to take part in an ongoing cognitive rehabilitation interventional trial [12], signed an informed consent to additionally undergo perfusion brain SPECT scanning at the baseline assessment phase. These patients were assessed with a comprehensive battery of neuropsychological tests and provided consent to be additionally evaluated by single-photon emission computed tomography (SPECT) with the brain perfusion radiopharmaceutical technetium-99m hexamethyl-propylene amine oxime (Tc-99m HMPAO). This process was conducted in the nuclear medicine department of the University hospital of Patras. Demographic and clinical characteristics of the 17 RRMS enrolled patients are provided in Table 1.

Variable	Mean	SD	Range
Age (years)	47.71	8.97	26 - 60
Education (years)	11.76	3.09	6 - 18
Gender			
Males	5		
Females	12		
EDSS - median	3.00	-	1.5-5.0
Disease duration (years)	14.00	7.01	3 - 28
MMSE	26.94	1.39	25 - 29
WASI IQ	102.80	8.10	
Fatigue (FSS)	4.10	1.55	
Depression (BDI-FS)	5.00	2.66	2-9

Table1. *Demographic and clinical characteristics of participants*

All patients met the revised McDonald criteria for the diagnosis of MS [13]. Additional study inclusion criteria were: (i) patient age between 21 and 60; (ii) educational level of at least 6 years (primary school graduates (iii) RRMS; (iv) expanded disability status scale (EDSS) score of between 0-5; (v) cognitive deficit on at least one domain of the Central Nervous System Vital

Signs neuropsychological screening battery [1]; (vi) native Greek speakers and (vii) normal or corrected hearing and vision. All patients provided written informed consent to participate in the study. Exclusion criteria were as follows: (i) ongoing major psychiatric disorders (e.g., psychotic symptoms or disorders, illegal drug or alcohol abuse); (ii) presence of another neurological disorder (e.g., dementia, stroke, epilepsy, traumatic brain injury resulting in a loss of consciousness for more than 30 minutes); (iii) Mini-Mental State Examination score ≤ 24; (iv) IQ score of ≤ 80 on the national validated Wechsler abbreviated scale of intelligence (WASI) [14]; (v) one or more exacerbations in the 3 months prior to enrollment, immunological or immunosuppressant treatment initiated within 4 months prior to enrollment, treatment with cognitive rehabilitation in the 12 months prior to enrollment; (vi) initiation of psychotropic medications or medications for spasticity, tremor, bladder disturbances and fatigue. If already taking such medications, doses and schedules had to be held constant during the study period; (vii) obese patients as determined by a clinical dietician in order to avoid compromised tissue oxygenation and reduced perfusion.

Clinical assessment

Clinical characteristics of MS patients were assessed by specialist neurologists with significant experience in the MS population. These neurologists provided the diagnosis of MS, type of disease course, disability rating (EDSS score) [15], and fatigue rating on the Greek validated Fatigue Severity Scale (FSS) [16]. They also screened patients to ensure eligibility of inclusion and exclusion criteria (except for cognitive criteria and mood). If deemed necessary patients were also assessed psychiatrically to ensure correct differential diagnosis of behavioral and mood disorders and to exclude patients with ongoing major psychiatric disorders.

Neuropsychological assessment

The procedure followed for the initial screening assessment of cognitive functions and intelligence level, comprehensive neuropsychological assessment, mood, and fatigue of the 17 RRMS patients included in the SPECT study has been described in detail elsewhere [12]. We utilized cognitive measures that assess domains normally impaired in MS individuals, independent of disease duration and disability status. The cognitive tests utilized in this study are incorporated in the Minimal Assessment of Cognitive Function in MS (MACFIMS) neuropsychological battery. MACFIMS is recommended by an expert panel for clinical monitoring and research in MS and has been validated internationally for this purpose. The sensitivity of the MACFIMS for distinguishing MS patients from healthy controls is (83%) [24].

All neuropsychological tests were administered by specialist neuropsychologists using standard procedures in single sessions. To minimize retest effects, alternative forms of the tests were used when available. Table 2 provides a summary of the utilized neuropsychological test battery arranged by cognitive domain assessed.

Cognitive functions/ domain assessed	Neuropsychological test used
Verbal memory	Selective Reminding Test (SRT) [17]
Visuospatial Memory	Brief Visuospatial Memory Test-Revised (BVMT-R) [18]
Verbal fluency	Greek Verbal Fluency Test (phonemic and semantic fluency) [19]
Attention/Processing speed	Symbol Digits Modalities Test (SDMT) [18] Trail Making Test Part A [20]
Executive functions	Response inhibition Stroop Neuropsychological Screening Test (SNST) – (colour word task) [21] Set shifting Trail Making Test Part B (TMT-B) [20]

Table 2. *Comprehensive neuropsychological battery that was administered arranged bycognitive function/domain assessed.*

rCBF SPET evaluation

The procedure that was followed for perfusion brain SPECT in this study has been described previously by our scientific group in patients with Parkinson's disease [22]. More specifically, SPECT was performed 30 minutes following intravenous injection of 740 MBq (20 mCi) Tc-99m HMPAO (Ceretec™, GE Healthcare Ltd., Buckinghamshire, United Kingdom) in a dimly lit, quiet room with the patients lying supine with eyes closed. Brain SPECT was performed with double-head gamma cameras (Millennium VG, GE Medical Systems, Milwaukee, WI, USA) equipped with low-energy, high-resolution, parallel-hole collimators in both institutions participating in the study. The head of each patient was held with fixation strips attached to a specially constructed carbon fiber head holder, which allowed the camera detector to rotate very close to the head. The data was collected into a 128×128 matrix, through 360° rotation at 3° steps for 20s per view. SPECT image reconstruction was achieved via filtered back-projection using ramp and filter Butterworth (order 10, cut-off 0.45 cycles.cm−1). Attenuation correction based on Chang's method was performed on each slice, with a uniform attenuation coefficient of 0.11.

Analysis of rCBF SPECT was performed by the NeuroGamTM software on a Xeleris 3 workstation (GE Healthcare). This involves intensity-thresholding and spatial normalization of the images to a standardized stereotactic (Talairach) 3-dimensional space. After anatomical standardization and voxel normalization to cerebellum perfusion mean values, NeuroGam compares each patient's rCBF SPECT against a database of gender- and age-matched healthy controls. A z-score map is also created, thus allowing for the objective demonstration of the extent and magnitude of rCBF changes. For the analysis of an individual study, the computer program calculates a voxel by voxel z-score using the following equation: z-score= ([control mean] [individual value]) / (control standard deviation [SD]). The z-score maps are displayed either in the standard cuts or by overlay on a 3D anatomical topographic representation by means of a specific color scale. Abnormal perfusion areas are defined as those with decreased uptake (below 2 SD of the normal mean uptake in area >50% pixels). Regions that were investigated for this study

included the right and left frontal, parietal, temporal and occipital lobes. We also investigated the predefined left and right Brodmann areas (BA), mainly in the prefrontal cortex; BA 9 (posterior lateral prefrontal cortex); BA 10 (medial prefrontal cortex); and BA 12 (orbitofrontal cortex).

Statistical analyses

We initially computed the basic descriptive statistics of the demographic, clinical and neuropsychological variables. We then computed the frequency of impaired MS patients on rCBF brain perfusion SPECT, using as criterion for impairment 2 SD below an age- and gender-adjusted normative database (NeuroGam™); and the frequency of impaired perfusion according to a hypoperfusion grading (see below). Next the normality assumption of the data was tested using the Shapiro-Wilk method. Percentage differences of impaired MS patients on rCBF brain SPECT between left and right hemispheres were investigated with the Chi square Test. Paired sample t-tests were used to compare cortical area perfusion differences between the left and right hemispheres. Cortical area perfusion differences between cognitively impaired and relatively cognitively impaired MS patients in both hemispheres were compared with the Mann Whitney U non-parametric test. We also examined correlations between rCBF brain SPECT and performance on cognitive measures using the non-parametric Kendall's tau test. A 5% level of significance was adopted

Results

rCBF SPET image analysis

The distribution of rCBF in our RRMS patients showed significant differences when compared to an age- and gender-adjusted normative database. More specifically, we found the highest impaired blood flow rates in the left posterior lateral prefrontal cortex (82.35%), right posterior lateral prefrontal cortex (64.70%), left frontal lobe (58.82%) and medial prefrontal cortex (64.70%). Smaller percentages of impaired perfusion were also noted in other predefined Brodmann areas and cerebral lobes. When we compared rCBF distribution between left and right hemispheres we found significant differences between the left and right frontal lobe, temporal lobe, posterior lateral prefrontal cortex and orbitofrontal cortex, with asymmetric left hemisphere dominance (Table 3).

In order to determine the perfusion severity level of our RRMS patients in both hemispheres of the cerebral lobes and predefined Brodmann areas, we introduced a perfusion grading system. This system graded hypoperfusion severity as follows: 0 to -1.6 SD, normal perfusion; -1.7 to -2.0 SD, borderline hypoperfusion; -2.1 to -2.5 SD, mild hypoperfusion; -2.6 to -3.0 SD, medium hypoperfusion; -3.1 to -3.5 SD, severe hypoperfusion. Based on this grading classification, (table 4) provides perfusion severity frequency of the cerebral lobes and predefined Brodmann areas respectively.

Brain Regions	(%) impaired		*Chi square test*
Lobar or Brodmann Area	Left	Right	
Frontal lobe	58.82	29.41	$x^2(1) = .160$, P<.001
Parietal lobe	29.41	23.52	ns
Temporal lobe	35.29	23.52	$x^2(1) = .255$, P<.05
Occipital lobe	23.52	17.64	ns
Brodmann Area (BA) 9	82.35	64.70	$x^2(1) = .345$, P<.001
Brodmann Area (BA)10	64.70	64.70	ns
Brodmann Area (BA)12	41.17	52.94	$x^2(1) = .398$, P<.01

***Notes**: Criterion for impairment was 2 standard deviations below age and gender adjusted normative data for rCBF SPET performance (NeuroGam software; GE Medical System,Segami Corp., Columbia, MD,USA); Brodmann Area (BA) 9:Posterior lateral prefrontal cortex; Brodmann Area (BA) 10: Medial prefrontal cortex; Brodmann Area (BA) 12: Orbitofrontal cortex; ns = non significant difference;*

***Abbreviations**: rCBF brain SPET: regional cerebral blood flow brain single photon emission computed tomography*

***Table 3.** Percentage of impaired RRMS patients on rCBF brain SPET.*

Hypoperfusion severity	BA 9 L/R		BA 10 L/R		BA 12 L/R		Frontal lobe L/R		Occipital lobe L/R		Parietal lobe L/R		Temporal lobe L/R	
Normal perfusion	11.8	23.5	29.4	29.4	52.9	35.3	23.5	29.4	52.9	82.4	52.9	64.7	52.9	70.6
Borderline hypoperfusion	5.9	11.8	5.9	17.6	5.9	23.5	23.5	41.2	23.5	11.8	17.6	11.8	23.5	5.9
Mild hypoperfusion	35.3	17.6	29.4	23.5	17.6	17.6	23.5	5.9	11.8	-	11.8	-	11.8	11.8
Medium hypoperfusion	17.6	29.4	11.8	11.8	-	-	11.8	5.9	-	-	5.9	-	-	5.9
Severe hypoperfusion	29.4	17.6	23.5	17.6	23.5	23.5	17.6	17.6	11.8	5.9	11.8	5.9	11.8	5.9

***Notes**: Criterion for impaired perfusion was 2 standard deviations below age and gender adjusted normative data for rCBF SPET performance (NeuroGam software; GE Medical System, Segami Corp., Columbia, MD, USA); Hypoperfusion grading: (0 to -1.6 SD, normal perfusion), (-1.7 to -2.0 SD, borderline hypoperfusion), (-2.1 to -2.5 SD, mild hypoperfusion), (-2.6 to - 3.0 SD, medium hypoperfusion), (-3.1 to -3.5 SD, severe hypoperfusion).*

***Abbreviations**: rCBF brain SPET: regional cerebral blood flow brain single photon emission computed tomography*

***Table 4.** Cerebral lobe and Brodmann area hypoperfusion severity rates of RRMS patients on rCBF brain SPET n (%).*

From Table 4 it is evident that severe hypoperfusion was recorded mainly in the left posterior lateral prefrontal cortex (29.4%) and left medial prefrontal cortex (23.5%) of our RRMS cohort. We also noted a medium degree of hypoperfusion in (29.4%) of our cases on the right posterior lateral prefrontal cortex. Furthermore, mild hypoperfusion was found primarily in (23.5%) of our sample

on the left frontal lobe. On the contrast the lowest rates of severe hypoperfusion were recorded on the right occipital, parietal and temporal lobes consecutively (5.9%) of our cases.

Comparison of cortical area hypoperfusion between the left and right hemispheres in the RRMS cohort

In order to determine hypoperfusion differences between the left and right hemispheres in our RRMS patients, we compared the mean perfusion rate of cortical areas that met the criterion for impaired perfusion of at least 2 standard deviations below age and gender adjusted data for rCBF performance based on the NeuroGam software. We found statistically significant perfusion differences between the left, M (SD) = -2.171 (.7951) and right, M (SD) = -1.918 (.7756) frontal lobes: [t = -4.167, 16, p <.001]; the left, M (SD) = -2.724 (.9464) and right, M (SD) = -2.353 (1.041) posterior lateral prefrontal frontal cortex: [t = -3.388, 16, p <.004]; and the left, M (SD) = -2.176 (.8363) and right, M (SD) = -1.912 (1.409) orbital frontal cortex: [t = -2.693, 16, p <.001].

Comparison of cortical area perfusion between cognitively impaired and relatively cognitively impaired RRMS patients

In order to compare cortical area perfusion between cognitively impaired and relatively cognitively impaired RRMS patients, we divided our RRMS cohort that underwent SPECT imaging into two separate groups. The first group (n=10), were cognitively impaired MS patients (CI) that failed ≥ 2 cognitive tests on the administered neuropsychological battery with performance ≤ 1.5 SD below normative data mean for the respective measure; the second group (n =7), were relatively cognitively impaired MS patients (RCI) that failed 1 cognitive test, with performance ≤ 1.5 SD below normative data mean for the respective measure. Based on this classification, we found statistically significant perfusion differences between CI and RCI RRMS patients on the left (z = -3.150, p < .001) and right frontal lobes (z = -3.290, p < .001); left (z = -3.640, p < .004) and right (z = -2.950, p < .001) posterior lateral prefrontal cortex; and left (z = -2.652, p < .001) and right (z = -2.858, p < .005) orbital frontal cortex. In these brain areas, the CI group demonstrated higher hypoperfusion compared to the RCI group and also abnormal perfusion as compared to the database controls (with the exception of the occipital lobes). Figures 1-3, clearly demonstrate these differences.

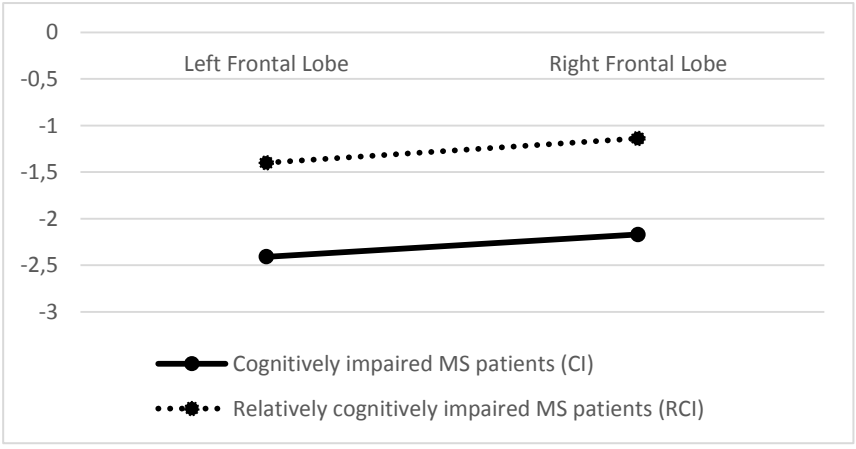

Figure 1. Comparison of cortical area perfusion between cognitively impaired and relatively cognitively impaired RRMS patients on the frontal lobes bilaterally

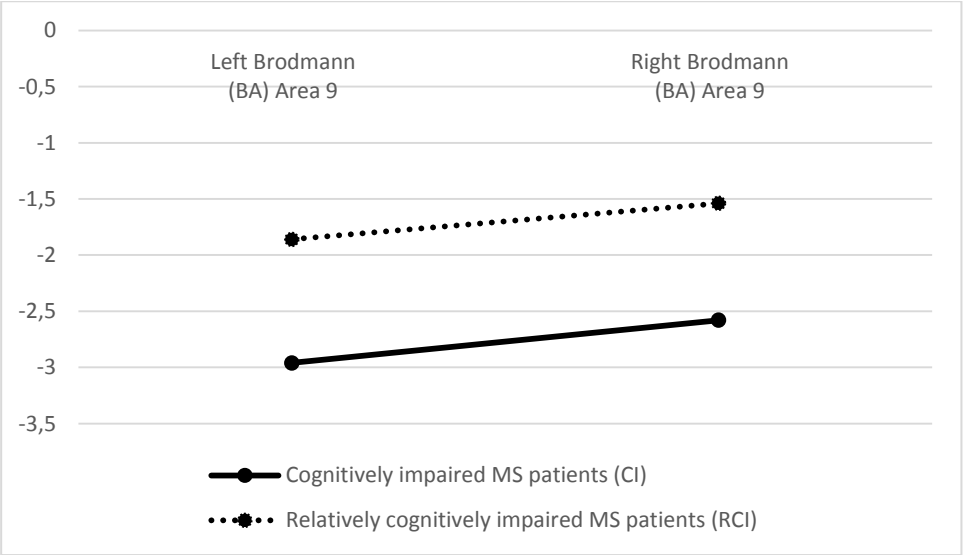

Figure 2. Comparison of cortical area perfusion between cognitively impaired and relatively cognitively impaired RRMS patients on the posterior lateral prefrontal cortex – BA (9) bilaterally.

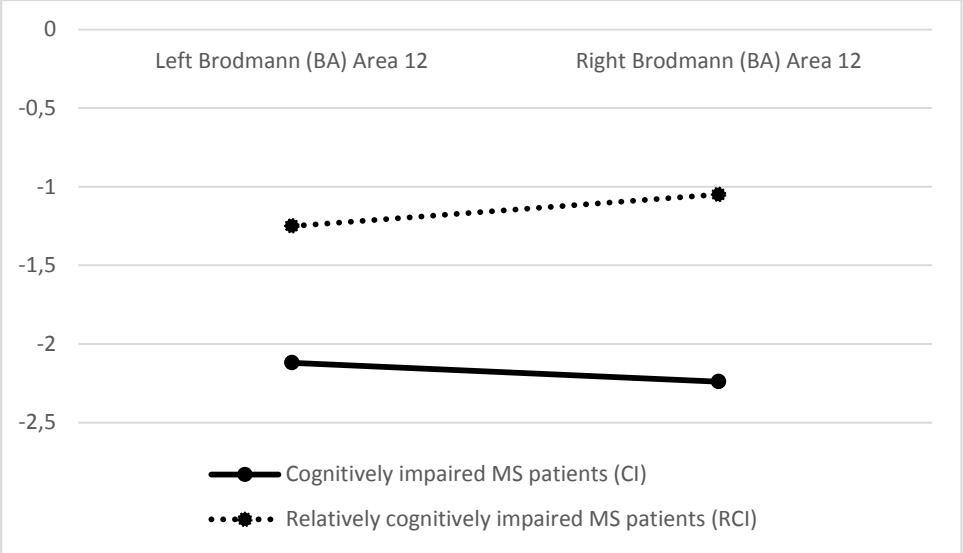

Figure 3. Comparison of cortical area perfusion between cognitively impaired and relatively cognitively impaired RRMS patients on the orbital frontal cortex – BA (12) bilaterally.

Associations between neuropsychological measures and rCBF brain SPET variables

We found statistically significant strong correlations between two measures of executive function and cerebral hypoperfusion i.e. the SNST (response inhibition) ($r = 0.815$, $p < .001$) and TMT part B (set shifting) ($r = 0.765$, $p < .001$) were associated with hypoperfusion in the left frontal lobe. Moreover, a moderate correlation was established between performance on a measure of phonemic verbal fluency (lexical retrieval) and hypoperfusion in the left posterior lateral prefrontal cortex ($r = 0.495$, $p < .001$); No other significant correlations were noted between our variables.

Discussion

Cognitive impairment in multiple sclerosis has a significant impact on disease outcome and health related quality of life. Considering the limited and indefinite literature on the association between cognitive functions and perfusion deficits in the MS population, the present study investigated perfusion detection rate and pattern, as well as the association between perfusion rates and cognitive dysfunction in cognitively impaired RRMS patients. Our results showed reduced regional cerebral blood flow in both cerebral hemispheres and various brain regions in our RRMS cohort compared to NeuroGam, which is a demographically matched normative database. The most notable and frequent perfusion deficits were recorded in the posterior lateral prefrontal cortex, medial prefrontal cortex and frontal lobe, but with asymmetric left hemisphere predominance. These findings are in accordance with older studies [7,8] that reported predominantly frontal hypoperfusion in their MS cohort. Furthermore, they are in keeping with the findings of a case study [23], which reported greater left hemisphere hypoperfusion in their MS patient.

This study however, not only confirms older reports, but extends the relative literature, firstly, by providing data regarding the severity of hypoperfusion in the cerebral lobes and several predefined Brodmanns areas and secondly, by comparing cortical area perfusion between RRMS patients with different levels of cognitive impairment.

Regarding perfusion deficit severity in the cerebral cortex, based on the grading system that we introduced, we detected severe hypoperfusion mainly in the left posterior lateral prefrontal cortex and left medial prefrontal cortex. Less severe perfusion deficits were also recorded on the right posterior lateral prefrontal cortex and left frontal lobes of our patients. Moreover, by comparing our RRMS cohort with different levels of cognitive dysfunction on cerebral perfusion, we found that level of cognitive severity actually influences cortical area hypoperfusion. More specifically, MS individuals with heavier cognitive impairment demonstrated more severe hypoperfusion on the frontal lobes, posterior lateral prefrontal cortex and orbital frontal cortex bilaterally. Equally significant findings of the study are the important associations that were established between results of perfusion reduction and neuropsychological performance. In this respect, strong associations were demonstrated between two measures of executive functions, i.e. the Stroop neuropsychological screening test (SNST), which mainly assesses response inhibition, and the Trail Making Test part B (TMT B), which loads mainly on set-shifting ability, and reduced cerebral blood flow in the left frontal lobe. Moderate associations were also noted between a measure of lexical retrieval and reduced perfusion in the left posterior lateral prefrontal cortex.

The strong associations recorded between performance on the executive function measures and impaired blood flow rates in the dominant for language left hemisphere and particularly the left frontal lobe, are not surprising, as both TMT B (contains alphabetic and number sequences) and SNST (verbally reading names) have a strong language component. These associations, although not necessarily causal, confirm the presence of executive dysfunction in patients with clinically stable RRMS, with mild- to- moderate cognitive impairment severity and relatively low disability status. This finding may imply that MS patients become less

cognitively flexible and have difficulties planning, organizing and completing everyday mentally demanding, effortful tasks or with interpersonal and social relationships, and overall activities of daily living.

The significant and critical role of the fronto-parietal network in executive function has been well established in the literature. Major theories of executive function postulate that the fronto-parietal network implements control by modulating processing in other brain regions. In this respect, connectivity between brain regions may possibly play a crucial role on how the fronto-parietal network impacts executive function. However, the investigation of executive function from the perspective of brain connectivity has received less attention. Specifically for MS, due to the significant variability of lesion patterns among patients, it is difficult to associate existing biomarkers to clinical symptoms and their progression. The topographic variability of lesions in patients with multiple sclerosis, therefore, lends itself to be studied via the lens of network analyses. Recent research into multiple sclerosis has adopted such a network approach through the utilization of functional connectivity [25,26].

In summing up our findings, our study demonstrated the presence of widespread blood flow reduction in several predefined Brodmann areas and lobes of the brain, relative to demographically matched healthy controls according to an established normative database. However, we noted blood flow reduction, mainly in the frontal lobes and other related prefrontal areas, involving both hemispheres, but with asymmetric left hemisphere predominance. Furthermore, RRMS patients with more severe cognitive decrements recorded higher rates of significant hypoperfusion relative to patients with less severe cognitive decline and also a different hypoperfusion pattern. It is therefore evident from our findings that brain perfusion SPECT was able to detect decreased cerebral perfusion of variable severity in our RRMS patients with mild to moderate disability status. Moreover, associations between measures of response inhibition, set shifting, lexical retrieval and hypoperfusion severity in the left frontal lobes and prefrontal cortex were also established.

Despite the interesting and novel findings demonstrated in our study, from a clinical viewpoint, there are two points which may limit the generalization of our report. Firstly, brain SPECT images provide lower spatial resolution compared to PET and MRI studies potentially limiting its clinical value in some cases. Secondly, our results are limited to RRMS patients and should not be generalized to MS patients with a progressive disease course.

In conclusion, our data suggest that cerebral hypoperfusion is an integral feature of MS pathology and that executive dysfunction is associated with robust cerebral perfusion deficits in the frontal and prefrontal cortex of cognitively impaired RRMS patients.

The authors declare that they have no conflicts of interest.

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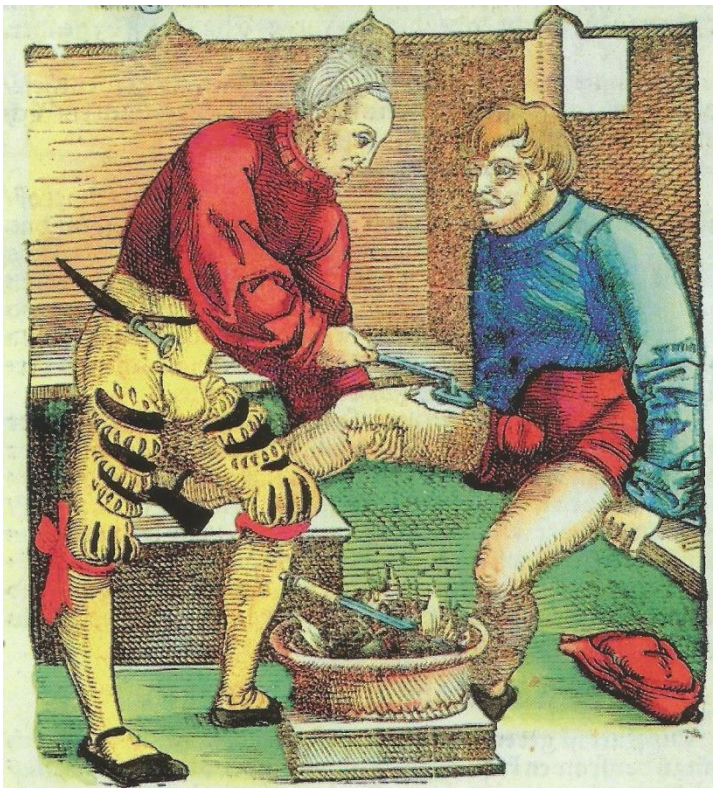

Anonymous. Lithograph. Cautery of the wound.

Short Communication

Are left angular gyrus and amygdala volumes important for financial capacity in mild cognitive impairment?

