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Institution: San Camillo S.R.L.

Principal Investigator

The study will be conducted by Dr. Giorgio Arcara

Study sites and principal investigators

The coordinating institution for the study will be San Camillo IRCCS s.r.l., under the supervision of Dr. Giorgio Arcara.

The affiliated institutions participating in data collection and their respective principal investigators are:

1. Institution: San Camillo IRCCS s.r.l. Via Alberoni, 70, Lido, VE
   Principal investigators: Giorgio Arcara; Rachele Pezzetta; Cristina Scarpazza
2. Institution: Università Campus Bio−Medico di Roma, Via Álvaro del Portillo, 21 − 00128 Roma
   Principal investigators: Francesco Motolese, Vincenzo di Lazzaro
3. Institution: IRCCS Centro San Giovanni di Dio Fatebenefratelli Via Pilastroni, 4, 25125 Brescia BS, Italy
   Principal investigators: Cristina Festari, Michela Pievani
4. Institution: Fondazione IRCCS Istituto Neurologico Naz.le Mondino. Via Mondino, 2, 27100 Pavia PV, Italy
   Principal investigators: Valentino De Franco, Alfredo Costa

5. Institution: Istituto Neurologico "Carlo Besta" | Fondazione IRCCS. Via Giovanni Celoria, 11, 20133 Milano MI, Italy
   Principal investigators: Monica Consonni; Giuseppe Lauria–Pinter
6. Institution: Ospedale IRCCS Neuromed. Via Atinense, 18, 86077 Pozzilli (IS)
   Principal investigators: Luana Gilio, Mario Stamponi Bassi, Diego Centonze, Lorena Belli
7. Institution: IRCCS Ospedale San Raffaele (MI). Via Olgettina, 60, 20132 Milano MI, Italy
   Principal investigators: Massimo Filippi, Federica Agosta, Elisa Canu
8. Institution: IRCCS Ospedale Policlinico San Martino (GE). Largo Rosanna Benzi 10 – 16132 Genova.
   Principal investigators: Matteo Pardini, Antonio Uccelli
9. Institution: Università degli Studi G. d'Annunzio di Chieti – CH
   Principal investigators: Michela Balsamo
10. Institution: Università degli Studi di Foggia – FG
    Ricercatori responsabili: Leonardo Carlucci
11. Institution: Università degli Studi di Padova – PD
    Ricercatori responsabili: Marco Marino, Cristina Scarpazza

Study Design

A multicenter experimental study involving cognitively healthy older adults, older adults with subjective memory complaints, older adults with mild cognitive impairment, and older adults with dementia.

Study Description

This study aims to validate the instrument known as the *dynamic Neurocognitive Adaptation Scale* (dNA), derived from the identical scale (*dynamic Neurocognitive Adaptation* – dNA) previously validated in English on a sample of 815 subjects residing in the United States. All the clinical centers involved will recruit approximately 265 subjects, administer the aforementioned scale, and collect demographic and medical history information. This information will be necessary and sufficient for the first part (Stage #1) of the instrument's validation. This phase will be followed by a second part (Stage #2) focused on exploring the correlation between the dNA score and neuropsychological variables (particularly those related to memory and executive functions), with the aim of investigating a measure of adaptation that is primarily cognitive in nature. This adaptation will provide a measure of cognitive efficiency, commonly referred to in the literature as cognitive reserve or resilience. In this phase, adherence to a Mediterranean-style diet (i.e., the Mediterranean diet, assessed via a specific questionnaire) will also be explored as a protective factor against general inflammatory processes and cognitive decline associated with Alzheimer's disease (AD). During the third phase (Stage #3), a measure of neural adaptation will be explored, collecting data on neural efficiency. In the literature, this is a form of resilience or adaptation often described as neural reserve. This component will be explored using structural magnetic resonance imaging (MRI) and functional magnetic resonance imaging (fMRI).