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Keywords: Neuropsychological assessment - Brain volume - aMCI - Financial capacity

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Abstract

Objective: The aim of this study was to investigate the importance of specific brain areas for financial capacity in patients suffering from amnestic Mild Cognitive Impairment (aMCI) over time. **Patients and Methods**: Fifteen aMCI patients underwent assessment of cognitive and mood functioning, as well as quantitative magnetic resonance imaging (for three times a 3 Tesla MRI). We used a detailed neuropsychological examination and a new instrument measuring financial capacity, the Legal Capacity for Property Law Transactions Assessment Scale (LCPLTAS), during a 12-month period. **Results**: Spearman and Kendall correlations revealed a number of statistically significant positive relationships at synchronous testing between financial capacity and brain volumes, while repetitive testing revealed that the right amygdala volume correlated with measured financial capacity (r first-third time=.908, P=.000), and the left angular gyrus volume difference showed a similarly strong correlation. In contrast to that, a number of neuropsychological tests correlated with financial capacity, but only MMSE seems to have the strongest correlation (r=.899, P=.000). **Conclusion**: Financial capacity in Greek aMCI patients strongly correlates with right amygdala and left angular gyrus volumes, a finding that supports that emotion as well arithmetic skills are involved in financial capacity, while the importance of MMSE as the only neuropsychological test with the strongest positive correlation is discussed.

Introduction

Financial capacity is a complex cognitive capacity that takes the form of a broad continuum of activities and specific skills. According to the most widespread approach regarding the conceptualizing financial capacity, it cannot be represented as a unitary construct, as performance skills (e.g. counting coins/currency, paying bills etc.) and judgment-decision making skills are both involved [1]. In addition to that, although general cognitive capacity is claimed to change in elder patients in time, we still know little about the role of brain volumes and more specifically of the longitudinal changes in brain volume and financial capacity in MCI. Previous

studies are scarce, but have mentioned that the predictive value which the medial frontal cortex is claimed to have on financial capacity in elders [2], while others put emphasis on the angular gyrus for the financial capacity performance of MCI patients [3].

The aim of this study was to explore the role that a plethora of brain areas may play on financial capacity changes in aMCI over time, by investigating if the volume of different brain areas correlates with financial performance as measured by a new assessment instrument [4].

Neuropsychological tests	First examinatic (baseline)	Second examinati (six months)	Third examination (twelve months)
MMSE	28.13 (1.40)	28.40 (1.84)	28.00 (1.69)
ADAS	24.06 (9.33)	21.00 (8.34)	21.60 (7.57)
GDS	0 (.00)	1.46 (1.55)	1.73 (1.86)
CDR (sum of boxes)	-	5.00 (.00)	5.00 (.00)
NPI	-	6.20 (2.21)	4.86 (2.41)
Digit Cancellation Test	20.20 (5.41)	22.20 (6.90)	20.93 (5.28)
RAVLT-immediate	39.93 (11.20)	34.40 (6.46)	37.33 (13.25)
RAVLT-delayed	6.73 (3.47)	5.20 (3.70)	6.13 (3.94)
RAVLT-recall	13.66 (1.34)	13.06 (1.53)	13.66 (1.39)
Clock drawing	4.60 (.73)	4.60 (.63)	4.40 (.63)
Clock copy	4.73 (.45)	4.73 (.45)	4.86 (.35)
Trail Making Part A	70.93 (42.89)	63.20 (31.25)	57.73 (26.33)
Trail Making Part B	194.33 (112.33)	211.53 (147.35)	213.46 (167.73)
WAIS-R Digit	25.20 (9.63)	28.33 (13.78)	28.60 (12.88)
Boston Naming Test	21.06 (5.36)	23.13 (4.88)	21.86 (5.02)
Digit Forward	6.00 (.92)	5.53 (.91)	5.53 (.99)
Digit Backward	3.93 (1.57)	3.93 (1.43)	4.06 (.88)
Letter Fluency	30.66 (9.10)	31.00 (12.49)	34.33 (12.84)
Category Fluency	42.06 (11.65)	45.33 (15.76)	45.26 (13.25)
WMS-III Logical Memory-immediate recall	8.40 (3.58)	10.86 (3.24)	9.13 (3.70)
WMS-III Logical Memory-delayed recall	7.60 (3.43)	9.80 (3.23)	8.00 (3.35)

Table 1*. Means and standard deviations for the administered neuropsychological tests at base-line, six and twelve-month examination.*

Patients and Methods

A homogeneous group of fifteen patients (10 women) with a diagnosis of aMCI without depression (Mean age=70.0, SD=8.31; Mean education=10.00, SD=3.70; MMSE score=28.00, SD=1.69, GDS=1.73, SD=1.76 at the time of their third assessment) from Northern Greece participated in the study. The patients underwent for three times a 3 Tesla MRI and a detailed neuropsychological assessment during a 12-month period. The neuropsychological assessment included tests such as the Mini-Mental State Examination (MMSE), the Geriatric Depression Scale (GDS), the Clinical Dementia Rating (CDR Sum of Boxes), the Alzheimer's Disease Assessment Scale (ADAS), the Neuropsychiatric Inventory (NPI), the Digit Cancellation Test, the Rey Auditory Verbal Learning Test (RAVLT-immediate, delayed and recall conditions), the Clock Drawing Test (CDT-immediate drawing and copy), the Trail-Making Test Part A and B, the WAIS-R Digit, the Boston Naming Test (BNT), the Digit Span Memory Test (Forward and Backward Conditions), the Verbal Fluency Test (letter fluency and category fluency), and the Wechsler Memory Scale-3rd ed. (WMS-III) Logical Memory Immediate and Delayed Recall (see mean

scores and standard deviations for all the tests in Table 1).

Analyses using Statistical Parametric Mapping (SPM 12) included volume data regarding the white matter, the grey matter, the cerebrospinal fluid, the total intracranial volume, and 16 more brain areas including the right angular gyrus, the left angular gyrus, the right amygdale, the left amygdala, the right precuneus, the left precuneus, the right hippocampus, the left hippocampus, the right parahippocampal gyrus, the left parahippocampal gyrus, the right thalamus, the left thalamus, the right medial superior frontal cortex, the left medial superior frontal cortex, the right medial frontal cortex, and the left medial frontal cortex. The above areas were selected on the basis of previous bibliographically based relevance with financial capacity [3]. Patients were additionally examined with a relevant Greek neuropsychological test, the Legal Capacity for Property Law Transactions Assessment Scale (LCPLTAS) [4].

Results

Results revealed numerous statistically significant positive correlations for the three times of the MRI measurements between different brain areas and the final LCPLTAS scores, but there were only two statistically significant parametric and non-parametric correlations between volume difference (first-third MRI measurement) and financial capacity (see Table 2).

Brain volumes	LCPLTAS scores Spearman ρ	LCPLTAS scores Kendall τ
Left angular gyrus	.716 (P=.013)	.559 (P=.013)
Right angular gyrus	.730 (P=.011)	.674 (P=.005)
Right medial superior fronta cortex	.749 (P=.008)	.636 (P=.008)
Left parahippocampal gyrus	.703 (P=.016)	.520 (P=.031)
Total intracranial volume	.796 (P=.003)	.674 (P=.005)
White matter	.786 (P=.004)	.597 (P=.013)
Right medial frontal cortex	.689 (P=.019)	.520 (P=.031)
Left medial frontal cortex	.619 (P=.042)	.482 (P=.046)
Left hippocampus	.656 (P=.028)	.482 (P=.046)

Table 2. *Brain volumes at the last MRI and Spearman and Kendall correlations withLCPTAS scores.*

The difference in volume between the first minus the third measurement of the right amygdala correlated with LCPLTAS scores ($r_{\text{first-third time}}$=.908, P=.000), and the left angular gyrus volume difference showed a similarly strong correlation with LCPLTAS ($r_{\text{first-third time}}$=.829, P=.003).

In addition to that, it is of interest that not all neuropsychological tests correlated with LCPLTAS. Statistically significant correlations were found between LCPLTAS total score and Trail-Making Part B (r=-.726, P=.011), and LCPLTAS and Boston Naming Test (r=.778, P=.005) at first examination. At second examination LCPLTAS total score was correlated with Digit Cancellation Test (r=.683, P=.020), Clock Drawing (r=.724, P=.012), WAIS digit (r=.651, P=.030), Boston Naming Test (r=.735, P=.010), Digit Backward Span (r=.742, P=.009), and MMSE (r=.731,

P=.011). Finally, at third examination LCPLTAS total score was correlated with Digit Cancellation Test (r=.670, P=.024), Trail Making Part B (r=- .723, P=.012), WAIS-R Digit (r=.648, P=.031), Boston Naming Test (r=.746, P=.008), and MMSE (r=.899, P=.000).

Discussion

Although neuropsychological tests evaluate problems in brain functioning, it seems that the MMSE, a brief measure of global cognitive function, is the only measurement that shows the strongest statistically significant correlation with this new financial capacity assessment scale.

In conclusion, this is the first study examining changes in MRI volumes, general cognitive functioning, and financial capacity in time. Although findings from the only concurrent study in aMCI patients indicates that impaired financial abilities correspond with volume of the angular gyri as mediated by arithmetic knowledge [3], this preliminary research suggests that repetitive brain imaging may show changes that influence financial capacity in a year's time and that there exist a number of specific brain volume regions that may influence cognitive performance for financial capacity. Furthermore, except for the expected finding of the left angular gyrus volume changes across time and its significance for financial capacity in MCI, we had an unexpected new finding which reveals the important role in declining financial skills of the right amygdala volume which may be explained by the claimed role that the right amygdala plays in the association of time and places with emotional properties through nonconscious processing [5, 6]. Although none of the participants reported prior financial hardship which could serve as a potent stressor and influence their hippocampal and amygdala volumes [7], the right amygdala may strongly engage in fast, shallow or gross analysis of affect-related information related to purely financial matters.

Future research should clarify further the relationships between brain volumes changes and financial capacity in patients who suffer from other types of dementia [8-10] and take into account possible brain subcortical vascular changes that apart from the easily measured MRI volumes may influence cognitive performance.

Acknowledgments

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The authors declare that they have no conflicts of interest.

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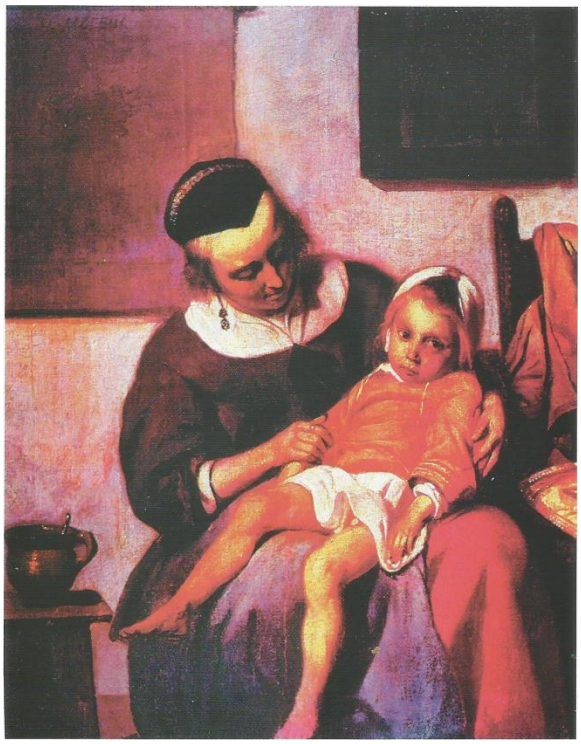

Gabriel Metsu. The sick child (1660).

Perspective

Removing muscle artifacts from EEG data of people with cognitive impairment using high order statistic methods

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Keywords: Subjective Cognitive Impairment - Mild Cognitive Impairment - Electroencephalography - Independent Component Analysis - Canonical Correlation Analysis

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Abstract

Objective: Often, people with Subjective Cognitive Impairment (SCI), Mild Cognitive Impairment (MCI) and dementia are underwent to Electroencephalography (EEG) in order to evaluate through biological indexes the functional connectivity between brain regions and activation areas during cognitive performance. EEG recordings are frequently contaminated by muscle artifacts, which obscure and complicate their interpretation. These muscle artifacts are particularly difficult to be removed from the EEG in order the latter to be used for further clinical evaluation. In this paper, we proposed a new approach in removing muscle artifacts from EEG data using a method that combines second and high order statistical information. **Subjects and Methods**: In the proposed system the muscle artifacts of the EEG signal are removed by using the Independent Vector Analysis (IVA). The latter was formulated as a general joint Blind Source Separation (BSS) method that uses both second-order and higher order statistical information and thus takes advantage of both Independent Component Analysis (ICA) and Canonical Correlation Analysis (CCA). Diagonalization methods for IVA in the proposed system were reworked based on SCHUR decomposition offering a faster second order blind identification algorithm that can be used on time demanding applications. **Results**: The proposed method is evaluated in both simulated and real EEG data. To quantitatively examine the performance of the new method, two objective measures were adopted. The first measure is the Root Mean Square Error (RMSE) while the second is the Signal-to-noise-ratio (SNR). **Conclusion**: The proposed method overcomes with the need of removing muscle artifacts on both realistic simulated EEG data and brain activity from people with cognitive impairment.

Introduction

The EEG is a recording of the electrical activity of the brain and reflects the summation of postsynaptic potentials of groups of cortical neurons arranged perpendicular to the scalp. The EEG is frequently contaminated by electrophysiological potentials associated with muscle contraction due to biting, chewing and frowning. These muscle artifacts, obscure the EEG and complicate the interpretation of the EEG or even make the interpretation unfeasible. Hence, there is a clear need to remove these artifacts from the EEG. A simple technique is to uses low-pass filters. However, as the frequency spectrum of the muscle artifacts projects with the frequency spectrums of that of brain signals, frequency filters not only remove the muscle artifacts but also necessary EEG information. Regression methods, investigated for eye movement artifact removal are not adapted for use, because no reference channel is available [1,2].

A more recently solution to this problem is the Independent Component Analysis (ICA) which separates the EEG into statistical independent components [3,4]. This method was already successfully applied to ocular artifact removal [5]. However, cross-talk can be observed when the separation of brain and muscle activity is considered. Furthermore, when using the ICA, identification of the components containing artifacts such as muscle activity, is not obvious, thus further user attention is needed [6]. ICA techniques that try to solve this problem, such as constrained ICA (cICA), cannot be applied for muscle artifact removal since this method locates only that component that is most common to a specific reference signal [7].

The ICA is a standard Blind Source Separation (BSS) method, which works under the assumption that sources are mutually independent, and that the mixing procedure is linear and instantaneous. Applications of BSS techniques can include speech enhancement, robust speech recognition, analyzing EEG and fMRI signals, feature extraction, image de-noising, etc. The most common ICA algorithms used in EEG data analysis are Infomax ICA [8,9], SOBI [10], and FastICA [11]. However, signals are often mixed in a convoluted manner. One common way to extend the instantaneous ICA to the convoluted model is the frequency domain blind source separation (FDBSS) approach. In FDBSS, observed signals are transformed to time-frequency (T-F) domain using short time Fourier transform (STFT). Although FDBSS has many advantages, it suffers from the well-known “permutation problem” that occurred when separated data must be aligned to make sure that each output signal only contains data from the same source [12,13].

The Independent Vector Analysis (IVA) was developed as an extension of ICA. Sources in the IVA model are considered as vectors instead of scalars. IVA utilizes not only the statistical independency among different sources, but also the statistical inner dependency of each source vector. The largest advantage of IVA is that the permutation problem is automatically avoided, and therefore there is no need for a post processing step after ICA for source alignment [14].

In the proposed system the muscle artifacts of the EEG signal are removed by using the IVA. This proposed method uses both second-order and higher order statistical information and thus takes advantage of both ICA and Canonical Correlation Analysis (CCA). During process, we assume that a linear mixing model exists in each dimension separately, and that the latent sources are seperated from others. In contrast to the ICA method, the sources can be random vectors, and therefore the elements of the later are closely related. In IVA, the goal is mixture

identification or signal separation for a collection of disjoint but coupled data sets.

Subjects and Methods

The proposed system is illustrated in Figure 1. EEG datasets are considered as contaminated signals that must be pre-processed. Primary, a signal filtering component was design for performing a band pass filtering. The produced filtered data is then leaded to the IVA-BSS component which will separate muscle artifacts from the signal. Pure EEG signals are then evaluated using the performance evaluation component. Filter implementation, IVA-BSS analysis and performance evaluation were developed using Matlab V2013a.

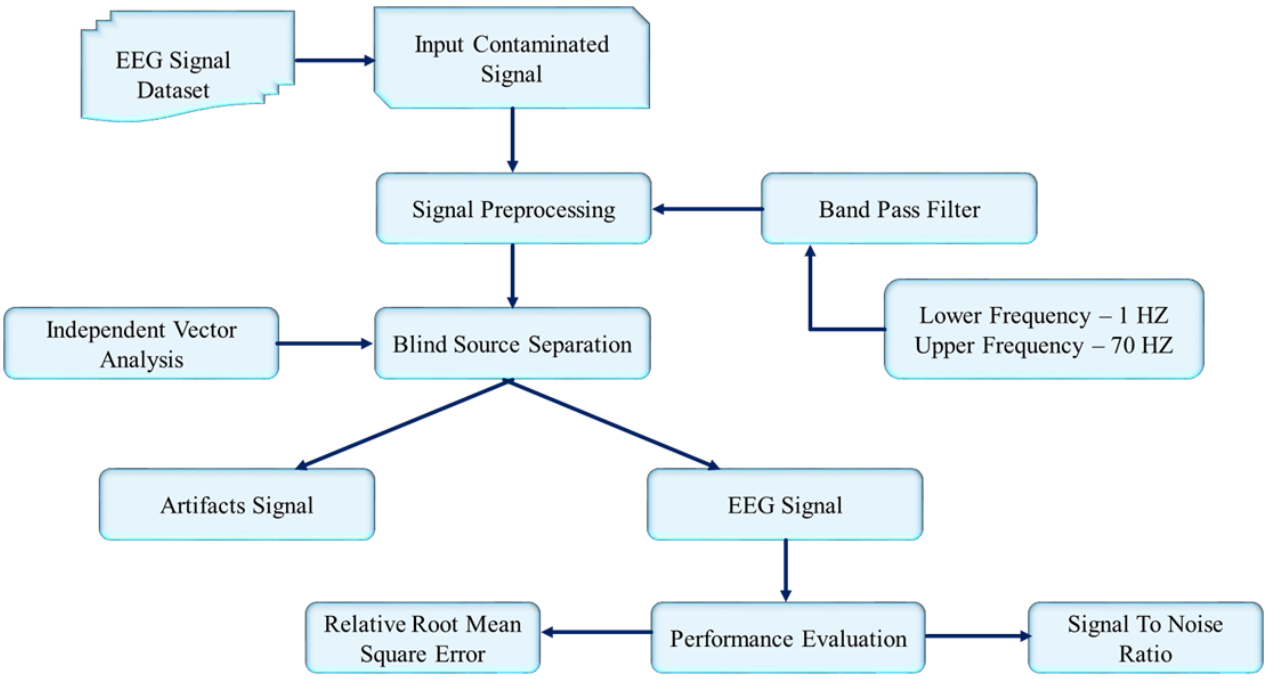


Figure 1. The proposed system using the IVA as BSS technique

EEG Signal Datasets

We first validate and evaluate our methods on several different realistic simulated data. The method for generating realistic simulated data was proposed by Xun [15]. We then apply them to real EEG recordings recruited from the Day Care Center of the Greek Association of Alzheimer Disease and Related Disorders "Saint ioannis", Thessaloniki. These real EEGs were acquired by using the Nihon Kohden EEG-1100C V01.00 system. The sampling frequency was at 500 Hz while 19 electrodes (FP1, FP2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, Fz, Cz, Pz) were placed according to the 10-20 system. Additional validation and evaluation was performed on these real EEG data.

Signal Filtering

In order to de-noise the contaminated EEG signal, filtering was applied to the input signal. A band pass filter was designed and applied to remove the noises from the signal. The band pass filter allows signals between two specific frequencies (cut-off frequencies) to pass, but that discriminates against signals of other frequencies. This filter module allows to pass signals between 1 and 70 Hz, since typical brain signal rhythms are located between this frequency frame [16].

Blind Signal Separation

BSS, also known as blind source separation, is the separation of a set of source signals from a set of mixed signals, without the aid of information about the source signals or the mixing process. In EEG, the interference from muscle activity masks the desired signal from brain activity. BSS, however, can be used to separate these two so an accurate representation of brain activity can be achieved [17].

IVA was formulated as a general joint BSS framework to ensure that the corresponding sources extracted from different data sets are maximally dependent while the sources within each data set are independent of each other. IVA is a generalization of ICA from one to multiple data sets, and was originally designed to address the permutation problem in the frequency domain for the separation of acoustic sources [18]. That is to say, source independence within one data set and corresponding source dependence across multiple data sets are maximized simultaneously [19].

Performance Evaluation Model

In order to quantitatively measure and evaluate the performance of our IVA method, two objective measures were adopted. The first measure is the RMSE, and the second is SNR that is often encountered in electrophysiology [20]. For each filtered signal in both realistic simulated data and real EEG datasets, we perform individually the ICA, CCA and IVA techniques while we keep track of the respectively RMSE and SNR values.

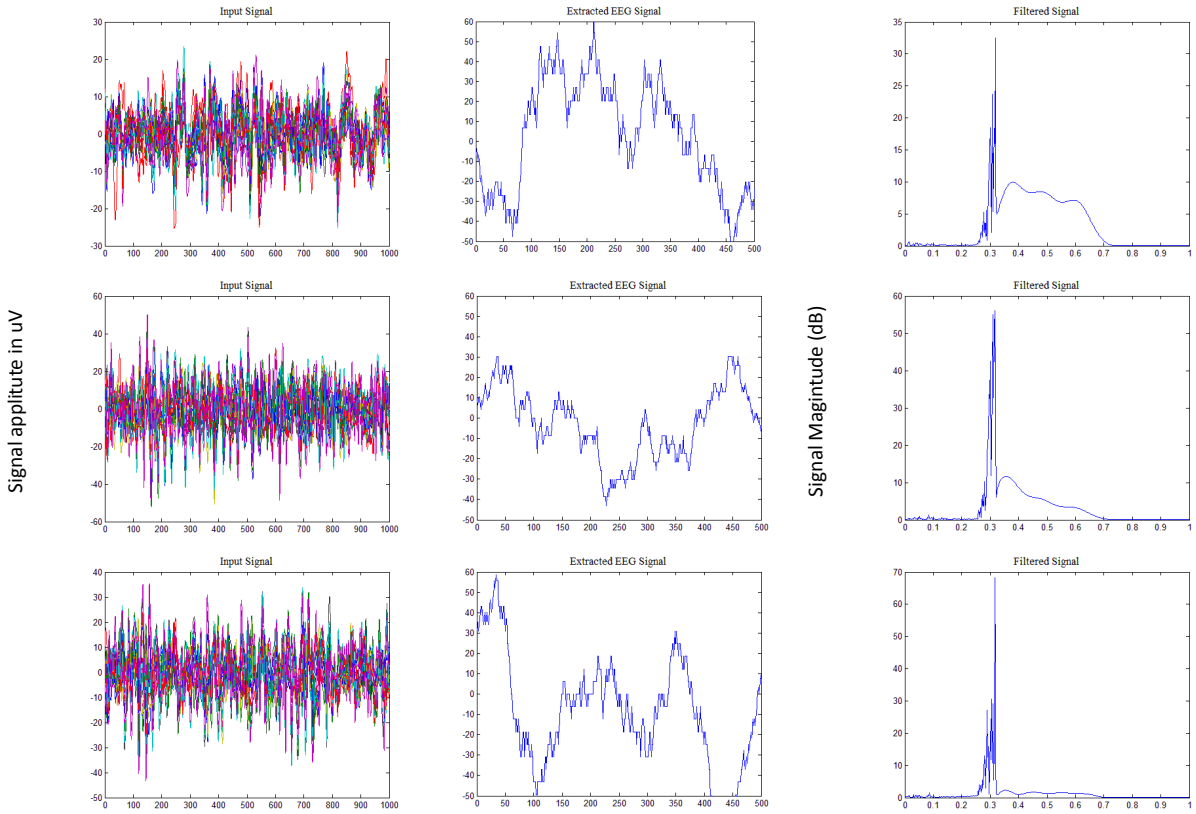
Results

Table 1 illustrates the Root Mean Square Error (RMSE) and Signal-to-noise-ratio (SNR)value from all five realistic simulated datasets when performing ICA, CCA and IVA methods individually (Table 1). These values were calculated by the performance evaluation module.

	Independent Vector Analysis (IVA)		Independent Component Analysis (ICA)		Canonical Component Analysis (CCA)	
Realistic Simulated Dataset	Characteristic Value					
	RMSE	SNR	RMSE	SNR	RMSE	SNR
1	0.3249	0.0756	1.6600	0.0220	6.6161	0.0005
2	0.3289	0.1415	1.0521	0.0035	6.3999	0.0005
3	0.3349	0.1007	0.6702	0.0060	2.6235	0.0013
4	0.3373	0.2084	0.6469	0.0066	3.1564	0.0011
5	0.3379	0.1698	0.7139	0.0056	2.9767	0.0011

Table 1. The results of the performance evaluation module on realistic simulated data

For each realistic simulated dataset, Figure 2 illustrates the input data, the signal after applying the band pass filter and the extracted, pure EEG, signal after muscle artifact rejection using the IVA method.



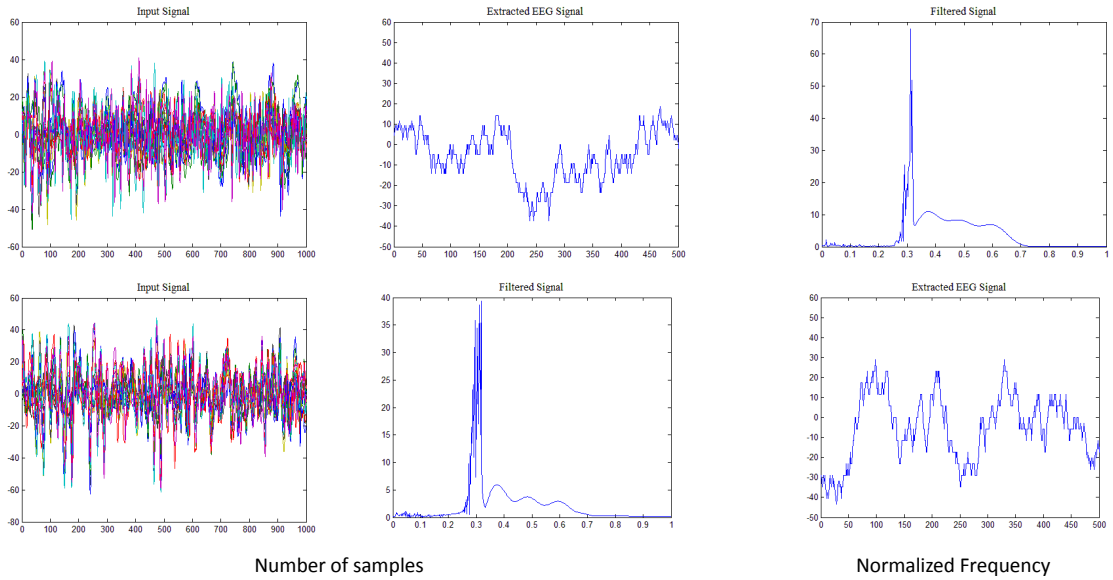


Figure 2. The five different, realistic simulated datasets. First column presents the input signal while second column shows the signal after applying the band pass filter. Third column presents the extracted, puree EEG signal after muscle artifact rejection using the IVA method.

The proposed IVA method was also applied to a selected real EEG recording recruited from the Day Care Center of the Greek Association of Alzheimer Disease and Related Disorders "Saint Ioannis". We have selected a dataset that is annotated with a patient body movement (Figure 3).

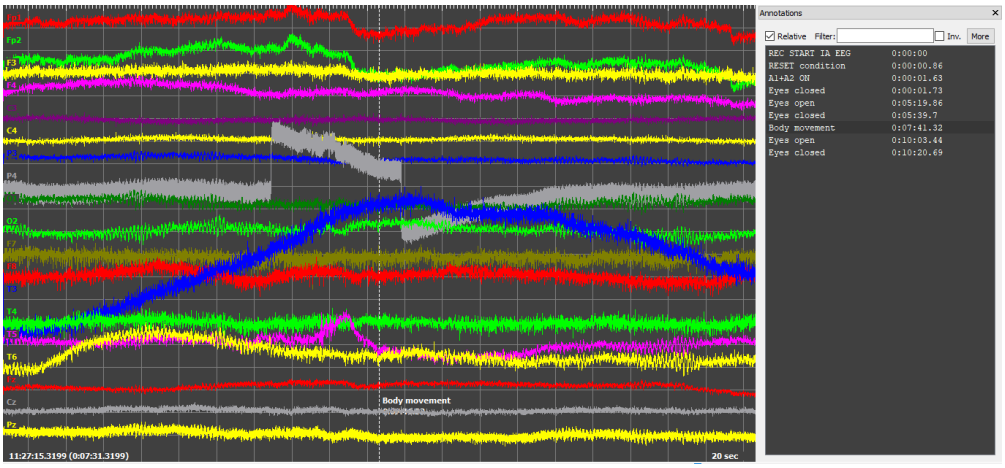


Figure 3. Selected real EEG data with body movement annotation. The whole recording consists almost of 10 minutes and 20 seconds while the body movement is annotated around 7 minutes and 41 seconds after recording started.

After applying to the dataset the IVA method we can extract the pure EEG signal free of muscle artifacts (Figure 4).

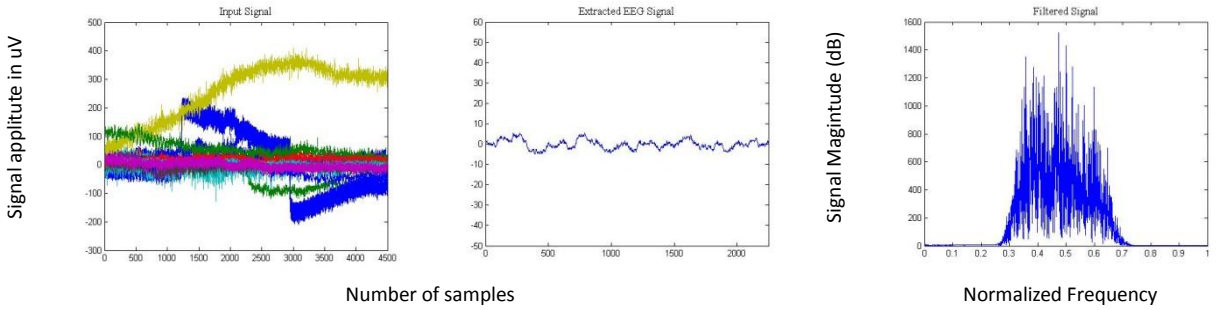


Figure 4. Applying the proposed IVA method into a selected, body movement annotated, EEG dataset. The figure illustrates the selected input signal, the pure EEG extracted signal and the produced signal after band pass filtering

Performance evaluation module, calculates the two objective measures that were adopted. Table 2 illustrates the RMS Error and SNR Value for the selected real EEG dataset.

Independent Vector Analysis (IVA)		**Independent Component Analysis (ICA)**		**Canonical Component Analysis (CCA)**	
Characteristic Value					
RMSE	SNR	RMSE	SNR	RMSE	SNR
0.3333	0.0130	0.3474	0.0349	24.7643	0.0001

Table 2. The results of the performance evaluation module on the selected real EEG signal

Discussion

The results are in agreement with the studies that IVA method is better on suppressing muscle artifacts from EEG recordings, without removing significant underlying EEG information. This is occurred due to the fact that IVA method takes advantages of both CCA and ICA but also solves the “permutation problem” that occurred when separated data must be aligned [21] in order that the output signal must contains data from the same source.

Another advantage of the proposed system is that the latter is significant faster, in terms of time execution, in respect with classical ICA and CCA approaches. This is occurred due to fact that the diagonalization methods for IVA have been replaced, within the proposed system, with the SCHUR decomposition, a faster and more effective way in diagonalization [22]. That offers a faster IVA - BSS that can be used on time demanding applications such as Brain Machine Interfaces (BMI).

Furthermore, the proposed system modules, due to their simplicity, can be used on portable and energy efficient computational systems.

Conclusion

In case of EEG recordings from people with Subjective Cognitive Impairment (SCI), Mild Cognitive Impairment (MCI) and dementia, the necessity of removing muscle artifacts is of the essence since these recordings must be used for further evaluation. Often, these EEGs involve patient's movement and thus muscular activity. In order to measure the functional connectivity between brain regions and activation areas during cognitive performance, these muscle artifacts must be removed. Our study indicates that the proposed IVA – BBS overcomes with this need since both objective measures of RSME and SNR are significant low and muscle artifacts are removed successfully from the original recordings.

The authors declare that they have no conflicts of interest.

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The port of Aigina

Commentary

Cerebellar pathology in Alzheimer's disease

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Keywords: Cerebellar pathology - Alzheimer's disease - Dementia - Memory impairment

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Abstract

Alzheimer's disease (AD) is one of the main causes of dementia in the western world. It is clinically characterized by memory impairment, deterioration of intellectual faculties and loss of professional skills. AD brains exhibit significant atrophy, predominantly in the temporal and parietal lobes, while light microscopy reveals deposition of senile plaques and neurofibrillary degeneration initially in the entorhinal cortex, the hippocampus, and in the acoustic and visual cortices, in the frontal lobe and the cerebellum in the advanced stages. Dendritic and spinal pathology, as well as loss of synapses are also key neuropathological features.

The cerebellum is a critical part in the distributed neural circuits participating not only in motor function but also in autonomic, limbic and cognitive behaviours. Lesions of the motor cerebellum, mostly in lobules III-V in the anterior lobe and the secondary sensorimotor region in lobule VIII result in dysmetria of movement, however lesions of the cognitive and limbic cerebellum in the posterior lobe, represented in lobules VI, VIIA (including lobules VIIAf and VIIAt at the vermis, and crus I and crus II in the hemispheres) and VIIB, and possibly lobule IX, are followed by dysmetria in the realms of intellect and emotion. Cerebellar functional topography has been demonstrated by tract tracing studies in non-human primates and in physiological and behavioural studies in rodents, cats and monkeys.

Further studies revealed the existence of a mosaic of intrinsic connectivity networks that match the topographically precise cerebrocerebellar connections, while topographic organization of cerebellum is also evident in task-based functional MRI in healthy controls, and in clinical neurology, neuropsychology and neuropsychiatry studies in patients with cerebellar lesions.

Although the cerebellum has not been extensively studied in AD, recent studies have revealed evidence of a unique pathological pattern of the cerebellar cortex, including loss of Purkinje cells, synaptic alterations in the mossy fibres, granule cell dendrites, parallel fibres and Purkinje cell dendrites with substantial loss of dendritic spines, and considerable alterations in ultrathin sections.

Introduction

Alzheimer's disease (AD) is one of the main causes of dementia in the western world. It is clinically characterized by memory impairment, deterioration of intellectual faculties and loss of professional skills [1,2]. AD brains are characterized by significant atrophy, being most prominent

in the temporal and parietal lobes [1]. Light microscopy reveals deposition of senile plaques and neurofibrillary degeneration initially in the enthorhinal cortex, the hippocampus, and in the acoustic and visual cortices, in the frontal lobe and the cerebellum in the advanced stages [1-5]. Dendritic and spinal pathology, as well as loss of synapses are also key neuropathological features [6].

The cerebellum is a critical part in the distributed neural circuits participating not only in motor function but also in autonomic, limbic and cognitive behaviours. Lesions of the motor cerebellum, mostly in lobules III-V in the anterior lobe and the secondary sensorimotor region in lobule VIII result in dysmetria of movement, however lesions of the cognitive and limbic cerebellum in the posterior lobe, represented in lobules VI, VIIA (including lobules VIIAf and VIIAt at the vermis, and crus I and crus II in the hemispheres) and VIIB, and possibly lobule IX, are followed by dysmetria in the realms of intellect and emotion [7,8]. Cerebellar functional topography has been demonstrated by tract tracing studies in non-human primates and in physiological and behavioural studies in rodents, cats and monkeys [7,9,10].

Further studies revealed the existence of a mosaic of intrinsic connectivity networks that match the topographically precise cerebrocerebellar connections, while topographic organization of cerebellum is also evident in task-based functional MRI in healthy controls [11], and in clinical neurology, neuropsychology and neuropsychiatry studies in patients with cerebellar lesions [8].
The cerebellum has not been studied extensively in AD, however recent studies have revealed certain changes in the cerebellum specific for Alzheimer's disease [12].

As we have shown in a previous study, the cerebellar cortex is characterized by a unique pattern of Alzheimer-type pathology, while there are only diffuse neuritic plaques and no neurofibrillary changes [13]. Furthermore, a loss of Purkinje cells and synaptic alterations in the mossy fibres, granule cell dendrites, parallel fibres and Purkinje cell dendrites with substantial loss of dendritic spines, and considerable decrease in number of granule and Golgi cells in the granule cell layer have been reported [2,3,14].
In the present study we aim to review the already sizeable existing literature on the neuropathological, structural and functional neuroimaging studies of the cerebellum in Alzheimer's disease.

Cerebellar Volume

Stereological studies have shown an overall reduction of 12,7% of the cerebellar volume in Alzheimer disease patients [15].

Guo et al in their recent study used MRI to study the disease-specific regional atrophy in the cerebellum and cerebral cortex in AD (use abbreviations) and three subtypes of frontotemporal dementia. They explored how the patterns of atrophy mapped onto previously described intrinsic connectivity networks, and they applied seed-based functional connectivity MRI to healthy brains from the normative human connectome database to explore intrinsic connectivity networks of those areas most affected by focal atrophy in the AD and FTD cases [16].

Moreover, they report eddisease-specific regional cerebral cortex atrophy in each of the entities, and found a respective atrophy in the areas of the cerebellum which are connected to the atrophied cerebral areas. In AD they reported a regional atrophy in the cerebellum involving crus I and II bilaterally and in their correlated cerebral areas with the angular gyrus, the middle temporal gyrus, precuneus and dorsal medial prefrontal cortex [16].

Wiegel et al in an holistic volumetric approach of the cerebellar cortex in Alzheimer's disease, reported a reduction of 24% and 22% in the volume of the molecular and granular layers of the cerebellum in comparison to normal controls [3].

Neuronal Density. Dendritic Changes and Spines. Amyloid.

Andersen et al used stereological methods to estimate the density of Purkinje and granule cells in ten cerebella from elderly female subjects with severe AD and ten age- and gender-matched controls. They divided the cerebellum into five regions and the found no significant difference in Purkinje or granule cells density in Alzheimer disease in comparison to normal control brains [15].

Fukutani et al using immunohistochemistry to investigate the pathological differences between ten cases of familiar and ten sporadic Alzheimer's disease cases in the cerebellum, they found significantly decreased Purkinje cells density in both sporadic and familial cases with substantial astrocytosis in the Purkinje cells layer, granular cells layer and white matter [14]. Further studies using Nissl staining method and stereological techniques revealed substantially reduced numbers of Purkinje cells in the cerebellar vermis and the anterior lobe of the cerebellar hemispheres [13,17], while Wiegel et al described a loss of Purkinje and granular cells as high as 32% and 30% in Alzheimer's disease [3].

Dendritic Changes

The study of the dendritic arborisations of Purkinje cells using either traditional staining methods such as Golgi method, or the modern immunohistochemical methods and 3D reconstruction software is one of the most challenging approaches of the central nervous system. Mavroudis et al, using Golgi method and 3D neuronal reconstruction techniques showed significant decrease in the dendritic tree density of the Purkinje cells from the cerebellar vermis and the anterior lobe of the cerebellum, with a significant loss of distal dendritic branches. In addition to the loss of dendritic branches, the authors reported a significant shrinkage of the dendritic field in Alzheimer's disease. Using Sholl's concentric circles analysis they found that the dendritic peak was at 90 μm, and 85 μm from cells soma in the Purkinje cells from the anterior cerebellar lobe and vermis respectively, whilst the dendritic peak for normal controls were at 120 μm and 130 μm for the aforementioned cerebellar areas [13,17,18].

Dendritic Spines

Substantial changes regarding the density and the morphology of the dendritic spines of the Purkinje cells have been also reported by a number of studies. Mavroudis et al reported a 50%

decrease in the number of the dendritic spines in the Purkinje cells from the cerebellar vermis in AD brains, and by 43% in the anterior lobe of the cerebellum. Additionally to spinal loss, the authors reported a possible reconstitution of dendritic spines in AD patients, while the vast majority of the remaining spines were of the short stubby type, with the majority of them being of the long neck type in normal controls. Numerous filopodia and dystrophic dendritic spines were also reported in the Purkinje cells from Alzheimer's disease brains [13,17].

Amyloid deposition and Neurofibrillary tangles

Cerebellar cortex is characterized by a unique neuropathological pattern in Alzheimer's disease. Numerous studies using either immunohistochemistry, or silver staining methods, failed to identify neurofibrillary changes or amyloid plaques and only a limited number of studies reported small figures of diffuse plaques in the cerebellar cortex of Alzheimer's disease brains [3,13]. Moreover, leptomeningeal and cortical amyloid angiopathy in the cerebellum was equivalent in Alzheimer's disease brains and normal controls [3].

Discussion

The cerebellum is a critical part in the distributed neural circuits participating not only in motor function but also in autonomic, limbic and cognitive behaviours. Lesions of the motor cerebellum result in dysmetria of movement, however lesions of the cognitive and limbic cerebellum in the posterior lobe, are followed by dysmetria in the realms of intellect and emotion (cerebellar affective syndrome).

Although cerebellum was thought to be spared by Alzheimer's disease, accumulating evidence showed a number of pathological changes, including loss of distal dendritic segments, decrease of the total number of dendritic spines, ubiquitin-immunoreactive dystrophic neurites and spines, microglial proliferation of the Purkinje and significant volume loss cells, even in the absence of the typical Alzheimer disease-like pathology [13,19-21].

This pattern of pathology suggests that two factors might be considered in the etiopathogenesis of cerebellar atrophy, first transneuronal degeneration and neuronal loss resulting from primary pathologic changes in cerebral structures and second parenchymal cerebellar ß-amyloidosis.

The network theory of neurodegeneration builds upon the Hebbian notion that neurons that are functionally and anatomically connected may also degenerate and die together. Interconnected neural networks in the non-human primates and human brain were identified with physiological techniques and functional neuroimaging studies [22].

Prusiner proposed that neurodegeneration occurs within interconnected networks as a result of self-propagation/prion-like spread of neurotoxic agents along neural pathways linking distributed nodes into functional modules [23.] The cerebellum might undergo focal atrophy in concert with interconnected cerebral nodes within the same functional module [12].

Diffuse senile plaques that are present in small figures in the cerebellum are characterized by the presence of beta protein, also called A4 protein, in a dispersed form and the

apparent lack of associated dystrophic neurites or reactive glial cells.

Diffuse plaques can be detected by modified Bielschowsky silver stain and are not recognised by antibodies to neurofilaments, tau, and PHF, all of which detext dystrophic neuritis, and furthermore no association of reactive astrocytes or microglial cells with diffuse plaques was ever observed [24].

Typical neuritic plaques were never detected in this location, making the cerebellar molecular cortex a useful site for the study of diffuse plaques because diffuse plaques in the cerebral cortex are intermingled with neuritic plaques.

Soluble Ab peptide and Ab oligomers unsettle neuroplasticity imbalance, resulting in an impairment of synaptic stabilization [25,26] and loss of dendritic branches, dendritic spines and synapses in Alzheimer's disease brains. Hyperphosphorylation of tau protein may also cause deleterious effects of neuroplasticity and may underlie its role in the aetiology of Alzheimer's disease [27,28].

The neuropathological, structural and functional changes of the cerebellum that have been described in Alzheimer's disease, may contribute to an unknown yet extend to the overall cognitive decline that occurs with disease progression.

Conclusion

The cerebellar cortex provides an excellent background for the study of the neuropathology and pathophysiology in Alzheimer's disease.

Other mechanisms, such as oxidative damage, vascular pathology, blood brain barrier abnormalities may also contribute in the neuronal degeneration in Alzheimer's disease. Furthermore self-propagation/prion-like spread of neurotoxic agents along neural pathways linking distributed nodes into functional modules could also explain the pattern of neurodegeneration in the cerebellum in Alzheimer's disease.

Morphological changes of the Purkinje cells is one of the pathological features of AD

Compared with cerebral Ab plaques in AD, cerebellar Ab plaques could be considered to possibly represent an earlier form of plaque evolution or even an attenuated stage in the process of plaque maturation.

A cerebellar cognitive affective syndrome to the cognitive profile of Alzheimer's disease should also be considered.

The authors declare that they have no conflicts of interest.

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Commentary - Case Study

The self in Alzheimer's Disease: A Case Study with Implications for Life Quality

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Keywords: Alzheimer - Dementia - Self Sense of Self - Self-hood - Person-hood - Life Quality Psycho-social - Intervention

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Abstract

In neurodegenerative disorders such as dementia of the Alzheimer's type the person gradually loses skills and aspects of everyday living. Does that indicate that he/ she lose the self? In the past it was widely accepted that the self diminishes in dementia, until it is lost in the latest stage, yet in the recent years this premise has been challenged. Accepting that the self is 'lost' can be problematic theoretically in the approach of self-hood, and practically in the care of individuals. Currently a novel perspective is 'gaining ground' supporting the existence of the self in dementia, and the importance of enhancing the self-hood, in order to improve the life of the person. A case study is presented where the self emerged along with a need to target it in order to improve the life of the person. A man with Alzheimer's disease was expressing rapid cognitive decline, along with emotional agitation and behavioral disturbances, and began having sessions. An intervention was structured that used all art modalities as a stimulus, and focused on the existing skills, on the narrative of the person, and on self- qualities, in order to improve life quality. It was composed of activities, each of which had a cognitive, an emotional, a behavioral and a somatosensory aspect, all of which were linked to the self in the past, present and future. The intervention appeared to be efficient in delaying the progression of the disorder (as much as possible), in stabilizing the emotional state of the person and in harmonizing the behavior. All the above were made apparent through regular assessments with a Mini Mental State Examination (MMSE), a Geriatric Depression Scale (GDS), and through quantitative and qualitative measures regarding the self, and life quality. More research is required to be able to generalize the results, yet a focus on the self and all its aspects appears to be efficient in the psycho-social treatment of Alzheimer's, and manages to improve life quality.

Introduction

Dementia is a condition with a long past- having appeared already in the ancient eras. Philosophers (like Plato), doctors (like Galen) or legal thinkers (like Solon) recognized that old age could often be accompanied by troubles in memory, attention or judgment [1]. In the recent years, individuals with dementia represent a growing population: in Europe more than 7.3 million

individuals suffered in 2011, and more than 160 thousand in Greece alone, with the numbers moving solely upwards, and expected to triple in the upcoming decades [2]. Based on statistics by Alzheimer's Disease International, in 2017 more than 50 million individuals lived with Alzheimer's and in 2015 the global cost of caring reached 818 billion dollars (2017).

The past and the present of dementia indicate that it is a condition in need of an efficient management and systematic efforts for improving the life quality of those who suffer.

Dementia and the Self Intervention.

Dementia as a condition has an odd relation with the sense of self, due to neurodegeneration. Until recently, it was widely accepted that the self is 'gone' in dementia, along with the cognitive and meta-cognitive abilities. In a first glance, this makes sense: the person gradually loses more and more skills related to the self (such as autobiographical memory or consciousness), while in the latest stages the person seems to have lost every characteristic that made him 'himself'. This has been supported through researches which have examined the material, the social and the spiritual self and have concluded that the stage of the disorder is directly linked to the level of self-concept for each person [3].

The 'abscence' of the self in dementia though is problematic. In the theoretical-philosophical level, if the self is gone, what is left? The person becomes a 'non- person' fact which leads to a theoretical dead end, and an 'ontological null-point. If the person has no self, then he/ she does not have the same human rights as others (or as in the past, in the pre-dementia state). In practice, this belief would lead to generalized, impersonal, even careless caring for the person, which has lost its meaning and importance (both by health and mental health professionals who experience frustration, and by family caregivers who often grieve for the person while he/ she is still alive) [4,5].

Recently a new perspective was borne, supporting both in theory and through research the maintaining of the self in dementia. Theoretically, the person, despite the level of progression of the disorder, remains unique in his thought, feelings and behaviors, and continues to interact (influence and be influenced by) with the environment. Moreover, the self changes throughout the course of lifetime depending on the developmental stage, its demands and capabilities, and in a similar manner, the self can adapt to dementia [4]. In research it has been shown that individuals with dementia can characterize themselves, and express their uniqueness in several ways, in a manner similar to healthy elderly individuals [5]. Finally, the self cannot be localized in one part of the brain, thus even if some parts of the self, and of the brain respectively, are affected by dementia, not all of them are entirely harmed, thus the self can still be maintained, even if altered (in the brain structures and functions that are less affected) [7].

The self may be influenced by the disease, but it is not brought to extinction due to the disease [6]. We could perceive the self as dynamic and multifaceted. It is dynamic since it has the ability to change and adapt to each developmental stage, its abilities and demands, and to different life phases, it can rely on experience and knowledge and can deal effectively with challenges [4]. Moreover, the self is multidimensional, since it is composed of a somatosensory, a cognitive, and emotional and a behavioral (and social) aspect, all of which are combined, creating

the unique person that each individual is [8]. From this perspective, the self may be altered in dementia, but it remains 'alive' and active along with the person!

It is of great importance to maintaining the self in dementia. Caring (by professionals or the family) has meaning and a quality with a humanistic value, while the person can seek the meaning in the life with dementia (especially in the first stages, but also throughout the way) [5]. In this way, the life quality of the person is improved, fact which is the ultimate goal of all interventions [9]. This has been shown in research studies: individuals who maintain a sense of self tend to have a better quality of life than those with less sense of self [10]. It is worth noting that life quality is not approached as an objective measure based solely on the existence or absence of a disease, but as a term both objective and subjective. In this view, individuals with dementia can maintain a life of quality despite the condition [11].

The self, despite being an abstract construct, may become the goal of a psycho-social intervention. This can both delay the progression of the disease (which influences all aspects of the self), and improve the life quality of the person involved [8,9].

Intervention

Based on the definition of the self and the insufficient existing literature, an intervention that targets the self in order to delay the progression of the disorder and improve life quality is structured. Each element of the intervention is based on existing literature.

The intervention is composed of sessions of activities, and each activity has a somatosensory, a cognitive, an emotional and a behavioral aspect, so that all aspects of the self (and all aspects that the disorder influences) are targeted [8]. Also, each activity is linked to the self in the past, present and future (thus to the narrative of the person) and to self qualities (such as personality characteristics or self- knowledge). Finally, there is a focus in the 'here and now', through focusing in the existing skills of the person (instead of the ones already influenced by the disease), and in the changing needs of the person [9]. The activities use all art modalities as a stimulus [12].

Each activity is presented in a hard copy, printed (with a large fond so that it is easy to read) and offered to the person, in order for him/ her to be active in the management of the disease, and to be approached by the coordinator as an equal [5].

The intervention has an intense person- centered approach and a humanitarian rational, drawing from the person- centered theory in dementia, which supports the enhancement of the sense of self and the individualized care. According to the person- centered approach, the self is maintained in dementia, and a focus on it will promote the life quality of the person [13,14].

As mentioned, the intervention is based on existing literature. All its aspects have been found (separately) to be effective in enhancing the sense of self and in improving life quality in individuals with dementia (in group settings or in individual sessions). According to a meta-analysis of interventions regarding the self in dementia, the focus on the narrative of the person, on self qualities, and on the existing skills, as well as the use of the arts are means to enhance the self and delay the progression of the disease [9]. Moreover, according to a person- centered music therapy case study, the person- centered approached, especially when combined with the arts can lead to several benefits for the person, including enhancing the sense of self and improving the quality of life [15]. Furthermore, the combination of all art modalities has been found

effective in enhancing the sense of self in individuals with dementia in a group setting [12]. Finally, according to a case study in an individual with severe dementia, the focus on all the different aspects of the self (somatosensory, cognitive, emotional, behavioral) was effective in delaying the progression of the disorder, and improve the life quality of the person [16].

Overall, the intervention is highly individualized and structured specifically for each person and his/ her needs [5]. So it was structured in the current case study.

Case Study

An individual, male and 85 years of age, exhibited the first signs of dementia. Soon he was diagnosed with Alzheimer's disease, but he was in the very early stages. About a year later, his cognitive state began to decline rapidly along with an emotional turbulence while he expressed intense behaviors that seemed 'out of character'. Thus his family requested sessions to manage the situation and delay the progression of the disorder.

In the first meeting, the person was reluctant and negative. Although he recognized that he had some memory troubles he believed it was not his 'fault', but it was due to his environment that he became angry angry. He explained that everyone treated him 'differently' and in a strange manner: he was not allowed to go out as he used to, he was not brought his daily newspaper, he was not allowed to do the activities that he used to (like preparing his coffee), and he was addressed with phrases such as 'you don't know' or 'never mind, you can't do this or that'. 'Is it not reasonable that I get angry?' he had asked. When he was asked what he likes to do he stated that 'I have not done something that I like in such a long time that I have almost forgotten what I like to do', while the phrase 'I am losing myself' appeared more than twice in the same initial conversation. Finally, he was asked if he is satisfied with his daily living, and he stated no. So it was suggested that we start sessions in which we will try to improve his daily living, through 'improving the mind, the emotion and his interaction with others' (as it was explained to him). The initial hesitation was vanished with this suggestion, which found him positive. Thus, we began hourly sessions twice a week.

Results

Before the beginning of the sessions, the individual conducted an assessment through a set of questionnaires. Cognitively, he was tested with the Mini Mental State Examination (MMSE), which assesses several cognitive functions that dementia influences with validity and reliability, in a short time [17]. The highest score indicated higher cognitive level. Emotionally he was assessed with the Geriatric Depression Scale (GDS), to test for depression. This test, through fifteen questions with a dual answer can indicate the existence of depression (apparent through the higher scores) [18]. Moreover, his life quality was assessed through a questionnaire with thirteen questions with four potential answers. The questionnaire Quality of Life in Alzheimer's Disease can be administered to the person and the caregiver (separately) and higher scores indicate higher life quality [11,19]. It was chosen both because it is suitable for the person and the

caregiver, and because it includes a question about the self (that sees it as linked to life quality). Finally, the sense of self was assessed through the I-AM test, which requires the completion of ten sentences starting with 'I am...', and which are characterized for whether they are true or false, general or specific, positive or negative, and for the number of answers generated [6].