Demographic information, medical history, dietary habits, and neuropsychological data collected by the participating centers will be compiled and managed at the Universities of Chieti and Foggia, under the coordination and responsibility of Professor Michela Balsamo (CH) and Professor Leonardo Carlucci (FG), respectively, both of whom have established expertise in statistics and psychometrics, particularly in the validation of instruments within the field of psychology. The two universities will carry out the data analysis phase aimed at validating the scale (Stage #1), also accounting for key demographic variables (age, sex, education level). Subsequently, the second phase (Stage #2) will be conducted, in which scale scores will be examined in relation to the aforementioned dietary habits and neuropsychological variables, particularly

memory and executive functions. All participating clinical centers will collect demographic, medical history, dietary habit, neuropsychological, and, where available, imaging data (MRI, fMRI). These data will be used in the third phase (Stage #3), which will focus on identifying the neural correlates of adaptation (i.e., neural efficiency) during aging. Imaging data collected by the participating clinical centers will be transferred to the University of Padua, which will oversee data receipt and analysis under the coordination and responsibility of Dr. Marco Marino, whose research focuses on the neural substrates underlying neurocognitive adaptation during aging. In this phase, both structural (e.g., volumetric and cortical thickness) and functional (e.g., resting-state fMRI) analyses will be performed. This phase will receive scientific support from the University of Leuven (Dr. Dante Mantini) and the Cleveland Clinic (Dr. Filippo Cieri); however, these institutions and their respective researchers will not have access to the data.

Hypotheses

Hypothesis #1: Investigators hypothesize that the validation results will be similar to those obtained in the U.S. validation study, with similar factor structure/dimensionality, and reliability coefficients and indices consistent with the English version.

Hypothesis #2: Investigators hypothesize a correlation between dNA and cognitive efficiency, specifically a positive correlation between scores on the dNA scale and results on neuropsychological tests of memory and executive functions.

Hypothesis #3: Finally, investigators hypothesize a correlation between dNA and improved functional connectivity (FC) at the level of so-called task-negative neural networks, such as the Default Mode Network (DMN). Investigators expect this increased FC within the DMN to be associated with a concurrent deactivation of networks FC) at the level of so-called task-negative neural networks, such as the Default Network (DN). Investigators expect this increased FC within the DN (within networks) to be associated with a concomitant deactivation of task-positive neural networks (between networks), such as the Dorsal Attention Network (DAN) and the Frontoparietal Control Network (FPCN).

Expected Duration

The expected duration of this study is 36 months.

According to the recent report by the Lancet Standing Commission 2024: Dementia Prevention, Intervention, and Care, 45% of cases of Alzheimer's disease (AD) dementia could be prevented through the adoption of behaviors that protect against neurodegenerative mechanisms (Livingston et al., 2024). Among these behaviors, physical, cognitive, and social activity play a key role. Concepts of neurocognitive reserve, resilience, resistance, plasticity, and adaptation have led to important advances in the prevention of these diseases. Engagement in physical, cognitive, creative, and social activities acts as a protective factor against neuropathological mechanisms typical of AD. A scale called *Dynamic Neurocognitive Adaptation* (dNA) was recently validated in English on a sample of 815 individuals residing in the United States (Cieri et al., accepted). This tool explores individual engagement in physical, cognitive, social, and creative activities in a dynamic manner over time, across the entire lifespan, capable of promoting specific mechanisms of neurocognitive efficiency and plasticity. The present study proposes the validation of the American scale in Italian, exploring the possibility of greater resistance to age-related pathological aging in those individuals who maintain ongoing engagement in physical, cognitive, social, and creative activities. Engagement in such activities across the lifespan may enhance neurocognitive efficiency, thereby acting as a protective factor against the most prevalent form of dementia, for which no effective pharmacological cure currently exists. Understanding the mechanisms underlying these adaptive and efficiency processes is essential for developing specific behaviors and targeted interventions for at-risk populations. A second objective is to identify crucial periods during which this type of preventive and protective intervention of a non-pharmacological nature may be most effective. Recent literature underscores the importance of a transitional phase between middle age and older age (Dohm-Hansen et al., 2024; Shen et al., 2024), highlighting the nonlinear and dynamic temporal nature of age-related changes (Ahadi et al., 2020; Dec et al., 2023; Holzscheck et al., 2020; Li et al., 2020; Timmons et al., 2019). These changes in cognitive, physical, and physiological domains deserve particular attention (Cieri et al., accepted; Nie, C. et al., 2022; Schaum et al., 2020; Wagner et al., 2023).