In an initial assessment, the person exhibited the first stages of Alzheimer's disease (MMSE: 21/30) with problems mainly in orientation, and low levels of depression (GDS: 4/15). Despite that, he expressed a low life quality (QOL-AD: 26/52). He stated he was not satisfied with the way he lives now, and his inability to do things around the house, while he was not doing any fun activities, and was frustrated with his family for not being able to do so. At the same time, his wide stated that his life quality was mediocre (QOL-AD: 34/52), with the answers between the spouses to differ only in the judgment of the relation with the family, and the overall life quality. Finally, the person exhibited a sense of self, but more generic, and with a negative tone. He was able to complete seven sentences before getting angry and giving up, all of which were true in the present, with three being general (man, husband and father, retired), two negative (old, frustrated), and two specific (me, person who loves and takes care of others).

The person repeated the same assessment every six months. At the first six months there were some changes which were more intense in the next evaluation (one year after the initial one). His MMSE is steady [20,21], and his GDS remained the same [3,4]. Yet there was a change in the quality of life both according to him [28,33] and according to his wife [35,36], while the answers of the two became more congruent. More specifically there was a change in the way he approached his relation with his family, and in his general way of life. Finally, there was a change in the sense of self. In the next evaluation he was able to complete ten sentences without getting angry, out of which: three remained general (man, husband and father, retired), one was negative (old), two were positive (smart, strong personality), three were related to the 'here and now' (grateful today, here in our meeting with…, nice/ good looking today) and one descriptive (person who speaks 'straight' and talks to everyone, and everyone knows me). His answers were similar in the third assessment (man, husband and father, grandfather of those beautiful grand kids, retired, elderly, smart, strong, here in our typical meeting, happy, person who takes care of others).

The person is continuing his sessions, and his assessments in the same pattern.

The intervention appeared to be efficient in delaying the progression of dementia (fact which was made apparent through the steady scores of the MMSE), and in improving the quality of life (both according to the person, and to the caregiver). Finally, through the intervention the person expressed more intensely his sense of self (as was made apparent through the more, more precise, more positive and more present-oriented answers in the I-AM test). Overall, there were some small changes in the time span of one year in his life quality, which appeared to be gradually increasing, and more qualitative changes in the sense of self.

Discussion

A man of 86 years old, suffering from dementia of the Alzheimer's type, participated in a holistic psycho-social intervention aiming at improving his life quality and delaying the progression of the

disorder through enhancing the sense of self.

The results can be explained through the existing literature, which has begun to investigate the relation between sense of self and life quality in dementia (9,10). We can perceive the change in the sense of self and the quality of life through a psycho-therapeutic and a neuroscientific perspective. From the therapeutic scope, the person was the center of the intervention, his uniqueness was focused upon, and life with dementia became a little more simple and understandable for the person, providing him with meaning. In this way, a therapeutic alliance was developed between the person and the therapist, which in turn, promoted the goals of the intervention [5]. From a neuroscientific scope, the targeting of different aspects of the self lead to exercising different parts of the brain and different functions, fact which allowed the delaying of the progression of Alzheimer's disease, and the maintenance of functionality, thus by extend, the life quality of the person [7].

Conclusion

The current case study leads to several conclusions and implications, both theoretical and practical. The sense of self seems to be characterized by permanency and stability, while being multifaceted and dynamic at the same time. The person can maintain the self even through neurodegenerative conditions. This can function both as a goal of a psycho-social intervention, and as the rational of caring, through an effort for an individualized in essence approach [4].

The paper had some limitations though. For starters the QoL-ADQ does not exist in a Greek version, so the translation was not official. Despite that the phrasing is very simple and clear, thus the translation is accessible, it is not systematized in Greek. This brings forth the need to officially translate questionnaires in Greek. Moreover, the differences among the assessments could have been influenced by the therapeutic relation itself: the person was accustomed to the process and the professional, fact which could have influenced his ability (and desire) to reply, but this factor was not assessed in research terms.

This leads to suggestions for future research that arise through the case study. The self can be investigated in relation to dementia of several stages through both quantitative and qualitative methods, or even through imaging techniques. The life quality in relation to self-hood could be more systematically explored, and in a more holistic manner, drawing information both from the person and his/ her environment. Finally, there could be an effort to translate and systematize questionnaires in Greek, so that mental health professionals could have a wide variety of choices for their method of evaluation.

During one session, the person stated that he feels like he is 'finding himself again' through our meetings. When he was asked to explain, he said he could not, but in some way our meetings made his everyday life better. Let us hope that we will be able to explain his words scientifically, through linking the sense of self in dementia with life quality, through an effective intervention.

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Commentary

Biochemical diagnosis of vascular cognitive impairment associated with subcortical small vessels disease

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Abstract

Subcortical small-vessel disease (SSVD) is a disorder that has been fully described in clinical, neuropathological and imaging aspects. It is considered as the most prevalent ischemic CNS disorder and has been associated to arterial hypertension, diabetes mellitus, dyslipidemia and obstructive sleep apnea. The hallmark of SSVD is the ischemic white matter lesions which can be presented as lacunar infracts and global brain hypoperfusion in a common and homogeneous subtype of vascular cognitive impairment (VCI) which is often unrecognized. The special nature and course of SSVD offers the opportunity of collecting knowledge at all stages of its pathogenicity. Arteriosclerosis, hypoxic hypo-perfusion and inflammation act synergistically, causing myelin degeneration and blood brain barrier alteration. Clinical diagnosis of SSVD includes early executive dysfunction manifested by impaired capacity to use complex information, to formulate strategies, and to exercise self-control. Brain imaging has advanced substantially the diagnostic tools for SSVD. Diagnostic biomarkers for Alzheimer Disease include reduction of cerebrospinal fluid amyloid-β (Aβ)42 and of the ratio Aβ42/Aβ40 sometimes with increased total tau levels. However, biomarkers specific for the diagnosis at all stages of SSVD are needed, especially because of the unsatisfactory treatment options at its late stages to date.

Introduction

The term vascular cognitive impairment (VCI) is used in order to describe the cognitive consequences of the heterogeneous set of sporadic and hereditary cerebrovascular diseases [1]. VCI is the second most prevalent type of cognitive impairment in the elderly, just below Alzheimer Disease (AD). The two pathologies share some of their risk factors and pathogenetic

mechanisms, such as arterial hypertension, diabetes mellitus, dyslipidaimia and amyloid angiopathy [2,3] and coexist in most of the cases as mixed dementia, which seems to be more common than the "pure" forms of each one [4]. It has been argued that vascular disease can accelerate the progression of AD [5] and vascular damage of white matter may be the first stage on the AD course [6]. The global population aging and the progression of vessel disease result in an estimation for an expansion of VCI in the future [7].

SVD presents a typical cognitive impairment syndrome characterized by mental slowness, personality changes and executive deficits affecting cognitive functions like planning, attention, flexibility, working memory and abstraction capacity [8]. Gait pattern can also be slower, resembling the bradykinesia of an extrapyramidal syndrome. There has been a great difficulty in the diagnosis of cognitive disorders resulting from vascular disease, which can be illustrated by the numerous diagnostic criteria proposed [9]. The most recently updated definitions and criteria were set by the International Society of Vascular Behavioral and Cognitive Disorders (VASCOG) [10] and by the Vascular Impairment of Cognition Classification Consensus Study (VICCCS) group [11,12]. Based on ample evidence, the large vessel disease combined with cardiological problems can result in unpredictably large strokes with massive brain damage. In contrast, SVD has been lately recognised as a disease that causes cortical degeneration of vascular origin (lacunar infracts and progressive white matter damage) affecting the cortex and the subcortical white matter by a progressive and gradual course that offers useful information for clinical trials and treatment development [13].

Among the sporadic forms of VCI, particular attention has been recently paid to VCI associated with subcortical small-vessel disease (SSVD). SSVD is probably the most prevalent vascular neurological lesion [14], occurring as part of a systemic dysfunction of arteriolar perfusion, affecting richly vasculated tissues such as brain, retina and kidneys [15,16]. SSVD offers a possible target for the identification and study of homogenous patient groups for the development of biomarkers [17].

Pathogenesis in SSVD

SSVD pathology includes diffuse injury of the cerebral white matter and damage of its blood supplying vessels [18,19]. Deep white matter is a borderline region vulnerable to fall of blood flow and of oxygen supply [20]. The vasculature of subcortical white matter derives from the cortex surface. The vessels become thinner as they trespass the cortex, leading to deeper regions and by the time they reach the periventricular white matter, their diameter is very small and their flow becomes unsafe [21]. Arterial hypertension plays a major role in the narrowing of vessels mainly through the thickening of the outer wall. Arterioles are the first to be influenced by developing atherosclerosis, lipohyalinosis, and fibrous necrosis [22]. Smooth muscle in the walls of vessels is characteristically replaced by collagen, and perivascular spaces are commonly enlarged [23]. The narrowed vessel becomes thicker and harder and vasodilation is hindered. As a result, the autoregulation of brain blood flow becomes insufficient [24]. The above can lead to hypoxia factor -1α production and inflammation [25]. Endothelium is damaged and small bleeds can occur. The blood brain barrier is altered and permits the entrance of pro-inflammatory plasma proteins into the brain [26]. The combination of free oxygen radicals and oxidant stress sets a constant inflammatory demyelinating procedure in standby [27]. The hypoxic and toxic environment

increases the proteolytic action with the result of myelin and axon loss [28]. Similar changes to those in cerebral white matter are often seen in deep grey matter. Veins adjacent to the lateral ventricles often have thickened walls [29]. This, in combination with diminished blood flow and hypoxia [30], has been related to the presence of White Matter Hyper-intensities (WMHs), a very common finding in T2-Flair of aged people. The link between WMHs with arterial hypertension and SSVD is long known but the significance of venous pathology in SSVD has only lately been systematically studied and related to cognitive state.

CSF biomarkers

Biochemical analysis of blood and CSF provides useful information on the biochemical changes of patients since CSF is in direct contact with the extracellular space. The biochemical study of CSF offered a great amount of knowledge in the field of AD research with the discovery of cortical neuronal degeneration markers, tau and phospho-tau proteins and markers of amyloid pathology, such as β-amyloid Aβ40 and Aβ42. Tau protein is one of the major components of intracellular neurofibrillary tangles and is considered as a neuronal/axonal degeneration marker while phospho-Tau is the pathologic hyper-phosphorylated alternative which has been associated directly with tangle formation in AD. Both of them are increased in the CSF of AD patients [31]. Beta-amyloid peptides with 40 (Aβ40) and with 42 amino acids (Aβ42) are the major components of extracellular amyloid plaques found in AD pathology. Decreased Aβ42 presents a high sensitivity and specificity (>85%) as compared to cognitively intact old subjects and, together with the Tau and Phospho-Tau, they have been incorporated in the research criteria for AD since in combination they can achieve a specificity >90% for diagnosis [32-34]. In VCI patients, normal levels of the above biomarkers offer diagnosis of exclusion (excluding the co-occurrence of AD).

SSVD Biomarkers

CSF analysis is important for patients with white matter damages in order to exclude other pathologies e.g. an inflammation, a vasculitis or multiple sclerosis. Furthermore, SSVD is mostly a pathology that evolves for decades before it expresses symptoms, if any. The research in order to establish specific biomarkers (diagnosis of inclusion) for SSVD/VCI includes several candidates, the leading of which are listed below.

Elevated CSF/blood albumin ratio (BCB/BBB disruption)

The Blood-CSF and blood-brain barriers (BCB/BBB) are selective permeability barriers located in the CNS. Their significance has to do with the need of the brain to be protected from general circulation in order to avoid injury from irritating/ toxic elements. An increase of CSF/serum albumin quotient (QA) indicates increased permeability of BCB/BBB integrity [35]. Both aging and all subtypes of VCI are associated with increased BCB/BBB permeability. Altered BCB/BBB has consistently been reported in VCI-SSVD patients, and it is thought to contribute to the pathogenic process in AD [36,37].

Altered CSF matrix metalloproteinases (extracellular matrix breakdown)

Matrix metalloproteinases (MMPs) are a family of enzymes of the extracellular matrix, among which MMP -2, -3,-7,-9,-10 and -12 are mainly active in the brain. Some MMPs are normally present in the CSF, while others (mainly MMP-3 and MMP-9) are increased only when an inflammatory response is elicited [38]. Measurement of MMPs is a promising SSVD biomarker, and has high validity in discriminating VCI-SSVD from neurodegenerative cognitive impairment [39,40].

CSF neurofilament (axonal damage)

The neurofilaments (NFs) are neuron proteins. They consist of three subunits of low (NF-L), medium (NF-M), or high (NF-H) molecular weight [41]. NF-L subunit may by a significant increase be a sensitive marker for neuronal death and axonal loss in several neurovascular/neurodegenerative disorders [42,43]. High CSF-NF-L levels were positively associated with increasing severity of White Matter Lesions in non-demented subjects [44] and with acute cerebral infarctions [45]. It is therefore suggested that NF-L could function as a marker of white matter damage indicating a neurovascular pathology especially in the absence of neurodegeneration markers. Far less is known about the rest of NF subunits, indicating a field for further research.

Blood inflammatory cytokines and adhesion molecules.

Some of the many molecules of the inflammatory pathway have been found to participate in the VCI/SSVD pathophysiology. C reactive protein (CRP) is a biomarker of systemic inflammation which has been positively related with WML [46] and an elevation of peripheral CRP is raising the risk for VCI while decreased levels indicate a healthier white matter structure [47,48]. CRP is synthesized in the liver as a response to Interleukin-6 (IL-6) blood level increase. IL-6 is secreted as a pro-inflammatory cytokine by the blood vessels and belongs to the group of Interleukins (ILs) a family of cytokines participating in the regulation of the immune system. Although numerous studies reported a positive association [49], the usefulness of IL-6 and CRP as biomarkers of SSVD, remains to be fully established [50]. Other candidate cytokines that could serve as SSVD biomarkers are CSF TNF-α, TGF-β vascular endothelial growth factor (VEGF) and CSF α1-antichymotrypsin which could all be found elevated in VCI-SSVD patients [51,52].

Conclusion

VCI-SSVD is the most common and homogeneous type of VCI but is often under-recognized in clinical practice. Its (differential) diagnosis could be empowered by specific biochemical markers which could also be useful to cast light to the pathogenicity of neurovascular disease. Additionally they could be used for identifying preclinical SSVD, in order to monitor the advance of the pathology and permitting early medical intervention. Given that the majority of persons with dementia are located in a spectrum with pure subcortical vascular disease (i.e. Binswanger's disease) at one end, and pure AD at the other, further research is required to identify the

appropriate markers that, used in combination can contribute in better understanding and treating of VCI.

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Honore Daumier. Lithograph. The physician (1835).

Greek Section

Ελληνικό Τμήμα

Commentary

Technical characteristics of Alzheimer model based on organ technology (organoid)

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Abstract

Alzheimer's disease (AD) is a serious neurodegenerative disorder that manifests itself as progressive damage to memory and knowledge and is the main cause of dementia in the elderly. AD is characterized by extracellular deposition of amyloid-β plate (Aβ) and by the formation of neurofibrillary tangles, composed of hyperphosphorylated Tau protein. These modifications lead to neuronal cell death, vascular dysfunction and inflammatory disorders. Described as "elderly disease", AD is an escalating threat to developed countries as life expectancy is increasing. Because of its severity, AD has been the subject of extensive studies that address the pathogenesis of the disease. However, its main cause remains unknown. Most research on neurological conditions has been applied to animal models. However, due to their high cost and the uncertain translation of their results to humans along with moral concerns, in recent years, there has been a growing need for in vitro modeling to mimic the brain. The creation of the aforementioned models aims at a better understanding of the factors contributing to the onset of the disease and the faster development of the treatment of diseases affecting the nervous system. Given this need, in this review, new approaches to study neurodegenerative disease were recorded. A three-dimensional (3D) neurosphere-based microfluid chip has been reported and this model imitates the in vivo microenvironment of the brain and provides a steady flow of fluid that is observed in the brain's space. Uniform neurospheres, with cell interactions and contacts in all directions, were formed in a hollow microfuge and a steady interstitial flow rate was maintained using a small pump osmotic system. In this model it was possible to control the toxic effects of amyloid-β. At the end, it was observed that the deposition of amyloid-β through an osmotic micro-pump significantly reduced the viability of the neurospheres and caused destruction of the neuronal networks. Therefore, this model was proposed as an in vitro brain model for neurodegenerative disease and high-throughput drugs.

Η νόσος Alzheimer

Ο Παγκόσμιος Οργανισμός Υγείας ορίζει την άνοια ως σύνδρομο - συνήθως χρόνιας ή προοδευτικής φύσεως - στην οποία παρατηρείται επιδείνωση των γνωστικών λειτουργιών (δηλαδή της ικανότητας επεξεργασίας των σκέψεων) πάνω από αυτό που μπορεί να αναμένεται από τη φυσιολογική διαδικασία γήρανσης. Η γνωστική εξασθένηση συνήθως συνοδεύεται από

συμπεριφορικές και συναισθηματικές αλλοιώσεις. Η σοβαρότητα των συμπτωμάτων αυξάνεται σταδιακά με την πάροδο του χρόνου. Σύμφωνα με την Alzheimer's Disease International, η άνοια έπληξε 46,8 εκατομμύρια ανθρώπους παγκοσμίως το 2015, όπου η ασθένεια Alzheimer αντιπροσώπευε 60 έως 80% των περιπτώσεων άνοιας. Επιπλέον, ο αριθμός των ασθενών αναμένεται να διπλασιαστεί σε 20 χρόνια, φθάνοντας τα 74,7 εκατομμύρια το 2030 και τα 131,5 εκατομμύρια το 2050. Η άνοια αναμένεται να είναι μία από τις πιο προβληματικές ασθένειες στις ανεπτυγμένες χώρες, προκαλώντας τεράστιες κοινωνικές και οικονομικές επιπτώσεις. Το συνολικό κόστος της άνοιας το 2015 εκτιμάται σε 818 δισεκατομμύρια δολάρια ΗΠΑ. Συνεπώς, η αποτελεσματική θεραπεία θα πρέπει να διερευνηθεί και να εφαρμοστεί το συντομότερο δυνατό [1].

Παθολογία της νόσου

Τα βασικά νευροπαθολογικά στοιχεία της AD περιγράφηκαν από τον Alois Alzheimer το 1906 και τον ίδιο περίπου χρόνο από τον Oscar Fischer [2]. Σε μακροσκοπικό επίπεδο, υπάρχει ατροφία του εγκεφάλου. Σε μικροσκοπικό επίπεδο, τα χαρακτηριστικά της νόσου είναι η απόθεση αμυλοειδών πλακών σε συνδυασμό με νευροϊνιδιακά τολύπια (tangles) και η εκτεταμένη απώλεια νευρώνων. Είναι γνωστό ότι παρατηρείται απώλεια του όγκου του εγκεφαλικού φλοιού, το οποίο είναι πιο έντονο στους κροταφικούς και βρεγματικούς λοβούς, καθώς και στις περιορισμένες περιοχές του μετωπιαίου φλοιού και της κυπαροειδούς έλικας. Ο εκφυλισμός των προαναφερθέντων περιοχών μπορεί να εξηγήσει συγκεκριμένες πτυχές της άνοιας που σχετίζονται με την ανάπτυξη του AD [1].

Όργανα σε τσιπ

Ένα όργανο σε τσιπ είναι μια συσκευή μικροκυψελικής κυτταρικής καλλιέργειας που δημιουργήθηκε με μεθόδους κατασκευής μικροτσίπ το οποίο περιέχει συνεχώς διάχυτους θαλάμους αποτελούμενους από ζωντανά κύτταρα διατεταγμένα ώστε να προσομοιάζουν τη φυσιολογία ιστού και οργάνων. Ανακεφαλαιώνοντας τις πολυκυτταρικές αρχιτεκτονικές, τις διεπαφές ιστού-ιστού, τα φυσικοχημικά μικροπεριβάλλοντα και την αγγειακή αιμάτωση του σώματος, αυτές οι συσκευές παράγουν επίπεδα ιστού και λειτουργικότητα οργάνων που δεν είναι δυνατή με συμβατικά συστήματα καλλιέργειας 2D ή 3D. Παρέχουν επίσης δυνατότητα απεικόνισης υψηλής ανάλυσης, σε πραγματικό χρόνο και in vitro ανάλυση βιοχημικών, γενετικών, μεταβολικών δραστηριοτήτων ζωντανών κυττάρων σε λειτουργικό ιστό και πλαίσιο των οργάνων. Αυτή η τεχνολογία έχει πολλές δυνατότητες να προωθήσει τη μελέτη ανάπτυξης ιστών, της φυσιολογίας των οργάνων και της αιτιολογίας της νόσου. Στο πλαίσιο της ανακάλυψης νέων φαρμάκων, αποτελεί ιδιαίτερα χρήσιμη μέθοδο για τη μελέτη των μοριακών μηχανισμών δράσης, τη δοκιμή τοξικότητας και την αναγνώριση των βιοδεικτών.

Οι συμβατικές δισδιάστατες (2D) κυτταρικές καλλιέργειες αναπτύχθηκαν σχεδόν πριν από έναν αιώνα. Παρά την αποδεδειγμένη αξία τους στη βιοϊατρική έρευνα, δεν μπορούν να υποστηρίξουν τις διαφοροποιημένες λειτουργίες του ιστού από πολλούς τύπους κυττάρων ή να προβλέψουν με ακρίβεια in vivo λειτουργίες ιστού και φαρμάκων [3]. Μέχρι σήμερα, οι περισσότερες in vitro μελέτες των νευρολογικών ασθενειών βασίζονται σε μεθόδους διδιάστατης (2D) καλλιέργειας, οι οποίες δεν έχουν επαφές και αλληλεπιδράσεις κυττάρου-κυττάρου που αποτελούν βασικά χαρακτηριστικά του τρισδιάστατου (3D) εγκεφαλικού ιστού. Οι

επαφές κυττάρων και οι αλληλεπιδράσεις είναι σημαντικές όχι μόνο για τη μορφογένεση αλλά και για την κυτταρική σηματοδότηση. Πολλές μελέτες έχουν δείξει διαφορές μεταξύ των καλλιεργειών 2D και 3D, επισημαίνοντας τη σημασία των επιδράσεων 3D καλλιέργειας σε διάφορα νευρικά κύτταρα φαινοτύπων [4-7]. Δεδομένου ότι τα κύτταρα σε μια 3D καλλιέργεια διατηρούν επαφές και αλληλεπιδράσεις κυττάρου-κυττάρου προς όλες τις κατευθύνσεις που μιμούνται την in vivo κυτταροαρχιτεκτονική, το εξωκυτταρικό περιβάλλον αυτών των κυττάρων παρέχει τη δυνατότητα χωροχρονικών κυτταρικών ερεθισμάτων, μια κατάσταση αρκετά διαφορετική από εκείνη των κυττάρων που καλλιεργούνται χρησιμοποιώντας μεθόδους 2D καλλιέργειας. Οι ιδιότητες μηχανικής σηματοδότησης κυττάρων, είναι βασικοί παράγοντες στον προσδιορισμό της μορφολογίας και της διαφοροποίησης - μια ακόμη διαφορά μεταξύ των κυττάρων σε 3D- και 2D-καλλιέργεια- [8-12].

Αυτοί οι περιορισμοί έχουν οδηγήσει στην ανάγκη για δημιουργία πιο σύνθετων μοντέλων 2D, όπως αυτά που ενσωματώνουν πολλαπλούς κυτταρικούς τύπους ή περιλαμβάνουν τη διαμόρφωση κυττάρων και σε τρισδιάστατα (3D) μοντέλα τα οποία αντιπροσωπεύουν καλύτερα την χωρική και χημική πολυπλοκότητα των ζωντανών ιστών. Οι 3D κυτταροκαλλιέργειες, που αναπτύχθηκαν πριν από 50 χρόνια [3], βασίζονται συνήθως σε υδρογέλες, οι οποίες αποτελούνται είτε από φυσική εξωκυτταρική μήτρα (ECM) μορίων ή συνθετικά πολυμερή, τα οποία διεγείρουν τα κύτταρα ώστε να πολώνονται και να αλληλεπιδρούν με τα γειτονικά. Τα τσιπ μπορούν να λάβουν πολλές μορφές, συμπεριλαμβανομένων των κυττάρων τυχαία διασκορπισμένων σε ECM ή συσσωματωμένα σε αυτοσυναρμολούμενες κυτταρικές μικροδομές γνωστές ως οργανοειδή. Τα τρισδιάστατα μοντέλα ήταν πολύ χρήσιμα για τη μελέτη της μοριακής βάσης της λειτουργίας των ιστών και την καλύτερη καταγραφή των οδών σηματοδότησης και της απόκρισης του φαρμάκου σε ορισμένες καταστάσεις ασθενειών σε σύγκριση με τα 2D μοντέλα [3].

Η μοντελοποίηση της ολοκληρωμένης ανθρώπινης φυσιολογίας in vitro είναι ένας τεράστιος στόχος με τη δυνατότητα μετασχηματισμού της βιολογικής έρευνας και τελικά της υγειονομικής περίθαλψης. Οι τρέχουσες μελέτες στηρίζονται σε μεγάλο βαθμό σε απλές ανθρώπινες κυτταρικές καλλιέργειες ή μοντέλα τρωκτικών. Η αναγωγική προσέγγιση για τον προσδιορισμό της συμπεριφοράς σε επίπεδο οργάνου στην κυτταρική καλλιέργεια είναι άμεσα κλιμακούμενη και ισχυρή. Επίσης, η προβλεπτική της ισχύς περιορίζεται από την έλλειψη βιολογικών λειτουργιών. Αντίθετα, τα ζωικά μοντέλα αναπαράγουν τη λειτουργία οργάνου και πολλαπλών οργάνων αλλά είναι εγγενώς εσφαλμένη λόγω διαφορών μεταξύ της φυσιολογίας των ζώων και του ανθρώπου. Οι πλατφόρμες οργάνων-σε-τσιπ (OOC) επιδιώκουν να συνδυάσουν το καλύτερο και από τα δύο μοντέλα, καλλιεργώντας ανθρώπινα κύτταρα σε ειδικές διαστάσεις τρισδιάστατων ιστών, σχεδιασμένες να αναπαράγουν τα πολύπλευρα κυτταρικά και εξωκυτταρικά υποδείγματα - μοριακά, δομικά και φυσικά in vivo για ένα δεδομένο σύστημα οργάνου.