Imaging studies have identified a specific pattern of functional connectivity (FC) within the medial temporal regions, which make up the DN (Raichle et al., 2001), also referred to as the task-negative network. This network comprises brain regions that are active during the resting state condition, when the subject is at rest inside the scanner and not engaged in performing any cognitive or motor task. The regions within the DN are associated with episodic and autobiographical memory, which are linked to the individual's sense of self (Cieri, 2022; Cieri and Esposito, 2018; 2019; Qin and Northoff, 2011; Yeshurun et al., 2021). Importantly, posterior components of the DN (such as the posterior cingulate cortex and the precuneus) are among the

earliest sites of amyloid plaques, a hallmark of this form of dementia (Sperling et al., 2009), and are thought to be responsible for the characteristic cognitive decline, especially in mnemonic functions. In cognitively healthy individuals, spontaneous brain activity should exhibit a spatiotemporal activation pattern consistent with the DN organization. This activation pattern should also show a negative correlation (anti-correlation) with the activation of the so-called task-positive networks such as the DAN and the FPCN, which are typically active during attention-demanding cognitive tasks (Esposito et al., 2019). With advancing age, this coordinated pattern of activation and deactivation – and the balance between correlation and anticorrelation – tends to deteriorate (Spreng et al., 2016). Such disruptions are especially pronounced in individuals with mild cognitive impairment (MCI) (Esposito et al., 2019), and have been identified as a prodromal sign of Alzheimer's disease (AD).

Objectives

The project has the following objectives:

Objective #1: The primary objective of the study is the Italian validation of the scale known as dNA on an elderly population (+65 years); the dNA Scale as already been validated in English on an elderly population (+65 years) residing in the United States.

Objective #2: The second objective is to examine the association between the results derived from the scale and cognitive measures; specifically, episodic memory and executive functions which are commonly impaired in neurodegenerative conditions such as AD will be further explored in order to investigate the relationship between the dNA score and cognitive efficiency.

Objective #3: The third objective is to examine the association between the results derived from the scale and neurocognitive measures with neural network activations in the resting state condition (resting-state fMRI, rs-fMRI). Specifically, the state of FC of the DN network associated with the deactivation of the so-called task-positive networks such as the DAN and the FPCN will be explored in order to measure a form of neural efficiency.

The study—both the original American study and its Italian validation and extension, which is the subject of this application—focuses on exploring so-called protective factors in cognitive aging, especially pathological aging, particularly as it pertains to AD. The scale in question examines participants' engagement in activities

known to act as protective factors against the pathological mechanisms typical of AD. Although the investigation of dietary habits —specifically those typical of a Mediterranean diet—is of a more exploratory nature, the study also aims to investigate the role of adherence to a Mediterranean-style diet (typically Italian), which has demonstrated a protective role against inflammatory mechanisms also typical of AD.

The present study explores neurocognitive adaptation mechanisms commonly conceptualized in the literature as resilience and reserve. Investigators will explore this form of neurocognitive adaptation into an initial form of cognitive efficiency, which will be assessed through the use of neuropsychological testing focused on memory and executive functions. In this context, investigators hypothesize a correlation between dNA scores and greater cognitive efficiency. The second form of efficiency, of a neural nature, will be explored through the use of functional magnetic resonance imaging (fMRI). Specifically, investigators expect improved neurophysiological efficiency in terms of spontaneous activity in the DN, which is negatively correlated with activation of the DAN and FPCN, in association with dNA scores.

Participants recruited at the participating clinical centers will undergo a clinical interview, during which demographic and medical history information will be collected, and the dNA will be administered, along with the questionnaire regarding adherence to dietary habits typical of a Mediterranean diet (14-Item Mediterranean Diet Adherence Screener; MEDAS). Subsequently, a neuropsychological assessment will be conducted, in which the subjects' general cognitive function will be evaluated, with particular focus on episodic memory and executive functions. The subjects will complete the following tests: Mini-Mental State Examination (MMSE) (or, alternatively, Montreal Cognitive Assessment, MoCA), Rey Auditory Verbal Learning Test (RAVLT), Trial Making Test (TMT) Form B, Digits Forward and Backward subtest (WAIS or WAIS-III), and Stroop Test. These measures will provide a global cognitive assessment (MMSE, MoCA) and more specific measures of memory and executive functions. Participants will also complete a survey designed to assess depressive symptoms using the Geriatric Depression Scale (GDS) and anxiety symptoms using the Geriatric Anxiety Scale (GAS) (or STAI, State and Trait Anxiety Index). Finally, the Cognitive Reserve Index Questionnaire will be administered to estimate Cognitive Reserve (CRIq).