Ο στόχος των OOC δεν είναι να οικοδομηθεί ένα ολόκληρο ζωντανό όργανο, αλλά μια ελάχιστα λειτουργική μονάδα ικανή να αναπαράγει ορισμένες πτυχές της ανθρώπινης φυσιολογίας με ελεγχόμενο και απλό τρόπο. Για παράδειγμα, τα κύτταρα που καλλιεργούνται σε μεμβράνες μπορούν να αναδημιουργήσουν διασυνδέσεις μεταξύ διαφορετικών ιστών, όπως η διεπιφάνεια κυψελίδων-τριχοειδών ή αιματοεγκεφαλικού φραγμού (BBB), ενώ πολυκύτταρα πρότυπα μπορούν να σχεδιαστούν για να επιτρέψουν την επικοινωνία μεταξύ διαφορετικών

τύπων κυττάρων. Για τους περισσότερους ιστούς, τα OOC πρέπει να ενσωματώνουν φυσικές δυνάμεις- υδροδυναμικές, μηχανικές και ηλεκτρικές - ώστε να επιτραπεί η ειδική για τα όργανα λειτουργικότητα και η επακόλουθη ωρίμανση που είναι αναγκαία για τη φυσιολογική συνάφεια των μετρούμενων δεδομένων. Πολλά όργανα μπορούν να ενσωματωθούν συνδέοντας μεμονωμένα OOC μέσω διαύλων μικρορευστού με αναλογίες όγκου και κατανομή ροής που μιμούνται τη φυσιολογική σύζευξη in vivo για δημιουργία in vitro μοντέλων υποσυστημάτων του ανθρώπινου σώματος [3]

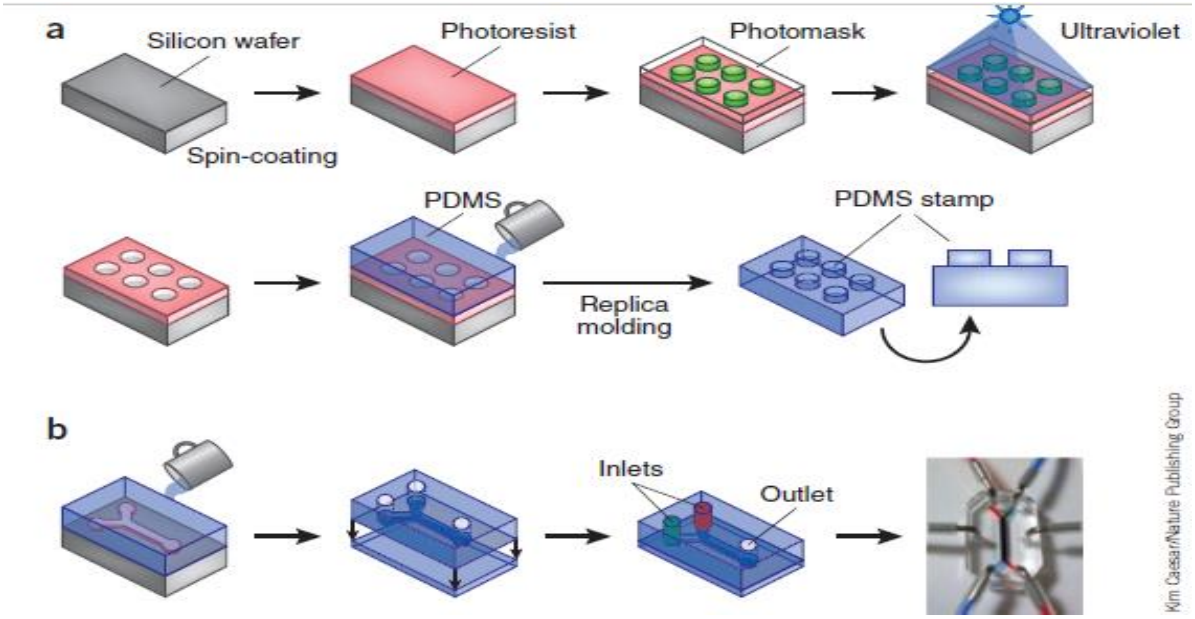
Organs-on-a-Chip: Αντιμετώπιση ανεκπλήρωτων αναγκών

Συνολικά, τα κίνητρα για τη μείωση του κινδύνου ανάπτυξης φαρμάκων και την εξατομίκευση της θεραπείας των ασθενών μπορούν να πραγματοποιηθούν μέσω της χρήσης OOC που καταγράφουν την ποικιλομορφία της ανθρώπινης γενετικής, της φυσιολογίας και της παθολογίας. Οι πρώτες προσπάθειες ενσωμάτωσης της κυτταροκαλλιέργειας σε μικρόρευστη επιφάνεια, με αποτέλεσμα τη δημιουργία των πρόδρομων OOC, εισήχθησαν το 2003 [13]. Οι σημερινές πλατφόρμες OOC, με τη χρήση μικροκυτταρικών και τρισδιάστατων κυτταροκαλλιεργειών για την ανάπτυξη μικροσκοπικών ανθρώπινων ιστών και οργάνων, μπορούν να βοηθήσουν στην επιτάχυνση της ανάπτυξης φαρμάκων με την επίλυση των αποκλίσεων στην ασφάλεια και αποτελεσματικότητα των φαρμάκων που παρατηρούνται μεταξύ των ζωικών μοντέλων, της κυτταρικής καλλιέργειας και των κλινικών μελετών. Τα ποσοτικά και μηχανιστικά στοιχεία που συλλέχθηκαν με τη χρήση των μοντέλων OOC θα μπορούσαν επίσης να φέρουν επανάσταση στις κλινικές δοκιμές, το πιο δαπανηρό και πιο επικίνδυνο στάδιο κατά το οποίο πολλά φάρμακα αποτυγχάνουν. Αντί της αντιμετώπισης των μελλοντικών ασθενών ως συλλογικής ομάδας, η χρήση κυττάρων ειδικών ασθενών επιτρέπει την καταγραφή των σημαντικών διαφορών που οφείλονται στη γενετική ποικιλομορφία, την εθνικότητα, το φύλο και την ηλικία των ασθενών. Η ίδια προσέγγιση φαρμάκων ακριβείας μπορεί να επιτρέψει την ανάπτυξη κλινικών δοκιμών *in vitro* για πληθυσμούς ασθενών με σπάνιες και παιδιατρικές ασθένειες ή να αναπτύξουν θεραπευτικά σχήματα που βελτιστοποιούνται για συγκεκριμένο ασθενή. Επιπλέον, η υιοθέτηση των OOCs από τη βιομηχανία θα διευκολύνει τις τρέχουσες προσπάθειες για τη μείωση, την τελειοποίηση και τελικά την αντικατάσταση των ζωικών μοντέλων με περισσότερες δεοντολογικές επιλογές [13].

Συσκευές καλλιέργειας μικρορευστών

Τα όργανα σε τσιπ είναι μικρορευστοί μηχανισμοί για την καλλιέργεια ζωντανών κυττάρων σε θαλάμους συνεχούς διάχυσης, με μικρομετρητές, προκειμένου να διαμορφωθούν φυσιολογικές λειτουργίες ιστών και οργάνων. Το απλούστερο σύστημα είναι ένας απλός εγχεόμενος μικρορευστοειδής θάλαμος ο οποίος περιέχει ένα είδος καλλιεργημένου κυττάρου (π.χ. ηπατοκύτταρα ή σωληνωτά επιθηλιακά κύτταρα νεφρού) το οποίο εμφανίζει λειτουργίες ενός τύπου ιστού. Σε πιο πολύπλοκα σχέδια, δύο ή περισσότεροι μικροδίαυλοι συνδέονται με πορώδεις μεμβράνες, επενδυμένοι σε αντίθετες πλευρές από διαφορετικούς κυτταρικούς τύπους, για να αναδημιουργούν διασυνδέσεις μεταξύ διαφορετικών ιστών (π.χ. διεπιφάνεια αλβιόλης-πνεύμονα ή αιματοεγκεφαλικού φραγμού). Αυτά τα συστήματα μπορούν να ενσωματώσουν φυσικές δυνάμεις, συμπεριλαμβανομένων των φυσιολογικών σχετικών επιπέδων τάσης διατμήσεως ρευστού, κυκλικής ροής και μηχανικής συμπίεσης, επιτρέποντας την ανάλυση

των αποκρίσεων εξειδικευμένων οργάνων, συμπεριλαμβανομένης της πρόσληψης κυττάρων του ανοσοποιητικού συστήματος, την αντίδραση σε φάρμακα, τοξίνες ή άλλες περιβαλλοντικές διαταραχές. Παρόμοιες αναλύσεις μπορούν να πραγματοποιηθούν με τσιπς που φέρουν κύτταρα από διαφορετικά όργανα τα οποία συνδέονται ρευστά με δύο τρόπους είτε απευθείας από ένα διάμεσο ιστό σε άλλο, είτε δυνητικά μέσω ενός δεύτερου διαύλου καλυμμένου με αγγειακό ενδοθήλιο, να μιμείται τις φυσιολογικές αλληλεπιδράσεις μεταξύ διαφορετικών οργάνων ή να μελετά την κατανομή του φαρμάκου *in vitro* [3].



Εικόνα 1: Μέθοδοι κατασκευής μικροφρεατικών τσιπ. (α) Δημιουργία αντιγράφων από πρότυπα καλούπια πυριτίου με φωτολιθογραφία. Σε ενα λεπτό ομοιόμορφο φιλμ ένα φωτοευαίσθητο υλικό επικαλύπτεται το τσιπ, το οποίο στη συνέχεια επικαλύπτεται με φωτοκασέτα (π.χ. διαφανή γυάλινη πλάκα με αδιαφανή στιβάδα χρωμίου) που φέρει μικροσκόπιο που παράγεται με λογισμικό σχεδιασμού στον υπολογιστή. Το φωτοκύτταρο προστατεύει μερικές περιοχές του τσιπ και εκθέτει άλλες στην υπεριώδη ακτινοβολία (UV) υψηλής έντασης. Το εκτεθειμένο στην υπεριώδη ακτινοβολία υλικό διαλύεται σε διάλυμα, αφήνοντας το μοτίβο χαραγμένο στο φωτοευαίσθητο υλικό. Τα ελαστομερή σφραγίσματα με επιφανειακή τοπογραφία συμπληρωματικά προς την χαραγμένη επιφάνεια δημιουργούνται με μία τεχνική χύτευσης με αντιγραφή, όπου το υγρό προπολυμερές PDMS χυτεύεται στην κορυφή του φωτοανθεκτικού υλικού και πολυμερίζεται. Η σφραγίδα PDMS μπορεί να χρησιμοποιηθεί για εκτύπωση μικροσυστοιχιών ECM μορίων σε οποιοδήποτε υπόστρωμα, συμπεριλαμβανομένων αυτών που βρίσκονται μέσα σε μικρορευστοειδείς συσκευές (που δεν φαίνονται). (β) Μικρο-ρευστή διάταξη ενός καναλιού κατασκευάζεται μέσω μιας PDMS σφραγίδας με δύο εισόδους, ένα κύριο κανάλι και μία έξοδος στεγανοποιημένη σύμφωνα με ένα επίπεδο υάλινο υπόστρωμα. Μια φωτογραφία μιας συσκευής μικροκυψελιδικής καλλιέργειας δύο θαλάμων, με κόκκινη και μπλε χρωστική, διαχέεται μέσω των άνω και κάτω καναλιών, φαίνεται στα δεξιά. Τα διαυγή πλευρικά κανάλια χρησιμοποιούνται για την εφαρμογή κυκλικής αναρρόφησης ώστε να παραμορφώνεται ρυθμικά η εύκαμπτη κεντρική μεμβράνη και τα προσκολλημένα κύτταρα [3].

Ζητήματα σχεδιασμού

Κατά το σχεδιασμό των ΟΟC, το πρώτο βήμα είναι να προσδιοριστεί το σύνολο των λειτουργικών χαρακτηριστικών του τροποποιημένου οργάνου.

Τύποι ανθρώπινων κυττάρων.

Οι τύποι ανθρώπινων κυττάρων που χρησιμοποιούνται στα ΟΟC προσδιορίζονται σε μεγάλο βαθμό από τη διαθεσιμότητα των κυττάρων και την ικανότητα σχηματισμού λειτουργικών ιστών. Στην ιδανική περίπτωση, όλες οι μονάδες οργάνων μέσα σε ένα ΟΟC δημιουργούνται από την ίδια πηγή κυττάρων. Τα πλεονεκτήματα και τα μειονεκτήματα της χρήσης πρωτογενών κυττάρων, κυτταρικών γραμμών και παραγώγων επαγόμενων πολυδύναμων βλαστοκυττάρων (iPSCs) μπορεί να ποικίλουν περαιτέρω από το σχεδιασμό ενός οργάνου στο άλλο.

Για τα περισσότερα ανθρώπινα όργανα, τα πρωτογενή κύτταρα είναι δύσκολο να ληφθούν καθώς είναι περιορισμένα σε ποσότητα και δεν μπορούν να επεκταθούν σε

καλλιέργειες, εμποδίζοντας την ανάπτυξη ΟΟC χρησιμοποιώντας κύτταρα από το ίδιο άτομο για να παρέχουν ένα γενετικά ομοιόμορφο υπόβαθρο. Το πλεονέκτημα των πρωτογενών κυττάρων είναι ότι είναι φαινοτυπικά ώριμα και λειτουργικά. Ενώ η λειτουργικότητά τους συνήθως μειώνεται με την πάροδο του χρόνου στην καλλιέργεια. Όμως, ορισμένες μέθοδοι μπορούν να βοηθήσουν στη διατήρηση του φαινοτύπου (όπως η συν-καλλιέργεια ή η διάχυση των κυττάρων. Αντίθετα, οι κυτταρικές σειρές είναι σχετικά εύκολο να καλλιεργηθούν και να επεκταθούν, αλλά τυπικά δεν έχουν τη χαρακτηριστική φαινοτυπική λειτουργία του οργάνου που προτίθενται να αντιπροσωπεύσουν. Ενώ η βελτίωση μπορεί να επιτευχθεί χρησιμοποιώντας τη διάχυση και / ή τη συν-καλλιέργεια, τα ΟΟC γενικά χρησιμοποιούν κυτταρικές σειρές όταν δεν υπάρχουν καλύτερες επιλογές.

Τα iPSCs θα μπορούσαν να είναι μια ιδανική και απεριόριστη πηγή κυττάρων για ΟΟCs καθώς προέρχονται από ένα μικρό δείγμα κυττάρων ή ιστών (όπως το αίμα), είναι εξειδικευμένα για τον ασθενή και μπορούν να επεκταθούν και να διαφοροποιηθούν επιλεκτικά σε πολλαπλές γενεές. Η χρήση μιας ενιαίας γραμμής iPSC για τη δημιουργία όλων των μονάδων ιστών σε ένα ΟΟC θα επέτρεπε τον διαχωρισμό των επιδράσεων του γονότυπου και του φαινοτύπου, καθώς οι μετρούμενες παθολογικές / φυσιολογικές αποκρίσεις θα εξαρτώνται από τη γενετική σύνθεση των κυττάρων. Η γενετική ομοιογένεια είναι επίσης επωφελής για τη μοντελοποίηση του τρόπου με τον οποίο τα φάρμακα θα επιδράσουν, συμπεριλαμβανομένων εκείνων που φέρουν μεταλλάξεις οι οποίες επηρεάζουν την αποτελεσματικότητα και την τοξικότητα του φαρμάκου. Περαιτέρω, η δημιουργία ισογονικών ελέγχων με επεξεργασία γονιδίων των iPSC για την εισαγωγή ή την αφαίρεση μίας μεταλλαγμένης από ασθένεια μετάλλαξης επιτρέπει μηχανιστικές μελέτες και ειδικούς στόχους για την ανάπτυξη φαρμάκων. Ωστόσο, δεν μπορούν να προέρχονται όλες οι κυτταρικές σειρές από την ίδια γραμμή iPSC και η ανάπτυξη ισχυρών πρωτοκόλλων για διαφοροποίηση και ωρίμανση iPSC παραμένει μια από τις προκλήσεις του τομέα. Σε γενικές γραμμές, οι iPSC κυτταρικές σειρές χάνουν το επιγενετικό σημάδι τους και δεν έχουν υποστεί την διαδικασία της ωρίμανσης. Η ωριμότητα και η φαινοτυπική σταθερότητα των κατασκευασμένων ιστών μπορεί να αυξάνεται με φυσική προετοιμασία και την ένταξη κυττάρων που υποστηρίζουν συγκεκριμένα όργανα όπως ινοβλάστες, μεσεγχυματικά βλαστοκύτταρα (MSCs) και ενδοθηλιακά κύτταρα [13].

Βιομιμητικές τιμές για τη μηχανική και την ωρίμανση λειτουργικών μονάδων ιστών και οργάνων.

Για κάθε ΟΟC, τα χαρακτηριστικά σήματα που υπάρχουν in vivo πρέπει να αναπαραχθούν *in vitro* μέσω μηχανικών μεθόδων που κατευθύνουν τη διαφοροποίηση των κυττάρων, τη συναρμολόγηση των ιστών και την λειτουργική ωρίμανση. Η πολυπαραμετρική κυτταρική καλλιέργεια βελτιώνει τις φυσιολογικές αποκρίσεις . Το μικροπεριβάλλον του ΟΟC μπορεί να σχεδιαστεί για να παρέχει παθητικά ή δυναμικά σήματα τεντώματος, ηλεκτρικά ή οπτικά, διάτμηση υγρού και βιοχημικές και ορμονικές αντιδράσεις. Η συμπερίληψη των στοιχείων περιβαλλοντικού ελέγχου που ανιχνεύουν και παράγουν τα βιοφυσικά ερεθίσματα θα επιτρέψουν τον έλεγχο ανατροφοδότησης των βιομιμητικών παραγόντων που οδηγούν τις φυσιολογικές αποκρίσεις. Η ικανότητα για εκτεταμένους χρόνους καλλιέργειας καθιστά δυνατή τη λήψη σημαντικών δεδομένων, καθώς πολλές παρενέργειες των φαρμάκων δεν είναι άμεσες. Παρομοίως, η δυνατότητα διεξαγωγής μελετών δόσης-απόκρισης μπορεί να παρέχει πληροφορίες για τον σχεδιασμό δοσολογιών του φαρμάκου [13].

Ηλεκτρονικές αναγνώσεις λειτουργιών συγκεκριμένων κυττάρων, ιστών και οργάνων.

Για δυναμικές μελέτες απόκρισης ιστών σε περιβαλλοντικά σήματα, οι διαδικτυακές αναγνώσεις της φυσικής, μεταβολικής και μοριακής κατάστασης των κυττάρων και οι ολοκληρωμένες αποκρίσεις ιστών παρουσιάζουν μεγάλο ενδιαφέρον. Τέτοιες μη καταστρεπτικές μέθοδοι επιτρέπουν τη διαχρονική μελέτη των ταυτόχρονων αποτελεσμάτων πολλαπλών μεταβλητών, τον πειραματισμό παραμέτρων σε μεγάλους χώρους και τη συνολική αξιολόγηση των θεραπευτικών παρεμβάσεων [13]. Η μέτρηση των λειτουργικών δεδομένων με οπτική απεικόνιση απαιτεί τη χρήση οπτικά διαφανών υλικά και λογισμικό επεξεργασίας εικόνας. Ιδανικά, οι ανιχνεύσεις απεικόνισης θα πρέπει να σχεδιάζονται για άμεση μετάφραση σε ρυθμίσεις βιομηχανίας υψηλής απόδοσης.

Διαμόρφωση και ενσωμάτωση των πλατφορμών ΟΟC.

Το πιο συχνά χρησιμοποιούμενο υλικό κατασκευής είναι το πολυδιμεθυλοσιλοξάνιο (PDMS), παρά τη σημαντική μη εκλεκτική απορρόφηση υδρόφοβων μορίων, συμπεριλαμβανομένου του οξυγόνου και πολλών φαρμάκων. Τα πλεονεκτήματα του PDMS περιλαμβάνουν τη βιοσυμβατότητά του, την ευκολία χρήσης για προσεγγίσεις μικροπαραγωγής, την οπτική διαφάνεια και την αυτόκαυστη αποστείρωση. Μέθοδοι που αναπτύχθηκαν για να συνεχίσουν τη χρήση του PDMS περιλαμβάνουν και θεωρήσεις σχεδιασμού που ελαχιστοποιούν την απορρόφηση φαρμάκων και την ακούσια ανάμειξη τους. Τα εναλλακτικά υλικά περιλαμβάνουν γυαλί, πολυανθρακικό και πολυουρεθάνη, καθώς και πολλά άλλα βιοσυμβατά πολυμερή [13].

Για την ευελιξία και την υψηλή απόδοση των αναλυτικών μετρήσεων, το εξωτερικό αποτύπωμα των ολοκληρωμένων πλατφορμών ΟΟC θα πρέπει να είναι συμβατό με μορφές που χρησιμοποιούνται συνήθως στην ανάπτυξη φαρμάκων (π.χ. πλάκα πολλαπλών κοιλοτήτων και γυάλινη ολίσθηση) καθώς ο εσωτερικός σχεδιασμός πρέπει να είναι συγκεκριμένος για τους ιστούς κάθε ΟΟC. Καθώς ρευστολογική ολοκλήρωση των πολλαπλών ΟΟC είναι κρίσιμη για να καταστεί δυνατή η «επικοινωνία» οργάνων-οργάνων, η ανάπτυξη μεθόδων για την ικανοποίηση αυτής της απαίτησης είναι απαραίτητη, διατηρώντας παράλληλα τη λειτουργικότητα των μεμονωμένων οργάνων που εξακολουθούν να αναπτύσσονται. Μια δημοφιλής και ευδιάκριτη προσέγγιση της χρήσης ενός κοινού μέσου ικανού να υποστηρίζει όλα τα ΟΟC μέσα στο ολοκληρωμένο σύστημα περιορίζεται σε ιστούς που έχουν ήδη ωριμάσει και είναι φαινοτυπικά σταθεροί. Εναλλακτικά, κάθε διαμέρισμα ιστού μπορεί να διαχωριστεί από την αγγειακή ροή από ένα ενδοθηλιακό φραγμό για να μιμηθεί το διαχωρισμό ιστού-ιστού στο σώμα. Με αυτόν τον τρόπο, θα μπορούσαν να διατηρηθούν ειδικά μέσα ιστού σε κάθε διαμέρισμα για να υποστηρίξουν και να ωριμάσουν τον κάθε ιστό με βέλτιστο τρόπο, ενώ παράλληλα επιτρέπουν τη διαβίβαση μεταξύ των ιστικών μονάδων μέσω αγγειακών συνδέσεων (π.χ. από κυτοκίνες και κυτταρο-εκκρινόμενα κυστίδια όπως εξωσώματα) [13].

Επειδή ο μεταβολισμός των κυττάρων μπορεί να αλλάξει από όργανο σε όργανο και με την ωριμότητα και τον χρόνο της καλλιέργειας, η βάση για τον προσδιορισμό του φυσιολογικού ρυθμού ροής κάθε οργάνου και ιστού δεν είναι απολύτως σαφής. Αρκετοί παράγοντες εξετάζονται, συμπεριλαμβανομένης της κατανομής της ροής του αίματος, των σχετικών μεγεθών των οργάνων και των μεταβολικών ποσοστών στο σώμα. Η διαμορφωσιμότητα της πλατφόρμας για να επιτρέπονται μεταβολές σε σχετικούς όγκους των μεμονωμένων ΟΟC, η σειρά με την οποία συνδέονται και οι ρυθμοί ροής ΟΟC είναι κρίσιμοι για τον προσδιορισμό της

διαμόρφωσης της ροής σε OOC πολλαπλών οργάνων [13].

Όργανα σε τσιπ και εφαρμογή τους στη νόσο Altzheimer

Παρά τα χρόνια μελέτης, πολλά παραμένουν άγνωστα για το ΚΝΣ (κεντρικό νευρικό σύστημα) - όπως ισχυρίζονται οι Pamies & al. [14]- πολύ περισσότερο από ό, τι για άλλα όργανα εξαιτίας της κυτταρικής οργάνωσης ειδικά για τον εγκέφαλο. Υπάρχει αυξανόμενη ανάγκη για *in vitro* μοντέλα νευροεκφυλιστικών ασθενειών όπως η νόσος του Alzheimer που θα επέτρεπαν την καλύτερη κατανόηση της αιτιολογίας και την ταχύτερη ανάπτυξη στρατηγικών θεραπείας. Ωστόσο, η ικανοποίηση αυτής της απαίτησης έχει ανασταλεί από την περιορισμένη ικανότητα μίμησης του in-vivo μικροπεριβάλλοντος σε ένα *in vitro* σύστημα. Έτσι, σχεδιάστηκε ένα μικρορευστό τσιπ που βασίζεται σε τρισδιάστατα (3D) νευροσφαιρίδια το οποίο μιμείται περισσότερο το in vivo μικροπεριβάλλον του εγκεφάλου παρέχοντας μια σταθερή ροή υγρού η οποίαπαρατηρείται εύκολα στον διάμεσο χώρο του εγκεφάλου. Τα ενοποιημένα νευροσφαιρίδια, με αλληλεπιδράσεις κυττάρου-κυττάρου και επαφές σε όλες τις κατευθύνσεις, σχηματίστηκαν σε κοίλες συστοιχίες μικροφρεατίων και διατηρήθηκε ένα αργό διάμεσο επίπεδο ροής χρησιμοποιώντας ένα σύστημα οσμωτικής μικροκατασκευής. Έχοντας ως βάση αυτό το σύστημα σαν πλατφόρμα, ερευνήθηκε η επίδραση της ροής στο μέγεθος νευροσφαιριδίου, το νευρικό δίκτυο και τη νευρική διαφοροποίηση.

Τα νευροσφαιρίδια που καλλιεργούνται με ροή ήταν μεγαλύτερα και σχημάτιζαν πιο εύρωστα και σύνθετα νευρικά δίκτυα από εκείνα που καλλιεργούνταν υπό στατικές συνθήκες, υποδηλώνοντας ένα αποτέλεσμα του ενδιάμεσου επιπέδου βραδείας και κυριαρχούσας ροής διάχυσης στη συνεχή μεταφορά θρεπτικών, οξυγόνου και κυτοκινών σε συνδυασμό με την απομάκρυνση των μεταβολικών αποβλήτων. Εκλέχθηκαν επίσης οι τοξικές επιδράσεις του αμυλοειδούς-β, ο σημαντικότερος παράγοντας στην εμφάνιση της νόσου του Alzheimer. Η προσθήκη αμυλοειδούς-β μέσω μιας οσμωτικής μικρο-αντλίας βρέθηκε ότι μείωνε σημαντικά τη βιωσιμότητα των νευροσφαιριδίων και προκάλεσε μεγαλύτερη καταστροφή των νευρωνικών δικτύων, σε σύγκριση με την προσθήκη αμυλοειδούς-β υπό στατικές συνθήκες. Έτσι, το τρισδιάστατο αυτό μοντέλο με την προσθήκη μικροπεριβάλλοντος τύπου *in vivo*, προτάθηκε με βάση την καλλιέργεια ως ένα in vitro μοντέλο εγκεφάλου για νευροεκφυλιστική ασθένεια και υψηλής διαλογής φαρμάκων.

Μέχρι σήμερα, οι περισσότερες *in vitro* μελέτες των νευρολογικών ασθενειών βασίζονται σε μεθόδους διδιάστατης (2D) καλλιέργειας, οι οποίες δεν έχουν επαφές και αλληλεπιδράσεις κυττάρου-κυττάρου που αποτελούν βασικά χαρακτηριστικά του τρισδιάστατου (3D) εγκεφαλικού ιστού. Ένας άλλος παράγοντας που παραβλέπεται στα τρέχοντα *in vitro* μοντέλα εγκεφάλου είναι η ροή του ενδιάμεσου υγρού. Στον εγκεφαλικό ιστό *in vivo*, το διάμεσο υγρό εξυπηρετεί την κρίσιμη λειτουργία της παροχής θρεπτικών συστατικών μέσω του εγκεφαλικού ιστού και εκκαθάρισης των μεταβολικών απορριμμάτων [15], [16]. Η διάμεση ροή στον εγκέφαλο είναι επίσης γνωστό ότι επηρεάζει την επικοινωνία κυττάρου-κυττάρου μεταξύ των μη συναπτικών νευρώνων [17]. Για τους λόγους αυτούς, *in vitro* μοντέλα εγκεφάλου σχεδιασμένα για να αντιπροσωπεύουν καλύτερα το *in vivo* περιβάλλον του εγκεφάλου δεν θα πρέπει να παραμελούν την 3D κυτοαρχιτεκτονική και τη διάμεση ροή. Οι επαφές κυττάρων και οι αλληλεπιδράσεις είναι σημαντικές όχι μόνο για τη μορφογένεση αλλά και για την κυτταρική σηματοδότηση. Πολλές μελέτες έχουν δείξει διαφορές μεταξύ των καλλιεργειών 2D και 3D,

επισημαίνοντας τη σημασία των επιδράσεων 3D καλλιέργειας σε διάφορα νευρικά κύτταρα. Δεδομένου ότι τα κύτταρα σε μια 3D καλλιέργεια διατηρούν επαφές και αλληλεπιδράσεις κυττάρου-κυττάρου σε όλες τις κατευθύνσεις που μιμούνται την *in vivo* κυτο-αρχιτεκτονική, το εξωκυτταρικό περιβάλλον αυτών των κυττάρων παρέχει τη δυνατότητα χωροχρονικών κυτταρικών διεγέρσεων, μια κατάσταση αρκετά διαφορετική από αυτή των κυττάρων που καλλιεργούνται χρησιμοποιώντας μεθόδους καλλιέργειας 2D. Ένας άλλος παράγοντας που παραβλέπεται στα τρέχοντα *in vitro* μοντέλα εγκεφάλου είναι η ροή του ενδιάμεσου υγρού. Στον ιστό του εγκεφάλου *in vivo*, το διάμεσο υγρό εξυπηρετεί την κρίσιμη λειτουργία της παροχής θρεπτικών ουσιών μέσω του εγκεφαλικού ιστού και την απομάκρυνση των μεταβολικών αποβλήτων. Η διάμεση ροή στον εγκέφαλο είναι επίσης γνωστό ότι επηρεάζει την επικοινωνία κυττάρου-κυττάρου μεταξύ των μη συναπτικών νευρώνων. Για τους λόγους αυτούς, in vitro μοντέλα εγκεφάλου σχεδιασμένα για να αντιπροσωπεύουν καλύτερα το in vivo περιβάλλον του εγκεφάλου δεν θα πρέπει να παραμελούν την 3D κυτοαρχιτεκτονική και τη διάμεση ροή [18].