Generalizability

The association of this scale with demographic and neurocognitive variables, on the one hand, and neural variables (neural structures and functions) on the other, provides crucial insights into the study of the pathophysiological mechanisms typical of AD. Lifetime engagement in the activities investigated by the scale under study will be useful for exploring concepts of neurocognitive adaptation, known in the literature as

cognitive reserve and resilience during aging. The association of this scale with cognitive variables (memory and executive functions) and subsequently neural variables (e.g., activation of neural networks via rs-fMRI) could reveal protective mechanisms of neurocognitive plasticity and efficiency which are vital to research in this field, identifying not only specific behaviors and targeted interventions but also crucial periods for preventive and protective, non-pharmacological interventions.

Methodology

Sample Description

The study plans to recruit 265 participants, divided into cognitively healthy individuals (HC), individuals with subjective memory complaints (SMC), individuals with mild cognitive impairment (MCI), individuals with AD (Alzheimer's disease), with possible AD, or probable AD. Each center will recruit approximately 30 subjects. The total number of centers is twelve (12); three (3) centers will not conduct clinical activities, namely the universities of Chieti, Foggia, and Padua, but will handle data analysis (Universities of Chieti and Foggia will handle behavioral data, while University of Padua imaging data), without participating in the recruitment phase. Eight (8) centers will actively participate in recruitment, enrolling approximately 30 subjects per center (total of approximately 265 subjects). The homogeneity of the sample (in diagnostic terms) is not relevant for validation purposes, provided that participants do not have established forms of dementia. Greater homogeneity among diagnostic groups is more desirable for the subsequent phases of the project, but even in this case, it is not decisive because the study's objective is the association of the scale with the mentioned variables, not the comparison between diagnostic groups, provided that the diagnostic categories are clear.

According to the literature, the number of participants required to validate a 20-item scale, which has already been validated in another language (specifically in English on a sample of 815 subjects residing in the United States), is approximately 250 subjects (Comrey and Lee, 1992). Approximately 265 participants will be recruited, and this number will be experimentally verified during the validation phase using a specific index (Kaiser-Meyer-Olkin; KMO) to confirm the appropriateness of the sample (Kaiser, 1974). As mentioned, the subjects used for the validation phase will be HC, people with SMC, subjects with MCI, or patients with AD, probable AD, or possible AD. The participating clinical centers may also recruit subjects with different diagnoses or suffering from other forms of dementia, but these patients will not be used for the first phase (Stage #1) of validation. However, these patients may be used for subsequent phases.

The subjects recruited for the validation phase (Stage #1) will be HC, individuals with SMC, individuals with MCI, individuals with AD, with possible AD, or probable AD (even without confirmation by cerebrospinal fluid (CSF) assays, imaging, or plasma assays; McKhann et al., 1984; https://www.centroalzheimer.org/area-familiari/la-malattia-di-alzheimer/malattia-di-alzheimer/criteridiagnostici/). For the next two phases (Stage #2 & Stage #3), patients with other forms of dementia may be recruited. In all three phases (Stage #1, Stage #2, and Stage #3), subjects with depressive or anxiety disorders, as assessed by the Geriatric Depression Scale (GDS) and the State-Trait Anxiety Inventory (STAI), will be eligible for recruitment, provided that the diagnosis is clearly specified during the clinical assessment, including the onset of symptoms.

Inclusion Criteria (Stage #1)

Cognitively healthy individuals (HC):

- age ≥ 65 years, residing in Italy;
- MMSE score between 24 and 30 inclusive (alternatively, MoCA: score between 26 and 30 inclusive);
- not depressed, not with MCI, and not affected by any form of dementia;
- episodic memory score adjusted for years of schooling (Wechsler Memory Scale Logical Memory II ≥9 for 16 years of schooling or more; ≥5 for 8–15 years of schooling, ≥3 for 0–7 years of schooling; or alternatively for Prose Memory Test with scores ≥9 for ≥16 years of schooling → ≥5 items in immediate or delayed recall; ≥5 for 8–15 years of schooling → ≥3–4 items in immediate or delayed recall; ≥3 for 0–7 years of schooling → ≥2 items in immediate or delayed recall).