Με την ανάπτυξη των μικροτεχνολογιών, μικροσυστήματα που παρέχουν in-vivo μικροπεριβάλλοντα, όπως το όργανο σε τσιπ και τα τσιπ κυττάρων, έχουν προταθεί και αρκετές μελέτες έχουν παρουσιάσει μεθόδους τρισδιάστατης καλλιέργειας. Από τα συστήματα αυτά, οι κοίλες συστοιχίες μικροκυμάτων προσφέρουν τα πλεονεκτήματα του ομοιογενούς σχηματισμού σφαιροειδούς μικροσκοπίου και του ελέγχου μεγέθους. Χρησιμοποιώντας αυτό το σύστημα, αναφέρθηκε η επίδραση της 3D καλλιέργειας στον νευρικό ιστό. Πρόσφατες μελέτες έχουν αναφέρει την ανάπτυξη συστημάτων που βασίζονται σε μικρορευστό τα οποία παρέχουν ένα ενδιάμεσο επίπεδο ροής στα κύτταρα *in vitro*. Η διάμεση ροή στον εγκέφαλο είναι πολύ αργή, με ταχύτητες που κυμαίνονται από περίπου 0,1 έως 0,3 μL/min. Οι περισσότερες αντλίες που μπορούν να διατηρήσουν αυτό το εύρος ροής είναι περίπλοκες και δαπανηρές. Ωστόσο, μια οσμωτική αντλία που αναπτύχθηκε από τους Park et al. 2015, παρέχει τέτοια αργή ροή χωρίς τη χρήση περίπλοκων συσκευών. Αυτή η σχετικά απλή συσκευή μπορεί εύκολα να χρησιμοποιείται για τον έλεγχο της ταχύτητας ροής και μπορεί να λειτουργήσει σε έναν επωαστήρα κυτταροκαλλιέργειας για αρκετές εβδομάδες χωρίς εξωτερική πηγή ενέργειας [18]. Έτσι κατασκευάστηκε ένα μιμητικό μοντέλο μικρορευστού 3D εγκεφάλου με ένα διάμεσο επίπεδο ροής συνδυάζοντας κοίλες συστοιχίες μικροβυθισμάτων με ένα σύστημα οσμωτικής μικροκατασκευής. Χρησιμοποιώντας αυτό το μοντέλο εγκεφάλου, ερευνήθηκε η επίδραση ροής στον 3D μικροσφαιριδικό νευρικό ιστό (νευροσφαιρίδια). Η ροή που παρέχεται από το οσμωτικό σύστημα μικρής αντλίας ήταν περίπου 0,15 μL/min, συγκρίσιμη με το επίπεδο της διάμεσης ροής.

Για να ερευνηθεί το αποτέλεσμα της ροής, ετοιμάστηκαν δύο τύποι μοντέλων εγκεφάλου: ένα στατικό μοντέλο (νευροσφαιρίδια καλλιεργημένα χωρίς ροή) και ένα δυναμικό μοντέλο (νευροσφαιροειδή καλλιεργημένα με ροή).Οι μεταβολές στο μέγεθος νευροσφαιριδίου και ο σχηματισμός νευρωνικού δικτύου μεταξύ τους ερευνήθηκαν τόσο στατικά όσο και δυναμικά. Για να αποδειχθούν οι δυνατότητες αυτούως ένα in vitro μοντέλο εγκεφάλου για μελέτες νευρολογικών ασθενειών, πραγματοποιήθηκε πρώτη δοκιμή των επιδράσεων του αμυλοειδούς-β σε 3D νευροσφαιρίδια καλλιεργημένα με διάμεση ροή in vitro.

Είναι εντυπωσιακό ότι με την καλλιέργεια νευροσφαιριδίων παράλληλα με και χωρίς αμυλοειδές-β κατέστη δυνατή η μίμηση του εγκεφάλου της νόσου Αλτσχάιμερ σε μια ενιαία πλατφόρμα. Το προτεινόμενο 3D brain-on-a-chip παρέχει ένα διάμεσο επίπεδο ροής που μιμείται

το in-vivo μικροπεριβάλλον και επιτρέπει μακρόχρονη in vitro παρατήρηση χωρίς την ανάγκηγια περιφερειακές συσκευές. Συνεπώς, θα μπορούσε να αποτελέσει ένα πολύτιμο in vitro μοντέλο εγκεφάλου για μελέτες που αποσκοπούν στην καλύτερη κατανόηση της παθολογίας των νευρολογικών παθήσεων ή στην ανάπτυξη στρατηγικών για θεραπεία ασθενειών όπως η νόσος του Alzheimer. Αυτό το τσιπ μπορεί να βοηθήσει στην καλύτερη κατανόηση ή παρακολούθηση συγκεκριμένων οδών στις νευροεκφυλιστικές νόσους λόγω της απλότητας σε αντίθεση με σύνθετα ζωικά μοντέλα. Επιπλέον, με περαιτέρω ανάπτυξη, θα μπορούσε να υποκαταστήσει τα ζωικά μοντέλα σε εφαρμογές ανάπτυξης φαρμάκων.

Σχεδιασμός και λειτουργία

Ο σχεδιασμός και η λειτουργία του τσιπ εμπνεύστηκε από το προηγουμένως αναφερόμενο τεχνητό ηπατικό τσιπ - artificial lung on a chip-(ALC). Η εικόνα 1 απεικονίζει την πορεία σχεδιασμού των τσιπ. Το τσιπ περιέχει κοίλα μικροφρεάτια για το σχηματισμό ομοιογενών 3D νευροσφαιριδίων με ομοιόμορφο μέγεθος. Το ωσμωτικό σύστημα μικρής αντλίας συνδέεται στην έξοδο για να παρέχει συνεχή ροή μέσου με ρυθμό 0.15 μL/min. Η ώσμωση οδηγείται από τη διαφορά συγκέντρωσης μεταξύ καθαρού απεσταγμένου νερού και διαλύματος 0,05 M πολυ-αιθυλενογλυκόλης (PEG) διαχωρισμένου με ημιδιαπερατό φιλμ σελοφάν. Ένα σωληνάκι διαμέτρου 3 cm, τυλιγμένο με εύκαμπτο πολυτετραφθορο-αιθυλένιο(Σωλήνας PTFE) (εσωτερική διάμετρος 1,0 mm, εξωτερική διάμετρος 1,5 mm) συνδέεται στην έξοδο του τσιπ για να ενεργεί ως δεξαμενή καθαρού απεσταγμένου νερού προκειμένου να διατηρεί τη ροή με την πάροδο του χρόνου. Διάλυμα 0,05 M PEG,όγκου ~ 4 ml σε τρυβλίο Petri των 35 mm παρείχαν την επίδραση ώσμωσης. Παρέχοντας τρισδιάστατη κυτταρο-αρχιτεκτονική και διάμεση ροή, το τσιπ αυτό προσεγγίζει το μικροπεριβάλλον κανονικών και AD εγκεφάλων, διευκολύνοντας τη διερεύνηση των επιδράσεων του αμυλοειδούς-β στον 3D νευρικό ιστό[18].

Κατασκευή τσιπ

Η διαδικασία κατασκευής τσιπ ακολούθησε εκείνη του ALC. Πρώτον, η κορυφή θαλάμου (ύψος, 200 μm) και η κοίλη στρώση μικροκυψελών πυθμένα χρησιμοποίησε την επιφανειακή τάση του PDMS προπολυμερούς όπως περιγράφηκε σε προηγούμενες μελέτες [19], [20]. Ένα προπολυμερές πολυδιμεθυλο σιλοξανίου (PDMS) αποτελούμενο από ένα μίγμα 10: 1 προδρόμου PDMS (Sylgard 184) και παράγοντα σκλήρυνσης αποχύθηκε στα κυλινδρικά μικροφρεάτια ώσπου να γεμιστούν πλήρως. Στη συνέχεια, το προπολυμερές απομακρύνθηκε με γυάλινη ολίσθηση, με εφαρμογή μικρής πίεσης στη μαλακή πλάκα μικροϋποδοχών PDMS. Το εναπομένον PDMS προπολυμερές σε κάθε μικροφρεάτιο στη συνέχεια σχημάτισε έναν κοίλο μηνίσκο μέσω επιφανειακής τάσης. Η τελική κοίλη δομή σχηματίστηκε με θερμική σκλήρυνση του προπολυμερούς στο φούρνο (80 ° C για 2 ώρες), μετά την οποία οι οπές εισόδου και εξόδου στον άνω θάλαμο διατρήθηκαν με μια αιχμηρή βελόνα. Τέλος, το άνω και το κάτω στρώμα συνδέθηκαν με επεξεργασία με πλάσμα οξυγόνου για 20 s. Για την ωσμωτική μικρο-αντλία, κυβικοί θάλαμοι PDMS (1 x 1 x 1 εκ.) σχεδιάστηκαν με μεμβράνη κυτταρίνης (5 × 5 mm). Η μεμβράνη κυτταρίνης συνδέθηκε με τον θάλαμο PDMS χρησιμοποιώντας το PDMS προπολυμερές ως συγκολλητικό, ακολουθώντας μία προαναφερθείσα μέθοδο [21]. Οι νευρώνες ήταν πρωτογενείς φλοιώδεις νευρώνες απομονωμένοι από εγκεφαλικές περιοχές φλοιού εμβρύων αρουραίου. Μετά την κατασκευή ενός εγκεφάλου σε τσιπ, οι Park& al. προσάρμοσαν

το συγκεκριμένο μοντέλο για να προσομοιώσουν έναν εγκέφαλο AD [18].

Το αμυλοειδές-β είναι ένα πεπτίδιο που βρέθηκε ότι εμπλέκεται στη νόσο του Alzheimer ως το κύριο συστατικό που απαντάται στις πλάκες αμυλοειδούς στους εγκεφάλους των ασθενών. Για να διερευνήσουν σωστά τη νόσο του Alzheimer, σχημάτισαν τέσσερις ομάδες με διαφορετικές συνθήκες καλλιέργειας: η πρώτη αντιστοιχούσε σε ομάδα ελέγχου με νευροσφαιρίδια χωρίς ροή, η δεύτερη αντιστοιχούσε στις ίδιες συνθήκες αλλά με προσθήκη αμυλοειδούς β και τελικά η τρίτη και τέταρτη ομάδα ήταν καλλιέργειες νευροσφαιριδίων με ροή χωρίς και με προσθήκη αμυλοειδούς-β, αντίστοιχα. Ανάλογα με την ομάδα τους, τα κύτταρα καλλιεργήθηκαν σε ένα "κανονικό" νευροσταθμικό μέσο (Gibco) για 10 ημέρες ή 7 ημέρες ακολουθούμενες από 3 ημέρες σε ένα μέσο που περιείχε αμυλοειδές-β.

Επιδράσεις της ροής στα νευροσφαιρίδια

Για να προσδιοριστούν οι συνέπειες της προσθήκης συνεχούς ροής θρεπτικών ουσιών, κυτοκινών και οξυγόνου στα κύτταρα, συγκρίθηκε η στατική με τη μη στατική ομάδα. Η πρώτη προφανής διαφορά ήταν το σφαιρικό μέγεθος. Την ημέρα 0, τα νευροσφαιρίδια και στις δύο ομάδες είχαν το ίδιο μέγεθος κατά μέσο όρο. Μετά από δέκα ημέρες, έγιναν οι ίδιες μετρήσεις και διαπιστώθηκε ότι τα νευροσφαιρίδια που καλλιεργούνται με συνεχή ροή αυξήθηκαν (τα κύτταρα έγιναν μικρότερα προς την έξοδο), ενώ τα υπόλοιπα παρέμειναν αμετάβλητα. Μια δεύτερη διαφορά μεταξύ των δύο ομάδων που φάνηκε να προκύπτει άμεσα από την παρενθετική ροή ήταν το μοτίβο σχηματισμού δικτύου στο τσιπ. Στην πραγματικότητα, η ομάδα που υπόκειται σε ροή αποκάλυψε μεγαλύτερη επέκταση νευριτών στα νευροσφαιρίδια, τα οποία συνεπάγονται άμεσα έναν πιο ισχυρό σχηματισμό νευρικού δικτύου. Αυτό επιβεβαιώθηκε από την ανοσοχρωματοποίηση της συναψίνης IIa, μιας πρωτεΐνης που ανήκει στις συνάψεις, οικογένεια πρωτεϊνών που εμπλέκονται στην απελευθέρωση των νευροδιαβιβαστών στις συνάψεις. Η ένταση αυτού του συναπτικού δείκτη ήταν πολύ υψηλότερη στο δυναμικό μοντέλο, πράγμα που σημαίνει ότι η συνεχής ροή ενισχύει επίσης το σχηματισμό της συνάψεως. Πράγματι, η χρήση δύο άλλων δεικτών: Β-ΙΙΙ τουμπουλίνη ως νευρωνικός δείκτης και δείκτης νευρικών βλαστοκυττάρων, έδειξε ότι υπό συνεχή ροή προήχθη η διαφοροποίηση των κυττάρων νευρικών προγόνων σε νευρώνες. Οι Parks et al. παρατήρησαν ότι η διάμεση ροή είναι απαραίτητη για να φέρει θρεπτικά συστατικά, κυτοκίνες και οξυγόνο στα κύτταρα και ως εκ τούτου να συμμετέχει στο σχηματισμό του συναπτικού δικτύου [18].

Επίδραση του αμυλοειδούς-β

Η προσθήκη αμυλοειδούς-β στις μισές καλλιέργειες έδωσε την δυνατότητα μελέτης της επίδρασης των πρωτεϊνών στα νευροσφαιρίδια. Χρησιμοποιήθηκε θειοφλαβίνη S, η οποία δεσμεύει πλούσιες σε β-φύλλα δομές και έτσι κηλιδώνει το αμυλοειδές-β, για να σημανθούν τα νευροσφαιρίδια και να μελετηθεί η παρουσία και η διανομή του αμυλοειδούς-β. Πρώτα απ 'όλα, παρατηρήθηκε ότι η ποσότητα του αμυλοειδούς-β που παραμένει στα νευροσφαιρίδια ήταν μεγαλύτερη στο μοντέλο δυναμικής ροής. Μετά την ποσοτικοποίηση των νεκρών κυττάρων, φάνηκε, όπως αναμενόταν, ότι οι καλλιέργειες με αμυλοειδές-β παρουσίασαν λιγότερα βιώσιμα κύτταρα, τα οποία αντιστοιχούσαν στα νευροτοξικά και αποπτωτικά αποτελέσματα αυτής της πρωτεΐνης. Η ένταση του φθορισμού αυξήθηκε μετά την επεξεργασία με αμυλοειδές-β. Επιπροσθέτως, βρέθηκαν χαμηλότερα επίπεδα συνασπιδίνης, τουμπουλίνης και νεστίνης, τα

οποία επίσης συσχετίζονται με την καταστροφή των νευρικών δικτύων [18].

Συμπέρασμα - Συζήτηση

Συνοψίζοντας στις πρόσφατες προσεγγίσεις για την μελέτη τη παθοφυσιολογίας του εγκεφάλου φαίνεται ότι έχουν κατορθώσει χρησιμοποιώντας μια βιομιμητική προσέγγιση να αναπτύξουν ένα «brain-on-a-chip» που δημιουργεί 3D κυτταρο-αρχιτεκτονική και διάμεση ροή. Θεωρείται ότι δεν έχουν υπάρξει προηγούμενες αναφορές που να περιγράφουν ένα σύστημα που συνδυάζει και τα δύο αυτά σημαντικά χαρακτηριστικά του in vivo μικροπεριβάλλοντος του εγκεφάλου. Η 3D καλλιέργεια είναι απαραίτητη για τον καθορισμό των φυσιολογικών επαφών και αλληλεπιδράσεων μεταξύ των κυττάρων και η ενδιάμεση ροή παίζει σημαντικό ρόλο όχι μόνο στην παροχή θρεπτικών συστατικών και την εκκαθάριση των μεταβολικών αποβλήτων αλλά και στη νευρική διαφοροποίηση και μορφογένεση παρέχοντας ένα συνεχές συμπλήρωμα του μέσου που περιέχει θρεπτικά συστατικά και οξυγόνο. Σύμφωνα με αυτό, τα νευροσφαιρίδια που καλλιεργήθηκαν υπό δυναμικές συνθήκες ήταν μεγαλύτερα και δημιούργησαν πιο ισχυρό νευρωνικό δίκτυο από τα νευροσφαιρίδια που καλλιεργούνται υπό στατικές συνθήκες. Το συγκεκριμένο σύστημα χρησιμοποιήθηκε για την διερεύνηση των νευροτοξικών επιδράσεων του αμυλοειδούς-β, αποδεικνύοντας μειωμένη βιωσιμότητα των κυττάρων, αυξημένη νευρική καταστροφή και συναπτική δυσλειτουργία, τα οποία είναι παθοφυσιολογικά χαρακτηριστικά της νόσου του Alzheimer in vivo. Το in vivo-ομοιάζον μικροπεριβάλλον που παρέχεται από το μικρο-ρευστό με βάση την καλλιέργεια 3D σε τσιπ έχει μεγάλες δυνατότητες ως ένα in vitro μοντέλο εγκεφάλου. Ως εκ τούτου, η συγκεκριμένη πλατφόρμα θα μπορούσε να γεφυρώσει το χάσμα μεταξύ των παραδοσιακών in vitro μοντέλων καλλιέργειας νευρικών κυττάρων και των in vivo εγκεφαλικών μελετών, που χρησιμεύουν ως πιο αξιόπιστο εργαλείο για τη μελέτη νευρολογικών παθήσεων της νόσου, στρατηγικές θεραπείας καθώς και ανάπτυξη νέων φαρμάκων.

Στην παρούσα εργασία, αναλύθηκαν δύο συνθήκες εγκεφάλου - 3D κυτταρο-αρχιτεκτονική και διάμεση ροή υγρού-. Ταυτόχρονα διερευνήθηκαν οι επιδράσεις ενός ενδιάμεσου επιπέδου ροής και τοξικότητα αμυλοειδούς-β πρωτεΐνης στα νευροσφαιρίδια, αποδεικνύοντας τη δυνατότητα αυτού του in vitro μοντέλου εγκεφάλου ως μέσω εξέτασης φαρμάκων και εργαλείο δοκιμών κυτταροτοξικότητας.

Διαπιστώθηκε σε πρώτη φάση ότι το παρενθετικό επίπεδο ροής επηρέασε την κατανομή μεγέθους νευροσφαιριδίων. Κατά την διάρκεια των 3 πρώτων ημερών μετά τη σπορά, το μέγεθος των νευροσφαιροειδών που καλλιεργήθηκαν τόσο υπό στατικές συνθήκες (ομάδα I) όσο και δυναμικά (ομάδα II) μειώθηκε ως αποτέλεσμα της συσσωμάτωσης μέσω αλληλεπιδράσεων κυττάρου-κυττάρου [18]. Ωστόσο, παρατηρήθηκαν διαφορές στις κατανομές μεγέθους των δύο ομάδων. Τα νευροσφαιρίδια της ομάδας II ήταν μεγαλύτερα σε όλα τα σημεία από τα νευροσφαιρίδια στην ομάδα I και εμφανώς αυξήθηκαν σε μέγεθος μεταξύ της 4ης και της 10ης ημέρας, ενώ το μέγεθος στην ομάδα I παρέμεινε ίδιο από την ημέρα 3 έως την ημέρα. Η διαφορά αυτή υποδηλώνει ότι ένα διάμεσο επίπεδο ροής εμπλέκεται στην επιτάχυνση της διαφοροποίησης των προγονικών κυττάρων του νευρώνα σε ώριμους νευρώνες, μια διαδικασία συνοδευόμενη από νευριτογένεση, έκφυση νευριτών και συναπτογένεση [22]. Αποτέλεσμα ήταν η αύξηση του όγκου των νευροσφαιριδίων.

Παρατηρήθηκε επίσης η παρουσία περισσότερων αμυλοειδών-β και νεκρών κυττάρων σε νευροσφαιρίδια που καλλιεργούνται υπό συνθήκες ροής. Υπό στατικές συνθήκες, η πρόσβαση του αμυλοειδούς-β στο εσωτερικό των νευροσφαιριστών, και συνεπώς η ικανότητά του να προκαλεί νευροσφαιρική αποικοδόμηση, περιορίζεται από απλή διάχυση. Ωστόσο, με ένα διάμεσο επίπεδο ροής, το διαλυτό αμυλοειδές-β μπορεί να διεισδύσει πιο βαθιά στα νευροσφαιρίδια, προκαλώντας σε περισσότερα νευρικά κύτταρα να υποβληθούν σε απόπτωση. Αυτό το εύρημα φαίνεται ότι έρχεται σε αντίθεση με τις προηγούμενες αναφορές που δηλώνουν ότι η παρενθετική κίνηση ρευστού συμβάλλει στην απομάκρυνση των διάμεσων διαλυμάτων μέσω ανταλλαγής με εγκεφαλονωτιαίο υγρό [23], [24]. Ωστόσο, υπό την συνεχή παροχή αμυλοειδούς-β σε νευροσφαιρίδια κατά τις τελευταίες 3 ημέρες καλλιέργειας. Ως εκ τούτου, χωρίς παροχή φρέσκου μέσου για τη λειτουργία του εγκεφαλονωτιαίου υγρού, δεν παρατηρήθηκε απομάκρυνση πλακών αμυλοειδούς-β. Συνεπώς, αντί να αντικρουσθούν με τις προηγούμενες μελέτες, τα αποτελέσματα αυτά ενισχύουν τη σημασία της ανταλλαγής διάμεσου υγρού με εγκεφαλονωτιαίο υγρό. Πρόσθετες μελέτες, στις οποίες παρέχεται νέο μέσο ροής μετά την παροχή αμυλοειδούς-β, απαιτούνται για την επιβεβαίωση του αποτελέσματος.

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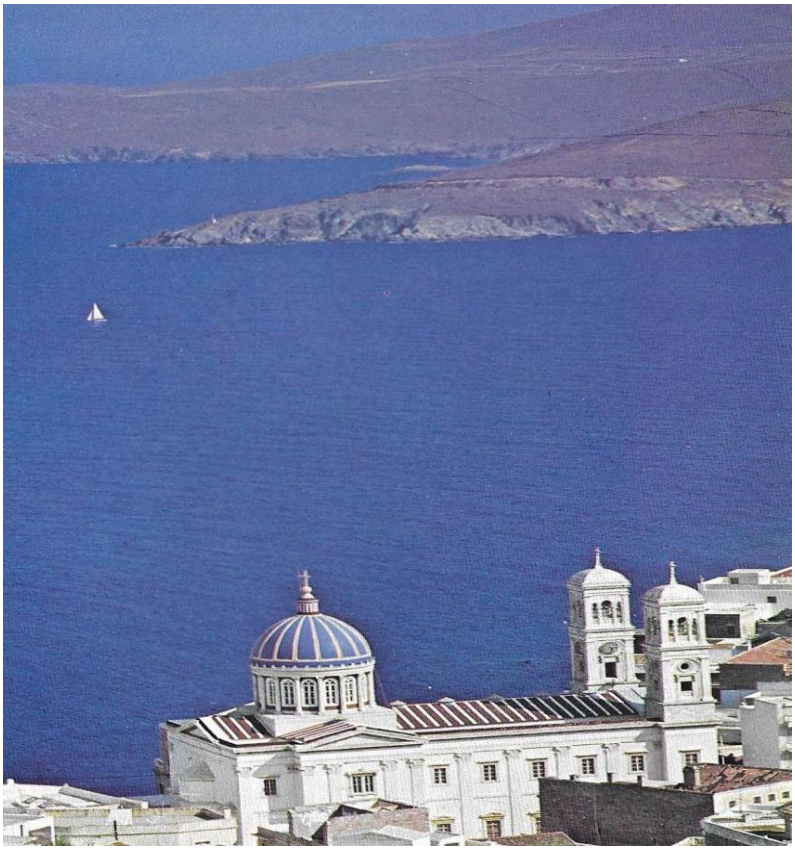

Syros

Commentary

Bioinks and *in vitro* neurovascular unit production – New prospects in Alzheimer's disease research

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Abstract

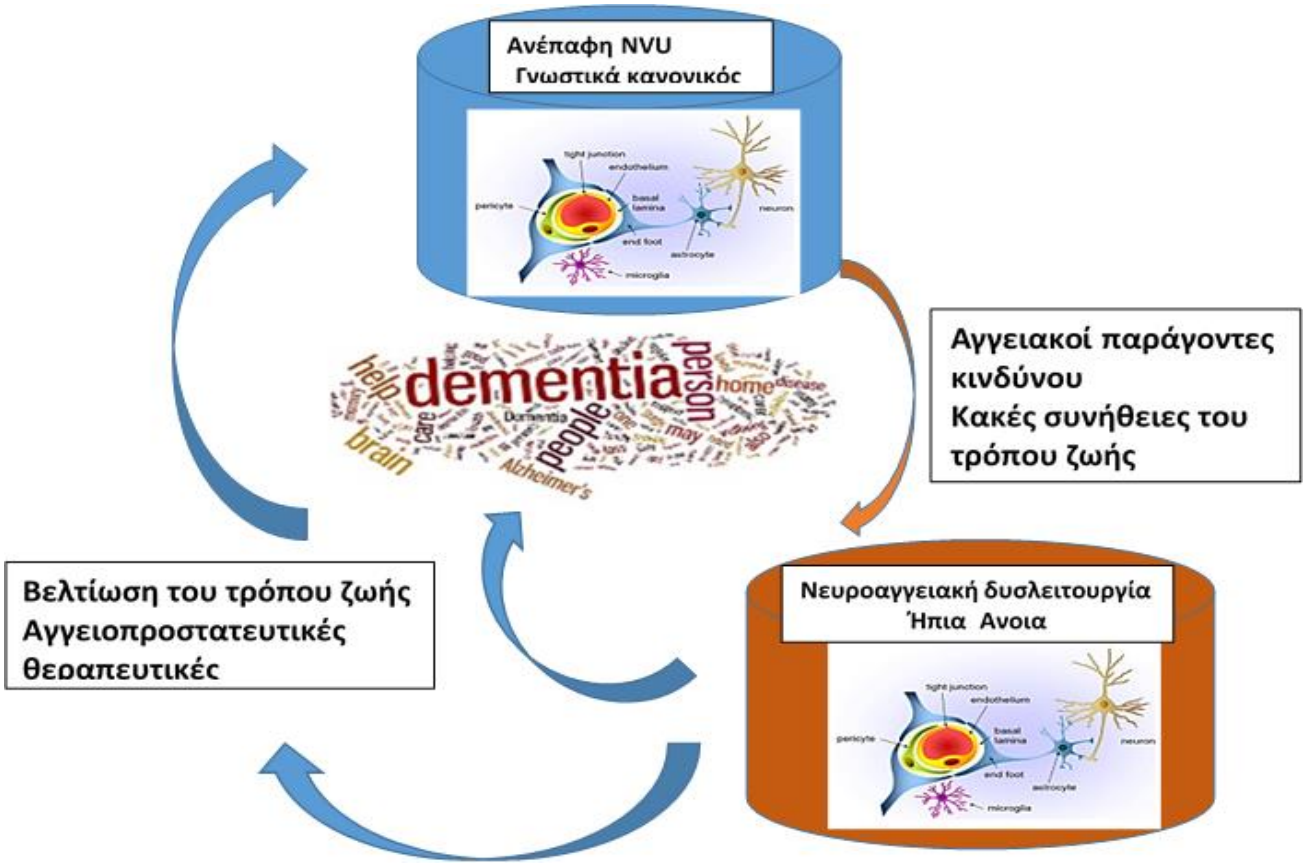
Neurovascular dysfunction is a central process in the pathogenesis of the stroke and most neurodegenerative diseases, including Alzheimer's disease. The multi-cell neurovascular unit (NVU) combines the components of the neural, vascular and extracellular matrix (ECM) into an important interface whose proper function is critical to maintaining brain health. Tissue engineering now offers new tools and information to promote understanding of NVU's operation.

A promising area for the development of NVU models is their bio-production through 3D bio-printing to produce a multi-layered NVU in which the contribution of the different cell types to neurovascular function and dysfunction can be studied at molecular and cellular levels. Nerve and vascular cells are encapsulated in a construct suitable for their viability and growth. This construct, called «bioink», is a pre-gelled biomaterial, usually with encapsulated cells, which can be bio-printed and gelled to successfully form a solid construct.

Bio-printing allows accurate placement of the neural and vascular cells to form appropriate interactions mimicking the in vivo state. Individual NVU cell types interact with the other cellular components of NVU through biochemical and physical markers, with direct and indirect interactions between neural and vascular components. The cell line sources, either derived from AD patients or healthy individuals, can be developed with the IPSCs technology. IPSCs can be obtained by different somatic cells via reprogramming strategies and further on differentiated into various cell lines that can be used to model disease, to discover new drugs and to treat cell replacement. Last but not least, the availability of 3D NVU models can also facilitate screening of drugs to correct neural dysfunction due to stroke, Alzheimer's disease and other dementia.

Εισαγωγή

Οι αγγειακές διαταραχές μπορούν να προκαλέσουν μια σειρά από μοριακά συμβάντα που οδηγούν σε νευροεκφυλισμό, γνωστική εξασθένηση και άνοια. Έχουν ήδη καταγραφεί κάποιοι κυτταρικοί και μοριακοί μηχανισμοί στα εγκεφαλικά αιμοφόρα αγγεία και τα παθοφυσιολογικά γεγονότα που οδηγούν σε εγκεφαλική διαταραχή της ροής του αίματος και διαταραχή της νευροαγγειακής μονάδας και του αιματοεγκεφαλικού φραγμού, τα οποία μπορούν όλα να συμβάλλουν στην εμφάνιση και εξέλιξη της άνοιας και της νόσου του Alzheimer (AD) [1]. Συγκεκριμένα, εξετάστηκαν επίσης η σχέση μεταξύ νευροαγγειακής δυσλειτουργίας και νευροεκφυλισμού συμπεριλαμβανομένων των επιδράσεων των γενετικών παραγόντων κινδύνου για AD στις αγγειακές λειτουργίες του εγκεφάλου, της κάθαρσης της τοξίνης του αμυλοειδούς-β πεπτιδίου του Alzheimer και της επίδρασης παραγόντων αγγειακού κινδύνου, που με τη σειρά τους μπορεί να επηρεάσουν τις συναπτικές, νευρικές και γνωστικές λειτουργίες (Σχήμα 1). Τέλος, έχουν αναφερθεί οι πιθανές πειραματικές θεραπείες για την άνοια και το Alzheimer με βάση το νευροαγγειακό μοντέλο και συζητήθηκαν μερικά κρίσιμα ερωτήματα που πρέπει να αντιμετωπιστούν από μελλοντικές μελέτες [1].



Σχήμα 1: Σχέση μεταξύ νευροαγγειακής δυσλειτουργίας και νευροεκφυλισμού συμπεριλαμβανομένων των επιδράσεων των γενετικών παραγόντων κινδύνου για AD στις αγγειακές λειτουργίες του εγκεφάλου και της επίδρασης παραγόντων αγγειακού κινδύνου αλλά και του καθημερινού τρόπου ζωής, που με τη σειρά τους μπορεί να επηρεάσουν τις συναπτικές, νευρικές και γνωστικές λειτουργίες.

Η νευροαγγειακή μονάδα είναι ένα οργανωμένο πολυκυτταρικό και πολυδύναμο δίκτυο που είναι σημαντικό για την υγεία του εγκεφάλου. Αυτή η μονάδα που εντοπίζεται στον εγκέφαλο αποτελείται από νευρικά και αγγειακά συστατικά, με τη διεπαφή και τις αλληλεπιδράσεις μεταξύ αυτών των συστατικών να είναι κρίσιμες για τη ρύθμιση της ροής του εγκεφαλικού αίματος μέσω της νευροαγγειακής σύζευξης, της λειτουργίας του αιματοεγκεφαλικού φραγμού (BBB), της νευροφλεγμονής και της νευρωνικής λειτουργίας. Η βλάβη της NVU μπορεί να οδηγήσει σε περιορισμένη μεταφορά οξυγόνου και θρεπτικών συστατικών στον εγκέφαλο, εξασθενημένη ικανότητα καθαρισμού τοξικών ενώσεων από τον εγκέφαλο και / ή να επιτρέψει τη διείσδυση επιβλαβών μορίων και ανοσολογικών κυττάρων σε όλο τον BBB, καθώς επίσης την πρόοδο της νευροαγγειακής δυσλειτουργίας και του νευροεκφυλισμού. Η σημασία των αγγειακών συνεισφορών σε μια σειρά ασθενειών του εγκεφάλου, συμπεριλαμβανομένης της νόσου του Alzheimer, της αγγειακής άνοιας, της νόσου του Parkinson και του εγκεφαλικού επεισοδίου, αναγνωρίζεται όλο και περισσότερο.

Στις προαναφερθείσες ασθένειες, η ακεραιότητα της NVU διακυβεύεται. Η αποικοδόμηση των συμπλεγμάτων των πρωτεϊνών σύνδεσης και η βασική μεμβράνη επιτρέπει τη διείσδυση συστεμικών ερυθροκυττάρων, λευκοκυττάρων και αντισωμάτων στον εγκέφαλο και την επακόλουθη διέγερση προληπτικά ενεργοποιούμενης απόκρισης των μικρογλοιακών κυττάρων και αστροκυττάρων. Η αντιφλεγμονώδης απόκριση προκαλεί περαιτέρω βλάβη του BBB και θάνατο νευρώνων ως αποτέλεσμα απομυελίνωσης και άμεση βλάβη στους νευρώνες. Αυτή η καταστροφή της NVU συμβάλλει στη νευροεκφυλισμό που είναι εμφανής στη νόσο του Alzheimer και σε άλλες μορφές άνοιας.

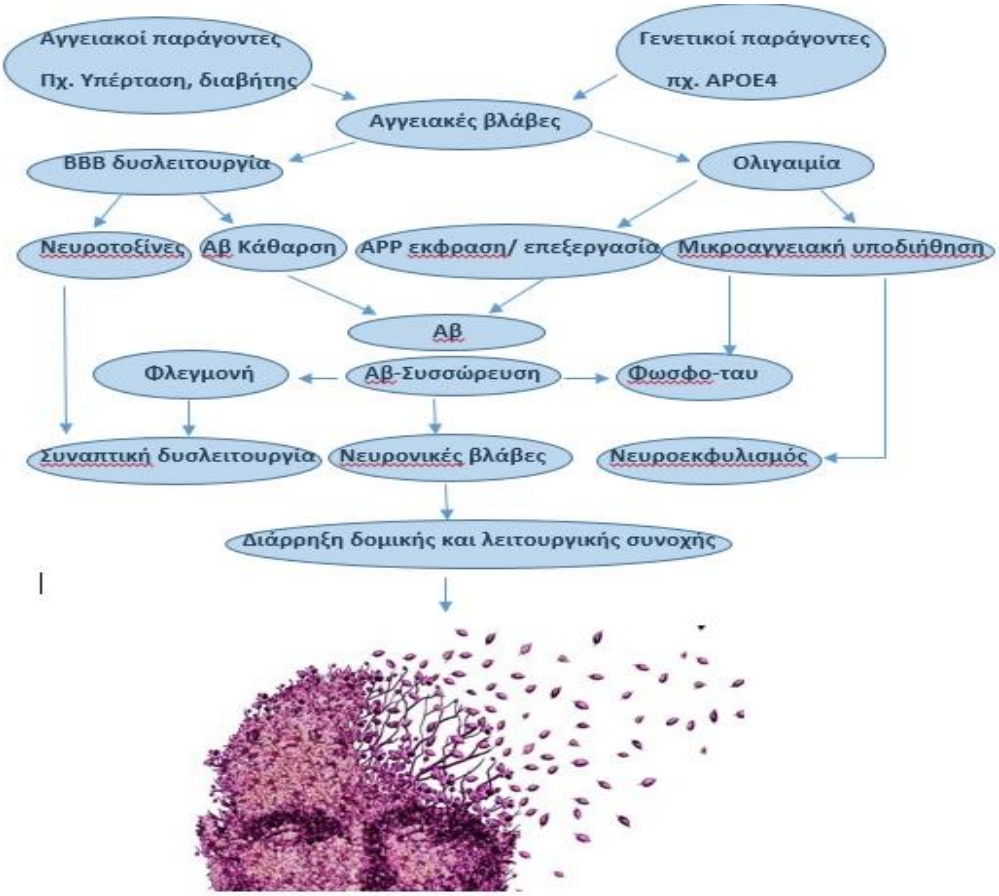
Αλτσχάϊμερ

Η AD (νόσος Alzheimer) είναι η πιο κοινή μορφή άνοιας. Τα παθολογικά χαρακτηριστικά της AD περιλαμβάνουν αυξημένο παρεγχυματικό και αγγειακό Αβ πεπτίδιο στον εγκέφαλο, υπερφωσφορυλιωμένα νευροϊνιδιακά tau πεπτίδια, γλοίωση και νευρωνική απώλεια. Επιπλέον, οι παράγοντες αγγειακού κινδύνου (π.χ. υπέρταση, διαβήτης) και ορισμένοι μείζονες γενετικοί παράγοντες κινδύνου για AD (π.χ. απολιποπρωτεΐνη Εε4 (APOE4)) οδηγούν σε εγκεφαλοαγγειακή βλάβη και εγκεφαλοαγγειακές διαταραχές που σχετίζονται με την AD [1].

Η two-hit vascular hypothesis (αγγειακή υπόθεση δύο-χτυπημάτων)

Η αγγειακή υπόθεση των δύο φάσων του AD δηλώνει ότι η εγκεφαλοαγγειακή βλάβη (φάση 1) είναι μια αρχική προσβολή που είναι ικανή να προκαλέσει νευρωνική βλάβη και νευροεκφυλισμό, αλλά μπορεί επίσης να προάγει τη συσσώρευση τοξίνης Αβ του Alzheimer στον εγκέφαλο (φάση 2). Η εγκεφαλοαγγειακή αποδιοργάνωση, συμπεριλαμβανομένης της διάσπασης του BBB και της μείωσης της ροής του αίματος στον εγκέφαλο CBF (cerebral blood flow), μπορεί να οδηγήσει στη συσσώρευση νευροτοξικών μορίων (π.χ., θρομβίνης, πλασμινογόνου, ινωδογόνου) και στην υποδιήθηση στον εγκέφαλο, αντίστοιχα, που μπορούν να προκαλέσουν άμεση νευρωνική βλάβη. Η αγγειακή δυσλειτουργία μπορεί επίσης να επηρεάσει την αμυλοειδογόνο οδό μειώνοντας την κάθαρση του Αβ και αυξάνοντας την παραγωγή του οδηγώντας σε αυξημένα επίπεδα Αβ στον

εγκέφαλο.



Σχήμα 2. Το αγγειακό μοντέλο δύο χτυπημάτων της νόσου του Alzheimer (AD). Οι αγγειακοί παράγοντες, όπως η υπέρταση και ο διαβήτης, και / ή οι γενετικοί παράγοντες κινδύνου για AD, όπως η απολιποπρωτεΐνη E4 (APOE4), μπορούν να οδηγήσουν σε εγκεφαλική βλάβη (φάση 1, πράσινα κουτιά). Εντός της ανεξάρτητης από αμυλοειδές-β πεπτίδιο (Αβ) οδού, η εγκεφαλοαγγειακή βλάβη οδηγεί σε δυσλειτουργία του αιματοεγκεφαλικού φραγμού (BBB) και συσσώρευση νευροτοξικών μορίων προερχόμενων από το αίμα από στην μια περίπτωση και ολιγαιμίας ή μειωμένου όγκου εγκεφαλικού αίματος στην άλλη. Επιπρόσθετα, μέσα στο μονοπάτι αμυλοειδογένεσης του Αβ, η δυσλειτουργία του BBB μπορεί να διαταράξει την κάθαρση του Αβ κατά μήκος του BBB και η ολιγαιμία οδηγεί σε υπερέκφραση και αυξημένη επεξεργασία της πρόδρομης πρωτεΐνης του Αβ (APP), η οποία μπορεί να προάγει την συσσώρευση Αβ στον εγκέφαλο. Οι συγκλίνουσες Αβ-ανεξάρτητες και Αβ-εξαρτώμενες οδοί μπορούν ανεξαρτήτως ή / και συνεργικά να οδηγήσουν σε συναπτική και νευρωνική δυσλειτουργία, νευροεκφυλισμό και διαταραχή της δομικής και λειτουργικής συνεκτικότητας του εγκεφάλου που τελικά οδηγεί σε άνοια [1].

Έτσι, οι Αβ-ανεξάρτητες και Αβ-εξαρτώμενες οδοί αλληλεπιδρούν και μπορούν ανεξάρτητα και / ή συνεργιστικά να οδηγήσουν στην έναρξη και εξέλιξη της άνοιας του AD. Είναι σημαντικό ότι αμφότερες οι οδοί επηρεάζονται από τους παράγοντες του αγγειακού, γενετικού, περιβάλλοντος και τρόπου ζωής (Σχήμα 2).

Χρήση διαφόρων κυττάρων, βιοϋλικών και βιοεκτύπωσης για την κατασκευή μιας 3D νευροαγγειακής μονάδας πολλαπλών συστατικών.

Η μοντελοποίηση της λειτουργίας και της δυσλειτουργίας της NVU είναι κρίσιμη για την κατανόηση της φυσιολογίας καθώς και για την αποκάλυψη των μοριακών και κυτταρικών μηχανισμών που υποκρύπτουν μια σειρά νευροαγγειακών και νευροεκφυλιστικών ασθενειών. Η ακριβής αναπαραγωγή των αλληλεπιδράσεων μεταξύ των διαφόρων τύπων κυττάρων NVU και των στοιχείων της ECM εντός του εγκεφάλου είναι σημαντική για να διασφαλιστεί η αποτελεσματικότητα ενός μοντέλου NVU.

Μια ελπιδοφόρα περιοχή για την ανάπτυξη των μοντέλων NVU είναι η βιοπαραγωγή τους μέσω της 3D βιοεκτύπωσης για την παραγωγή μιας πολυσύστατης NVU στην οποία η συμβολή των διαφόρων τύπων κυττάρων στη νευροαγγειακή λειτουργία και δυσλειτουργία μπορεί να μελετηθεί σε μοριακό και κυτταρικό επίπεδο. Σε αυτή την ανασκόπηση συζητούμε πώς μπορεί να επιτευχθεί αυτό με το συνδυασμό των διαφορετικών τύπων κυττάρων με τα κατάλληλα βιοϋλικά για να μιμηθούν την ECM και τα διάφορα πλεονεκτήματα και μειονεκτήματα της αξιοποίησης των προόδων της 3D βιοεκτύπωσης για την κατασκευή λειτουργικών μοντέλων 3D NVU.

Πρόσφατα, αποδείχθηκε ότι η εκφυλισμός των περικυττάρων οδηγεί σε νευροαγγειακή αποσύζευξη και νευροεκφυλιστικές μεταβολές. Η μικροαγγείωση δημιουργεί μια περιαγγειακή τάση, δίνοντας στα νευρικά βλαστοκύτταρα τα επιτρεπτά σημεία για νευρογένεση [2]. Οι παράγοντες που εκκρίνονται από τα ενδοθηλιακά κύτταρα, όπως ο αγγειακός ενδοθηλιακός αυξητικός παράγοντας και οι χημειοκίνες, υποστηρίζουν τη διόγκωση και διαφοροποίηση των νευρικών βλαστικών κυττάρων, καθώς και τη νευρική επιστράτευση και μετανάστευση. Στην NVU, τα αστροκύτταρα είναι ο βασικός τύπος κυττάρων που μεσολαβούν στη νευροαγγειακή σύζευξη. Τα αστροκύτταρα είναι τα πιο άφθονα νευρογλοιακά κύτταρα στον εγκέφαλο. Διαθέτουν προεκτάσεις που ονομάζονται αστροκυτταρικά πόδια μέσω των οποίων αλληλεπιδρούν τόσο με τα ενδοθηλιακά κύτταρα όσο και με τις συνάψεις στους νευρώνες, συνδέοντας φυσικά τους γειτονικούς νευρώνες με τα τριχοειδή αγγεία τους, ανιχνεύοντας αλλαγές στο περιβάλλον και προσαρμόζοντας ανάλογα τη μικροαγγειακή λειτουργία. Τα ανοσοκύτταρα, όπως τα μικρονευρογλοιακά κύτταρα, αν και δεν αποτελούν δομικό συστατικό του BBB, περιλαμβάνονται συχνά στο NVU καθώς επηρεάζουν τη λειτουργία του φραγμού ως απάντηση σε τραυματισμό και ασθένεια και έχουν ιδιαίτερη σημασία για τη μοντελοποίηση της NVU στο εγκεφαλικό επεισόδιο και τις νευροεκφυλιστικές νόσους όπου η φλεγμονή παίζει σημαντικό ρόλο στην έναρξη και την πρόοδο του νευροεκφυλισμού.

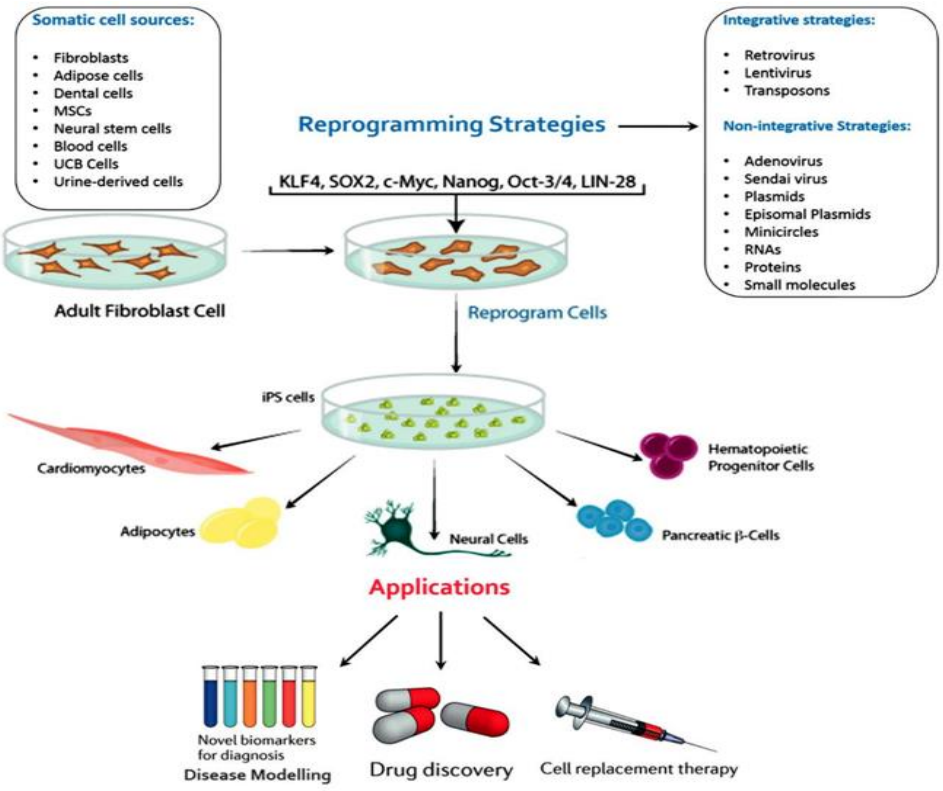
Πολλοί διαφορετικοί παράγοντες υπαγορεύουν την πηγή αυτών των κυττάρων για χρήση σε μοντέλα NVU. Αυτά περιλαμβάνουν τη διαθεσιμότητα, το κόστος, την ευκολία χρήσης και την ικανότητα αναπαραγωγής της (πάθο)φυσιολογίας που απαιτείται. Μέχρι σήμερα οι κυριότερες πηγές κυττάρων που χρησιμοποιούνται για την έρευνα NVU είναι αθανατοποιημένες κυτταρικές σειρές (π.χ., μικροαγγειακά ενδοθηλιακά κύτταρα bEnd.3 εγκεφάλου ποντικού ή κύτταρα SH-SY5Y ανθρώπινου νευροβλαστώματος) και πρωτογενή κύτταρα που ελήφθησαν από εγκέφαλο τρωκτικών (π.χ. νευρώνες) ή ανθρώπινη ομφαλική φλέβα (π.χ. ενδοθηλιακά

κύτταρα). Οι αθανατοποιημένες κυτταρικές σειρές προσφέρουν πλεονεκτήματα, όπως γρήγορη ανάπτυξη, ανθεκτικότητα, ικανότητα πολλαπλής διέλευσης και σχετικά χαμηλό κόστος για την απόκτηση και συντήρηση και είναι εξαιρετικά χρήσιμα για την αρχική βελτιστοποίηση ενός μοντέλου 3D NVU. Ωστόσο, τα αθανατοποιημένα κύτταρα συχνά δίνουν χαμηλότερες αποδόσεις σε λειτουργικές δοκιμασίες και δεν εκφράζουν πάντοτε τις ίδιες πρωτεΐνες μεταφοράς και πρωτεΐνες στενής σύνδεσης όπως in vivo και γενικά εμφανίζουν κακή λειτουργία φραγμού. Σε μοντέλα BBB, τα πρωτογενή κύτταρα έχουν αποδειχθεί ότι έχουν υψηλότερα λειτουργικά αποτελέσματα από τις αθανατοποιημένες κυτταρικές σειρές και μπορεί να αντανακλούν καλύτερα την κατάσταση in vivo. Ωστόσο, τα πρωτογενή κύτταρα είναι δύσκολο να καθαριστούν, μπορεί να αναπτυχθούν αργά και / ή να χάσουν τον φαινότυπο τους σε καλλιέργεια. Οι διαφορές των ειδών μπορούν επίσης να επηρεάσουν ένα μοντέλο NVU εάν, για παράδειγμα, ανθρώπινα ενδοθηλιακά κύτταρα συνδυάζονται με νευρώνες τρωκτικών.

Η βιοπαραγωγή ενός 3D μοντέλου NVU με την τεχνολογία της ιστομηχανικής, χρησιμοποιώντας bioinks με βάση την υδρογέλη με συγκεκριμένη χωρική κατανομή των διαφορετικών κυτταρικών τύπων και με διαφορετικούς συνδυασμούς των φυσιολογικών και νοσούντων κυττάρων NVU, θα επιτρέψει την ακριβή έρευνα της συνεισφοράς κάθε τύπου κυττάρου στη νόσο ειδικής δυσλειτουργίας, όπως η νευροαγγειακή αποσύζευξη, τα μη φυσιολογικά πρότυπα, καθώς και η διευκόλυνση της διαλογής φαρμάκων υψηλής απόδοσης για τη διόρθωση της νευροαγγειακής δυσλειτουργίας που οφείλεται στο εγκεφαλικό επεισόδιο, τη νόσο του Alzheimer και άλλες μορφές άνοιας.

IPSCs: Επαγόμενα πολυδύναμα βλαστικά κύτταρα στη νόσο του Alzheimer [3]

Η διαθεσιμότητα ανθρώπινων επαγόμενων πολυδύναμων βλαστικών κυττάρων (iPSCs, induced-pluripotent stem cells) επαναφέρει την προσέγγιση των επιστημόνων στα μοντέλα NVU, δίνοντάς τους την ευκαιρία να χρησιμοποιήσουν ανθρώπινα κύτταρα που δεν μπορούσαν στο παρελθόν (Σχήμα 3).



Σχήμα 3. Μια επισκόπηση της τεχνολογίας iPSC. Τα σωματικά κύτταρα μπορούν να ληφθούν από διάφορες πηγές, όπως το δέρμα, το αίμα και τα ούρα. Υπάρχουν πολλές στρατηγικές επαναπρογραμματισμού και οι καλύτερες είναι οι μη ολοκληρωμένες στρατηγικές. Τα iPSCs μπορούν να διαφοροποιηθούν σε διάφορες κυτταρικές σειρές που μπορούν να χρησιμοποιηθούν για τη μοντελοποίηση των νόσων, για την ανακάλυψη φαρμάκων και για τη θεραπεία αντικατάστασης κυττάρων (η εικόνα λήφθηκε από την Sharma [13]).

Οι πρόοδοι στην τεχνολογία των βλαστοκυττάρων καθιστούν δυνατή τη δημιουργία iPSCs από τους ενήλικες και τη διαφοροποίησή τους στους διάφορους τύπους κυττάρων (ενδοθηλιακά, περικύτταρα, αστροκύτταρα, νευρώνες και μικρονευρογλοιακά κύτταρα) που σχηματίζουν την NVU. Η διαθεσιμότητα εξειδικευμένων σε ασθένεια iPSCs που φέρουν την ακριβή μετάλλαξη DNA και άλλες γενετικές πληροφορίες που υπάρχουν στον ασθενή δότη χωρίς υπερέκφραση οποιωνδήποτε μεταλλαγμένων γονιδιακών προϊόντων, επιτρέπει να διερευνηθεί με ακριβή λεπτομέρεια η επίδραση της μετάλλαξης της νόσου στη δομή και λειτουργία της NVU. Μπορούν να δημιουργηθούν ισογονικοί μάρτυρες στους οποίους η ειδική για τη νόσο μετάλλαξη μεταβάλλεται στην αλληλουχία άγριου τύπου σε ένα συγκεκριμένο γονίδιο (ή αντίστροφα, όπου η αλληλουχία άγριου τύπου μεταβάλλεται στην μετάλλαξη της νόσου), επιτρέποντας την επίδραση των μεταλλάξεων στο άτομο τύπους κυττάρων που πρέπει να αξιολογηθούν. Πράγματι, η πιθανή χρησιμότητα των κυτταρικών μοντέλων iPSC που σχεδιάστηκαν για να μιμηθούν τη NVU αναγνωρίστηκε πρόσφατα ως ένα νέο βασικό ερευνητικό εργαλείο για τη μελέτη της νευροαγγειακής δυσλειτουργίας.