Individuals with Subjective Memory Complaints (SMC):

- age ≥ 65 years, residing in Italy;
- MMSE score between 24 and 30 inclusive (alternatively MoCA: score 26–30 inclusive);
- a significant memory impairment, reported by the subject, a family member, or the clinician;
- not depressed, not with MCI, and not affected by any form of dementia;
- episodic memory with a score on the Wechsler Memory Scale Logical Memory II adjusted for years of schooling (≥9 for 16+ years of schooling, ≥5 for 8–15 years of schooling, ≥3 for 0–7 years of schooling or, alternatively, on the Prose Memory Test with scores ≥9 for ≥16 years of schooling → ≥5 items in immediate or delayed recall; ≥5 for 8–15 years of schooling → ≥3–4 items in immediate or delayed recall; ≥3 for 0–7 years of schooling → ≥2 items in immediate or delayed recall).

Individuals with mild cognitive impairment (MCI):

- age ≥ 65 years, residing in Italy;
- MMSE score between 19 and 23 inclusive (alternatively MoCA);

- a decline in memory reported by the subject, a family member, or the clinician;
- not depressed, and not affected by any form of dementia, with preserved ability in activities of daily living;
- objective episodic memory loss on the Wechsler Memory Scale Logical Memory II adjusted for years of schooling (≥9 with 16 or more years of schooling, ≥5 for 8–15 years of schooling, ≥3 for 0–7 years of schooling).

Individuals with <u>probable Alzheimer's disease (AD)</u>:

- Insidious onset: symptoms developed gradually over the course of months
- A decline in performance compared to the previous level of functioning is evident, as also described by a caregiver (often a family member)
- Onset with memory disturbances, defined as difficulty learning new information or recalling it.

or

- Onset with non-mnemonic symptoms
  - > Onset with language symptoms, particularly difficulty finding the correct words
  - > Onset with visuospatial symptoms: perceptual deficits characterized by failure to recognize objects, people, or written words
  - > Onset with executive symptoms: difficulties with reasoning and critical thinking

Criteria for <u>possible Alzheimer's disease (AD)</u>:

- Atypical course
  - Some criteria for probable AD are met, but the onset of symptoms may have been sudden, or there is a lack of objective evidence of progressive cognitive decline
- Mixed etiology presentation
  - > All criteria for probable AD are met
  - > Concomitant cerebrovascular disorders, or
  - > Features typical of another dementia (e.g., Lewy body dementia), or
  - > Evidence of other neurological disorders or non-neurological comorbidities, or possible use of medications with effects on cognition.

Tests: MMSE < 23; MoCA < 25; objective episodic memory loss on the Wechsler Memory Scale Logical Memory II adjusted for years of schooling (≥9 with 16 or more years of schooling, ≥5 for 8–15 years of schooling, ≥3 for 0–7 years of schooling);

Exclusion criteria

- subjects under 65 years of age, not residing in Italy, suffering from other forms of dementia.

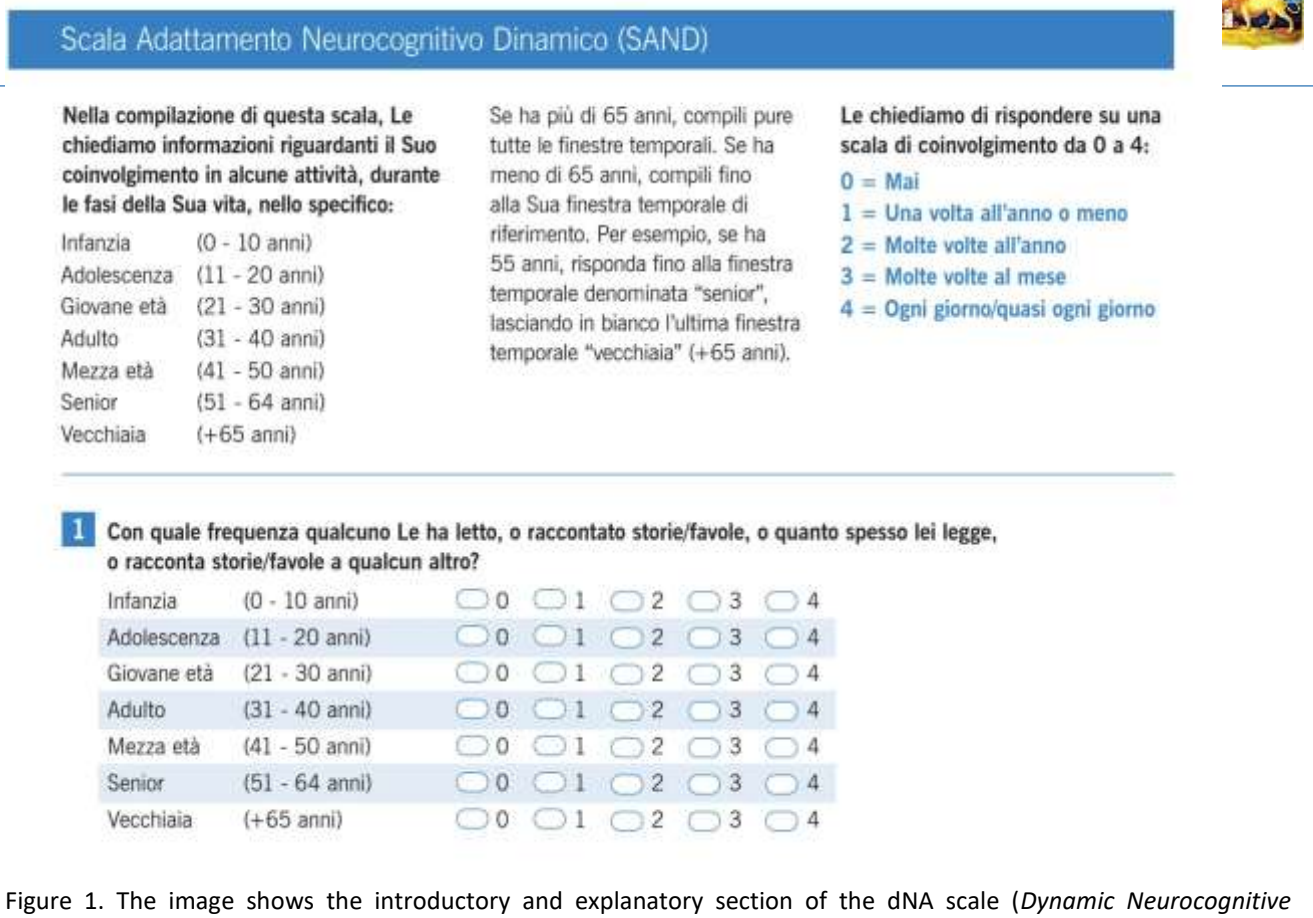
Inclusion criteria (Stage #2 & Stage #3)

- age ≥ 65 years, residing in Italy, residing in Italy;
- cognitively healthy, or affected by MCI/AD, other forms of dementia, or mental disorders, provided that these are described in detail by the clinician, including scores on scales such as anxiety and depression, and not included in the validation (i.e., in Stage #1).

If a subject is already enrolled at a recruitment center and has already undergone a demographic, medical history, neuropsychological, or MRI assessment for another research study, and their cognitive status has not changed over the past six months, the clinician may administer the dNA (and the Mediterranean diet adherence scale) and access the previously collected data, provided that the participant is informed of the study's objectives and gives informed consent.

Procedures

This study involves administering the dNA scale, along with a demographic and medical history assessment, a questionnaire investigating adherence to a typically Mediterranean diet, and a cognitive assessment using neuropsychological testing and neuroimaging via functional magnetic resonance imaging (fMRI). Among the demographic variables, particular importance will be given to years of schooling—a proxy typically used in the literature to define cognitive reserve—and diet, a protective factor against the inflammatory mechanisms present in AD-type dementia. This information will be collected during interviews and through the completion of demographic and medical history forms. Following the collection of this information, together with the dNA scale, each center will share these data with the Universities of Chieti and Foggia, which will analyze the data with the aim of validating the scale in Italian and associating the aforementioned variables, with the exception of MRI images, which will be analyzed by the University of Padua.