Πολλές ομάδες χρησιμοποίησαν και διεξήγαγαν αρκετές μελέτες σε *in vitro* μοντέλα με νευρικά και μη νευρωνικά κύτταρα που προέρχονται από iPSCs. Οι επιστήμονες κατέληξαν στο συμπέρασμα ότι οι νευρώνες που προέρχονται από iPSCs από ασθενείς με AD θα μπορούσαν να είναι αποτελεσματικοί στη διαλογή φαρμάκων, ώστε να αναπτυχθούν νέες θεραπείες που θα προστατεύουν τα κύτταρα από την τοξικότητα των Αβ πεπτιδίων στον εγκέφαλο AD [4]. Παρόμοιο αποτέλεσμα ελήφθη με νευρώνες που προέρχονται από iPSC σποραδικών AD

ασθενών και με ασθενή που φέρει την παθογόνο μετάλλαξη APP-E693Δ. Η μελέτη δείχνει ότι αυτές οι κυτταρικές σειρές παράγουν ενδοκυτταρικά Αβ ολιγομερή, καταλήγοντας σε ένα καλό κυτταρικό μοντέλο AD [5]. Τα iPSCs μπορούν να χρησιμοποιηθούν για την εισαγωγή νέων πιθανών βιοδεικτών της νόσου, όπως προτείνεται από τους Shirotani και συνεργάτες. που ανέπτυξαν μια καινοτόμο μέθοδο στους νευρώνες που διαφοροποιούνται από τα iPSCs [6].

Μια άλλη μελέτη ανέφερε τη δημιουργία ενός δικτύου πρωτεϊνών σύνδεσης που σχετίζεται με το Αλτσχάιμερ χρησιμοποιώντας iPSCs, αποδεικνύοντας ότι μπορούν να χρησιμοποιηθούν ως μοντέλο διαλογής φαρμάκων και να οδηγήσουν σε μείωση της πρωτεΐνης tau μετά από θεραπεία με αναστολέα της γ-σεκρετάσης [7]. Για τη δοκιμή φαρμάκων, είναι σημαντικό οι νευρώνες που προέρχονται από το iPSC να είναι καλά διαφοροποιημένοι, επειδή παρατηρήθηκε ότι μεταξύ των σταδίων πρώιμης και όψιμης διαφοροποίησης, τα κύτταρα έχουν διαφορετικές ευαισθησίες έναντι των φαρμάκων [8]. Η τεχνολογία επεξεργασίας του γονιδιώματος θα μπορούσε να χρησιμοποιηθεί και για τη διόρθωση των μεταλλάξεων, δημιουργώντας ένα ισογονικό έλεγχο. Για παράδειγμα, οι Pires και συνεργάτες ανέφεραν ότι η γραμμή A79V-iPSC σε συνδυασμό της σειράς A79V-GC-iPSC θα μπορούσε να χρησιμοποιηθεί για τη μελέτη παθολογικών κυτταρικών φαινοτύπων που σχετίζονται με μετάλλαξη A79V σε PSEN [9]. Είναι ενδιαφέρον το γεγονός ότι ο ρόλος των iPSCs στην έρευνα AD υποστηρίχθηκε επίσης από την ανάλυση των νευρώνων που προέρχονται από iPSCs ασθενών με σύνδρομο Down και οι οποίοι συνήθως έχουν υψηλό κίνδυνο ανάπτυξης AD νωρίς. Οι συγγραφείς διαπίστωσαν ότι τέτοια νευρικά κύτταρα αναπαράγουν το αρχικό κυτταρικό ενδεικτικό σήμα για το AD, το οποίο είναι χρήσιμο για τη μοντελοποίηση αυτής της παραλλαγής του [10].

Τέλος, και τα μη νευρωνικά κύτταρα που προέρχονται από iPSCs θα μπορούσαν να είναι πολύ χρήσιμα στην μοντελοποίηση ασθενειών και τον έλεγχο φαρμάκων. Πολλά παθολογικά ενδεικτικά σήματα βρέθηκαν να παρεκκλίνουν στα αστροκύτταρα που προέρχονται από iPSCs ασθενών με fAD και sAD υποδηλώνοντας ότι η αστροκυτταρική ατροφία θα μπορούσε να είναι ένας εύλογος μηχανισμός για πρώιμη γνωστική εξασθένηση και κατά αυτόν τον τρόπο ανοίγεται πεδίο νέων θεραπευτικών στρατηγικών για το AD [11]. Μια άλλη μελέτη ανέφερε μεταβολές στα αστροκύτταρα που προέρχονται από το iPSC που μεταλλάσσονται με PSEN1, αποκαλύπτοντας τον κύριο ρόλο αυτών των κυττάρων και επιβεβαιώνοντας τη σημασία της εφαρμογής της τεχνολογίας iPSC για τη μελέτη των νευροεκφυλιστικών ασθενειών [12].

Τύποι κυττάρων που χρησιμοποιούνται για NVU engineering in vitro.

Μια σημαντική παράμετρος κατά το σχεδιασμό ενός μοντέλου NVU είναι η επιλογή κυτταρικών στοιχείων. Τα ενδοθηλιακά κύτταρα και τα περικύτταρα είναι τα βασικά δομικά στοιχεία του αγγειακού συστατικού στην NVU. Τα ενδοθηλιακά κύτταρα στην εγκεφαλική μικροαγγείωση είναι μορφολογικά, βιοχημικά και λειτουργικά διακριτά από τα μη εγκεφαλικά ενδοθηλιακά κύτταρα. Αν και εκφράζουν συμβατικές συνδετικές πρωτεΐνες πρόσφυσης όπως η VE-cadherin τα ενδοθηλιακά κύτταρα του εγκεφάλου συνδέονται επίσης μεταξύ τους με σφιχτές συνδέσεις, οι οποίες μειώνουν την παρακυτταρική μεταφορά μεταξύ γειτονικών κυττάρων. Οι σφιχτές συνδέσεις σχηματίζονται από αλληλεπιδράσεις μεταξύ πρωτεϊνών όπως οι οκκλουδίνες, οι κλαυδίνες και τα συνδετικά μόρια πρόσφυσης. Τα περικύτταρα, μαζί με τα αγγειακά κύτταρα λείων μυών, είναι τα τοιχώματα που εντοπίζονται απευθείας στο τοίχωμα των τριχοειδών. Στον εγκέφαλο, τα περικύτταρα διαδραματίζουν ζωτικό ρόλο στο πλαίσιο της NVU υποστηρίζοντας

την αγγειογένεση, ρυθμίζοντας την τριχοειδή λειτουργία και συμμετέχοντας στο σχηματισμό και τη συντήρηση του BBB. Το ενδοθήλιο του εγκεφάλου έχει σημαντικά υψηλότερη κάλυψη περικυττάρων από τους περιφερικούς ιστούς, γεγονός που υποδηλώνει μια συγκεκριμένη εγκεφαλική λειτουργία για αυτόν τον τύπο κυττάρου.

Βιοϋλικά για NVU ικριώματα: Bioinks και μήτρες υδρογέλης

Ένα bioink είναι ένα βιοϋλικό το οποίο έχει προηγουμένως πηκτωματοποιηθεί, συνήθως με ενθυλακωμένα κύτταρα, τα οποία μπορούν να υποβληθούν σε bioprinting και να πηκτωματοποιηθούν για να σχηματίσουν επιτυχώς ένα στερεό κατασκεύασμα. Υπάρχουν πολλά διαφορετικά βιοπολυμερή που μπορούν να αναπτυχθούν σε ένα bionik για να σχηματίσουν μια μήτρα υδρογέλης για τα κύτταρα. Αυτά κυμαίνονται από φυσικές πρωτεΐνες και πολυσακχαρίτες, οι οποίοι είτε αποτελούν συστατικά της ECM είτε μιμούνται τις φυσικές ιδιότητες της ECM, σε συνθετικά βιοπολυμερή και πεπτίδια, τα οποία μπορούν να συντονιστούν ώστε να μιμούνται τη φυσική εξωκυττάρια μήτρα της νευροαγγειακής μονάδας. Ιδανικά η μήτρα υδρογέλης θα επιτρέψει στα κύτταρα να συνθέσουν και να αποθέσουν τη δική τους φυσική ECM, μιμούμενα έτσι τους φυσιολογικούς ρόλους της φυσικής ECM.

Για ένα μοντέλο NVU, το bioink πρέπει να είναι ικανό να διευκολύνει την κυτταρική μετανάστευση και προσκόλληση, αγγείωση / αγγειογένεση και νευρογένεση, καθώς και αλληλεπιδράσεις μεταξύ αγγειακών και νευρικών διεπαφών. Οι φυσικές ιδιότητες (που καθορίζονται από τη ρεολογία) είναι επίσης εξαιρετικά σημαντικές στην υπαγόρευση της αποτελεσματικότητας του bioink για την ανάπτυξη μιας ιεραρχικής NVU δομής, καθώς και στην παροχή των κατάλληλων φυσικών και μηχανιστικών χαρακτηριστικών που απαιτούνται για τα κύτταρα της NVU. Κατά την ανάπτυξη μιας 3D βιοεκτυπωμένης NVU, οι ρεολογικές παράμετροι πρέπει να εξισορροπούνται με τη λειτουργική δομή που απαιτείται από το μοντέλο. Για παράδειγμα, bioinks με υψηλότερη τάση διαρροής (και τυπικά υψηλότερη ακαμψία) τείνουν να εκτυπώνονται με καλύτερη ανάλυση από αυτούς με χαμηλότερα ιξώδη. Όμως η NVU υπάρχει μέσα σε ένα μαλακό *in vivo* περιβάλλον του 1 kPa. Η βιοεκτύπωση και η πηκτοματωποίηση σε στρώσεις παρέχουν την ευκαιρία για την προσαρμογή της διεπιφανειακής αλληλεπίδρασης μεταξύ διαφορετικών αγγειακών και νευρικών συστατικών στη NVU. Αν και ένας ταχύτερος ρυθμός πηκτοματωποίησης συσχετίζεται θετικά με τον ορισμό και την ανάλυση ενός εκτυπωμένου bioink, συσχετίζεται αντιστρόφως με την αγγειογένεση και την νευρογένεση. Οι βασικές δομικές ιδιότητες για bioinks κατάλληλα για NVU μπορούν να χωριστούν σε τέσσερις υποκατηγορίες: διασταυρούμενη σύνδεση, μηχανικές ιδιότητες, πορώδες και κυτταρική προσκόλληση.

Bioinks υδρογέλης για την ανάπτυξη NVU μητρών

Φυσικά πολυμερή

Μία κοινή στρατηγική για τη λειτουργικότητα των υδρογελών για τους σκοπούς της βιοεκτύπωσης της NVU είναι να συνδυάσει φυσικά δύο βιοπολυμερή (αντί να τα τροποποιήσει χημικά) για να παράγει ένα αναμεμιγμένο bioink με βάση την υδρογέλη, με τις πλεονεκτικές ιδιότητες κάθε πολυμερούς να συνεισφέρουν στις επιθυμητές ιδιότητες της NVU. Αυτή η τεχνική έχει χρησιμοποιηθεί εκτεταμένα για τη δημιουργία bioinks με βάση το κολλαγόνο.

Μια άλλη στρατηγική συνδυάζει περισσότερα από δύο πολυμερή για να παράγει μια υδρογέλη με πολλαπλές πλεονεκτικές ιδιότητες για μηχανική ιστών ενός μοντέλου NVU. Ένα παράδειγμα αυτού είναι ο συνδυασμός ινώδους, υαλουρονάνης (υαλουρονικού οξέος) και λαμινίνης για την παραγωγή ενός τρισδιάστατου μοντέλου για νευρικά και αγγειακά βλαστοκύτταρα. Αυτή η υδρογέλη χρησιμοποίησε τις ωφέλιμες ιδιότητες του ινώδους για τα νευρικά βλαστοκύτταρα, με διεισδυτικά δίκτυα υαλουρονικού οξέος για ενίσχυση της διασταυρούμενης σύνδεσης και για ενίσχυση των ομοιοτήτων του πηκτώματος με την φυσική ECM των νευρώνων. Τα βιοπολυμερή της υαλουρονάνης προάγουν τη μετανάστευση και τον πολλαπλασιασμό των κυττάρων και - ζωτικής σημασίας για την ανάπτυξη ενός μοντέλου NVU - προάγουν την αγγειογένεση και την νευρογένεση. Η προσθήκη λαμινίνης με τη δική της αλληλουχία IKVAV παρείχε στα κύτταρα λειτουργικές περιοχές προσκόλλησης. Αν και η υδρογέλη δεν ήταν βελτιστοποιημένη για βιοεκτύπωση, οι ρεολογικές ιδιότητές της θα μπορούσαν να προσαρμοστούν για να παράγουν ένα bioink και να χρησιμοποιούν τα ευνοϊκά για NVU συστατικά εντός της υδρογέλης.

Αντίθετα, μερικές υδρογέλες είναι βιοεκτυπώσιμες χωρίς τροποποίηση ή ανάμιξη, αλλά στερούνται της ενδογενούς περιοχής κυτταρικής-προσκόλλησης που απαιτείται για την ανάπτυξη αποτελεσματικών μοντέλων NVU. Το κόμμι Gellan είναι ένα παράδειγμα ενός πολυσακχαρίτη που χρησιμοποιείται συνήθως για την παραγωγή υδροπηκτών, τα οποία όταν δεν έχουν τροποποιηθεί και δεν έχουν τη λειτουργική περιοχή για κυτταρική προσκόλληση, αλλά μπορούν να τυπωθούν σε 3D με ένα στοιχείο διασταυρούμενης σύζευξης για να σχηματίσουν δομές NVU. Η χημική τροποποίηση του κόμμεος gellan για να περιέχει την επικράτεια κυτταρικής προσκόλλησης του IKVAV επέτρεψε τη χρήση του πολυσακχαρίτη για βιοεκτυπωμένα μοντέλα νευρικών κυττάρων του φλοιού του εγκεφάλου.

Συνθετικά βιοπολυμερή

Οι συνθετικές υδρογέλες επιτρέπουν τον πλήρη έλεγχο των παραμέτρων παραγωγής και λειτουργικών παραμέτρων, καθώς επιτρέπουν τον σχεδιασμό της ακριβούς δομικής σύνθεσης και των λειτουργειών της υδρογέλης στην προβλεπόμενη εφαρμογή. Αυτό παρουσιάζει ευκαιρίες για την ανάπτυξη bioinks κατάλληλων για NVU, καθώς οι ακριβείς ιδιότητες βιοεκτύπωσης και οι περιοχές κυτταρικής προσκόλλησης μπορούν να σχεδιαστούν στη δομή του βιοπολυμερούς. Μια κοινή τάξη συνθετικών bioinks βασιζόμενων σε βιοπολυμερή είναι τα αυτοσυναρμολογούμενα πεπτίδια, τα οποία σχηματίζουν οργανωμένα νανοϊνώδη β-φύλλα που αναπαράγουν τη δομή της φυσικής ECM. Τα εν λόγω πεπτίδια μπορούν να σχεδιαστούν ώστε να συναρμολογούνται γρήγορα και αυτόνομα μέσω αλληλεπιδράσεων φυσικής διασταυρούμενης σύζευξης ή, εναλλακτικά, μέσω επαγωγής φυσικών ή χημικών αλληλεπιδράσεων. Αυτή η βιομοριακή και κυτταρική αυτοσυναρμολόγηση είναι συνήθης *in vivo* και μπορεί να αναπαραχθεί σε κατασκευές υδρογέλης, όπου η μετανάστευση κυττάρων μέσω των πόρων στην υδρογέλη επιτρέπει αλληλεπιδράσεις κυττάρου-κυττάρου. Λόγω του συνθετικού σχεδιασμού τους, οι βιοεκτυπωτικές ιδιότητες μπορούν να προσαρμοστούν στη συνθετική υδρογέλη για να αναπτυχθεί ένα πλήρως λειτουργικό bioink και τέτοια υλικά έχουν χρησιμοποιηθεί για μελέτες κυττάρων NVU. Τα συνθετικά πεπτίδια μπορούν επίσης να αναπτυχθούν για να αναπαράγουν διάφορες πλευρές των ιστών, με πολυπεπτίδια που προσομοιάζουν αυτά της ελαστίνης σχεδιασμένα να ενσωματώνουν περιοχές αγγειογόνου πεπτιδίου για χρήση σε μελέτες 3D

καλλιέργειας αγγείων για την προώθηση της αγγειακής ανάπτυξης.

Βιοκατασκευή της NVU

Η NVU είναι πολύπλοκη, με πολλαπλά κύτταρα και αλληλεπιδράσεις κυττάρου-κυττάρου που παρουσιάζουν ιδιαίτερες προκλήσεις για την *in vitro* μοντελοποίηση. Για να απλοποιηθεί ένα μοντέλο NVU - ή για να εστιαστεί στο αγγειακό σύστημα - πολλά μοντέλα χωρίζουν τη NVU αναπτύσσοντας ένα μοντέλο μόνο για το BBB. Άλλες προσεγγίσεις είναι η χρήση μερικού συστατικού του BBB, δηλαδή, ενδοθηλιακών κυττάρων και αστροκυττάρων / πεπτιδίων / αγγειακών λείων μυϊκών κυττάρων, με νευρικά κύτταρα. Αυτές οι προσεγγίσεις δεν δίνουν το αποτέλεσμα της μοντελοποίησης ενός ολόκληρου NVU, αλλά μπορούν να είναι πολύ χρήσιμες για τη διερεύνηση του BBB και την παράκαμψη της δυσκολίας της συν-καλλιέργειας μέχρι πέντε τύπων κυττάρων σε ένα μοντέλο.

Στο πλαίσιο των εφαρμογών της ιστομηχανικής και αναγεννητικής ιατρικής, ο ορισμός της βιοκατασκευής (biofabrication) ως ερευνητικού πεδίου έχει διευκρινιστεί ως «η αυτοματοποιημένη παραγωγή βιολογικά λειτουργικών προϊόντων με δομική οργάνωση από ζωντανά κύτταρα, βιοενεργά μόρια, βιοϋλικά, συσσωματώματα κυττάρων όπως μικροιστούς ή κατασκευές υβριδικού κυτταρικού υλικού, μέσω βιοεκτύπωσης ή βιοσυναρμολόγησης και επακόλουθων διαδικασιών ωρίμανσης ιστών».

Η βιοσυναρμολόγηση χρησιμοποιεί την αυτοσυναρμολόγηση των κυττάρων για τη δημιουργία αλληλεπιδρούντων δικτύων και ιστών, όπου μπορεί να χρησιμοποιηθεί η 3D βιοεκτύπωση για την τοποθέτηση των κυττάρων σε κατάλληλη χωροχρονική θέση για συναρμολόγηση. Αυτό μπορεί να χρησιμοποιηθεί για την ανάπτυξη ενός αγγειακού συστήματος μέσω 3D βιοεκτύπωσης ή για την ανάπτυξη ιεραρχικών δομών χρησιμοποιώντας ένα θερμοπλαστικό υλικό ως δομικό υπόστρωμα μεταξύ της στρωματοποιημένης απόθεσης σχηματισμών κυττάρων προερχόμενων από ένα bioink. Η βιοσυναρμολόγηση είναι μια σημαντική στρατηγική σχεδιασμού για την ανάπτυξη μοντέλων NVU, όπου η αυτοματοποιημένη εναπόθεση κυττάρων που περιέχουν μονάδες (με τη μορφή υδροπηκτών) μπορεί να ξεκινήσει τη βιοσύνθεση των νευρωνικών αγγειακών διεργασιών και των αλληλεπιδράσεων κυττάρου-κυττάρου, οι οποίες είναι ζωτικής σημασίας για την ανάπτυξη ιεραρχικών μοντέλων NVU και την παραγωγή ενός BBB με λειτουργικό φαινότυπο.

Υπάρχουν πολλές διαφορετικές τεχνικές bioprinting, οι οποίες έχουν περιγραφεί σαφώς σε άλλα άρθρα. Δύο κύριες στρατηγικές έχουν χρησιμοποιηθεί για τη βιοεκτύπωση των μοντέλων NVU:

- έμμεση παρασκευή ικριώματος, όπου χρησιμοποιείται ένα αρνητικό καλούπι για να περικλείσει και να σχηματίσει bioinks με ενθυλακωμένα κύτταρα
- άμεση βιοεκτύπωση (bioplotting), όπου το βιολογικό στοιχείο και τα κύτταρα εκτυπώνονται απευθείας σε δομή 3D.

Και οι δύο αυτές προσεγγίσεις στο bioprinting έχουν τη δυνατότητα να παράγουν πολυκύτταρα μοντέλα 3D NVU, με καθορισμένα κυτταρικά διαμερίσματα, κρίσιμες κυτταρικές

αλληλεπιδράσεις και αγγεία.

Η τεχνική της βιοεκτύπωσης προσφέρει επίσης τη δυνατότητα εισαγωγής καναλιών μέσα στο μοντέλο NVU μέσω της χρήσης θυσιαζόμενων βιοϋλικών στην άμεση βιοεκτύπωση - επιτρέποντας την ενισχυμένη διάχυση μέσων, θρεπτικών ουσιών και οξυγόνου σε όλο το μοντέλο, μιμούμενο το τριχοειδές δίκτυο της *in vivo* NVU και αποφεύγοντας την ανάπτυξη νεκρωτικών περιοχών που παρατηρούνται σε οργανοειδείς καλλιέργειες και σε μερικές ιδιαιτέρως ινώδεις κατασκευές υδρογέλης. Αυτή η προσέγγιση εισάγει επίσης δυνητικά επιβλαβείς ή προστατευτικές ενώσεις και φάρμακα για την καταπολέμηση της δυσλειτουργίας της NVU μέσω μιας φυσιολογικώς σχετικής «συστημικής» διαδικασίας, η οποία μιμείται την εισαγωγή φαρμάκων μέσω ενδοφλέβιας χορήγησης ή από του στόματος χορήγηση.

Αποτελέσματα για μοντέλα 3D NVU

Για την αξιολόγηση της λειτουργικότητας των μοντέλων 3D NVU μπορεί να μετρηθεί μια σειρά από αποτελέσματα. Η λειτουργία του BBB μπορεί να μετρηθεί με ηλεκτρική αντίσταση trans-endothelial (TEER), η οποία χρησιμοποιεί ρεύμα μεταξύ δύο ηλεκτροδίων ως μέτρο διαπερατότητας BBB ή με κίνηση χρωματισμένης ή φθορίζουσας βαφής (π.χ. φλουορεσκεΐνη ισοθειοκυανικό-δεξτράνιο). Αν και οι τιμές TEER μετρήθηκαν *in vivo* σε εγκέφαλο αρουραίου μεταξύ 1200 και 1900 V cm^2,σε καλλιεργημένα κύτταρα το φράγμα διαπερατότητας θεωρείται αποτελεσματικό όταν η τιμή TEER είναι πάνω από ένα όριο (τυπικά 250 V.cm^2). Η ακεραιότητα των αλληλεπιδράσεων κυττάρου-κυττάρου μπορεί να μετρηθεί με μικροσκοπία ανοσοφθορισμού χρησιμοποιώντας αντισώματα έναντι ειδικών πρωτεϊνών, όπως κατά VE-καντερίνης, N-καντερίνη και Connexin 43. Εάν απαιτείται, τα βιοκατασκευασμένα μοντέλα 3D NVU μπορούν να υποβληθούν σε φυσιολογικές προσεγγίσεις ιστολογίας, όπως η ενσωμάτωση παραφίνης και ο τεμαχισμός ιστού, πριν από την ανοσοϊστοχημεία. Οι συγκεκριμένες πρωτεΐνες μπορούν να ποσοτικοποιηθούν με τη χρήση ποικιλίας τεχνικών ποσοτικού προσδιορισμού πρωτεϊνών, συμπεριλαμβανομένου του Western blot και ELISA, ενώ το mRNA μπορεί να ποσοτικοποιηθεί με ανάστροφη μεταγραφή qPCR. Μονοκυτταρικές αναλύσεις μπορούν να χρησιμοποιηθούν για να χαρακτηρίσουν συγκεκριμένους τύπους κυττάρων εντός της NVU, πέπτωντας τη μήτρα για απελευθέρωση των κυττάρων, καθαρίζοντας έναν ξεχωριστό τύπο κυττάρου από το υπόλοιπο με κυτταρομετρία ροής και στη συνέχεια χρησιμοποιώντας αλληλουχία RNA ή proteomics για να προσδιοριστούν αλλαγές ενδιαφέροντος υπό διαφορετικές συνθήκες.

Τελικές παρατηρήσεις και μελλοντικές προοπτικές

Η βιοκατασκευή ενός *in vitro* 3D μοντέλου NVU μέσω bioinks και στοιχείων βιοεκτύπωσης επιτρέπει την ανάπτυξη ενός πολυκύτταρου μοντέλου που μιμείται περισσότερο την κατάσταση in vivo, με την εισαγωγή της εκτύπωσης να επιτρέπει την παραγωγή διαδραστικών διεπαφών μεταξύ των διαφόρων κυτταρικών συστατικών. Ένα μοντέλο ιστομηχανικής που μπορεί να αναπαράγει το 3D μικροπεριβάλλον του ιστού της NVU είναι επιτακτική ανάγκη για να διασαφηνιστούν οι αλληλεπιδράσεις κυττάρου - κυττάρου και νευρώνων - αγγείων που είναι σημαντικές για τη φυσιολογική λειτουργία και τη νόσο μέσα σε μια *in vivo* NVU. Η δυνατότητα

δημιουργίας ενός *in vitro* νευρικού ιστού θα ανοίξει πολλά ερευνητικά πεδία που σήμερα δεν είναι προσεγγίσιμα, ανεξάρτητα από την ευκαιρία να μελετηθεί η 3D-χωρική σύνδεση μεταξύ διαφόρων νευρωνικών πληθυσμών και του τρόπου επικοινωνίας μεταξύ τους. Σε συνδυασμό με την τεχνολογία iPSC, μπορεί να δημιουργηθεί ένα φυσιολογικό μοντέλο για την κατανόηση των φυσιολογικών και παθολογικών μηχανισμών και την κατανόηση των μηχανισμών που πλήττονται από τις νευροεκφυλιστικές ασθένειες. Τέλος, ο συνδυασμός της τεχνολογίας της 3D βιοεκτύπωσης και της τεχνολογίας των iPSC θα ανοίξει όχι μόνο νέες δυνατότητες σε πολλά πεδία μελέτης, έλεγχο φαρμάκων, αντικατάσταση ακριβών in vivo πειραμάτων αλλά και στην εξατομικευμένη ιατρική, χάρη στη χρήση κυττάρων προερχόμενων από ασθενείς. Πιο σημαντικό, η δημιουργία ενός 3D νευρικού ιστού που αποτελείται από το κύτταρο του ασθενούς θα επιτρέψει τη λεγόμενη νευροαναγέννηση, ανοίγοντας τη δυνατότητα αντικατάστασης ενός εκφυλισμένου ιστού.

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