Scala Adattamento Neurocognitivo Dinamico (SAND)

Nella compilazione di questa scala, Le chiediamo informazioni riguardanti il Suo coinvolgimento in alcune attività, durante le fasi della Sua vita, nello specifico:

Infanzia	(0 - 10 anni)
Adolescenza	(11 - 20 anni)
Giovane età	(21 - 30 anni)
Adulto	(31 - 40 anni)
Mezza età	(41 - 50 anni)
Senior	(51 - 64 anni)
Vecchiaia	(+65 anni)

Se ha più di 65 anni, compili pure tutte le finestre temporali. Se ha meno di 65 anni, compili fino alla Sua finestra temporale di riferimento. Per esempio, se ha 55 anni, risponda fino alla finestra temporale denominata "senior", lasciando in bianco l'ultima finestra temporale "vecchiaia" (+65 anni).

Le chiediamo di rispondere su una scala di coinvolgimento da 0 a 4:

0 = Mai
1 = Una volta all'anno o meno
2 = Molte volte all'anno
3 = Molte volte al mese
4 = Ogni giorno/quasi ogni giorno

1 Con quale frequenza qualcuno Le ha letto, o raccontato storie/favole, o quanto spesso lei legge, o racconta storie/favole a qualcun altro?

Infanzia	(0 - 10 anni)	0	1	2	3	4
Adolescenza	(11 - 20 anni)	0	1	2	3	4
Giovane età	(21 - 30 anni)	0	1	2	3	4
Adulto	(31 - 40 anni)	0	1	2	3	4
Mezza età	(41 - 50 anni)	0	1	2	3	4
Senior	(51 - 64 anni)	0	1	2	3	4
Vecchiaia	(+65 anni)	0	1	2	3	4

Figure 1. The image shows the introductory and explanatory section of the dNA scale (*Dynamic Neurocognitive Adaptation Scale*), which takes approximately 15 minutes to complete. For the complete PDF of the dNA scale, please refer to the attachments included in the submitted documentation.

Neuropsychological variables

The neuropsychological assessment involves the administration of the following instruments: MMSE (or MoCA), Rey Auditory Verbal Learning Test (RAVLT), Trial Making Test (TMT) Form B, Digits Forward and Backward subtests (WAIS or WAIS-III), Wechsler Memory Scale Logical Memory II (or Prose Memory), and Stroop Test. These measures will provide an overall cognitive assessment (MMSE or MoCA) and more specific measures of memory and executive functions.

Neural variables

If the volunteer subject has not already undergone an MRI as part of another study conducted at IRCCS San Camillo, the neuroimaging assessment will involve the use of magnetic resonance imaging, through which structural (MRI) and functional (fMRI) data will be collected, specifically during the resting state (resting-state fMRI) without the use of any specific cognitive or motor task.

Participating centers that have the capability may also retrieve information from the patient's medical record regarding biomarkers of pathological aging (e.g., APoE). Similarly, if the behavioral, clinical, or instrumental

examination has already been performed at the same center, it will be possible to retrieve the data without repeating the examination, provided that the participant has given informed consent.

Statistics

Statistical Analyses

Analyses Required for Validation

For the primary objective analyses, a principal component analysis (PCA) and an exploratory factor analysis (EFA) will be conducted in parallel, serving as the basis for the subsequent confirmatory factor analysis (CFA) in the validation sample. Factors will be extracted without specifying the number and patterns of loadings between the observed variables and the latent factors. In CFA, on the other hand, the number, meaning, associations, and patterns of free parameters in the factor weight matrices before analyzing the data are specified (Morris et al., 1996). While in EFA, factors are extracted without specifying the factor loadings between the observed variables and the latent factor variables, in CFA the number, meaning, associations, and model of free parameters in the factor loading matrix before analyzing the data will be specified. EFA will be useful for determining the underlying factor structure and for identifying latent domains capable of explaining the common variance among activity items rather than simply summarizing the maximum amount of observed variance, as is done in PCA analysis (Negash et al., 2013). EFA will be used to identify and distinguish the key constructs of the dNA instrument. Descriptive statistics (means and standard deviations) will be used for continuous variables and percentages for categorical variables to characterize the study sample.

A PCA will be performed with permutation of the raw data to determine the number of components among the items. PCA factor loadings will be examined, retaining all factors with eigenvalues >1. Before performing EFA, investigators will assess the adequacy of the sample size using the Kaiser-Meyer-Olkin (KMO) statistic. Bartlett's sphericity test, separately for all seven time windows, will be used to test for homogeneity of variances. Cronbach's alpha (α) will be calculated to assess the internal consistency of the scale, using a threshold of > 0.8. Investigators will also use direct oblique rotation with Kaiser normalization and examine the consistency of the items to identify excessively high correlations ($r \geq .80$) suggesting multicollinearity or low correlations ($r < .30$) indicating insufficient common variance.

During the CFA phase, a structural equation modeling (SEM) approach will be used with a maximum likelihood estimation method to assess the fit of the factor structure hypothesized based on the EFA results. CFA allows for the direct comparison of alternative models of relationships between constructs, which is fundamental for establishing construct validity. Model fit indices will be calculated, including chi-square (p-value > 0.05),

root mean square error of approximation (RMSEA; cutoff ≤ 0.06), normed fit index (NFI), comparative fit index (CFI; cutoff ≥ 0.90), Tucker-Lewis index (TLI; cut-off ≥ 0.95), and standardized root mean square residual (SRMR; cut-off ≤ 0.08).

Finally, the coefficient of determination (R^2) will be evaluated, which explains how much of a factor's variability is caused by its relationship with another factor.

Behavioral Correlation Analysis

Correlation analyses will also be conducted among the various domains within the scale: cognitive, physical, creative, and social, using distance correlations, a set of measures that examines potential relationships between vectors without the assumptions required for classical product-moment correlations, remaining valid under multiple conditions, including when the variables have an implicit assumption of temporality that may violate the assumptions of traditional coefficients (Szekely, Rizzo, and Baklrov 2007; Szekely and Rizzo, 2009). The domains will also be examined across the seven time periods using RM-ANOVA with Greenhouse-Geisser sphericity corrections. Within-subject comparisons by time and all interactions between time, gender, and education level will be calculated. The role of gender, education, and their interaction will be examined. Investigators will calculate planned contrasts within domains for paired time comparisons and interactions if significant, and examine Bonferroni-Holm corrected paired comparisons between educational levels (as a measure of cognitive reserve). Finally, investigators will calculate the correlations between the scale and adherence to a Mediterranean diet, as well as the correlation between the scale and scores on cognitive tests of memory and executive functions. Moderation analyses will also be conducted to test the hypothesis that higher dNA scores may moderate negative effects on neuropsychological measures (lower scores in memory and executive functions) and neurophysiological measures (poor anticorrelation between DN and DAN/FPCN).

fMRI Analysis

Structural MRI scans will be acquired with a 24 cm field of view, resolution 256×256×170, for voxel dimensions of 1×1×1, 2 mm^3. A standard echo-planar imaging sequence will be used to acquire rs-fMRI data with 140 time points, TR/TE=3000/30 ms, flip angle=80 degrees, 48 slices, spatial resolution=3.3×3.3×3.3 mm^3, and imaging matrix=64×64. The first five volumes will be discarded. Preprocessing steps will include slice-timing correction, realignment, co-registration to T1-weighted images with skull stripping, and spatial normalization to the 2-mm MNI 152 reference space.

Seed-based analysis

The fMRI data will be preprocessed using standard procedures for functional connectivity analysis, including correction for head motion, co-registration of functional images to brain anatomy, bandpass filtering (0.01–

0.1 Hz), regression of white matter, cerebrospinal fluid, and spatial smoothing with a 6 mm full-width half-maximum kernel (Gavrilescu et al., 2008). Regions of interest (ROIs) will be defined based on the relevant literature on cognitive decline. By inverting the spatial transformation required to co-register the MRI image in MNI space, the coordinates of the ROIs will be located in individual space. The ROIs will be spherical, centered at the coordinates in individual space, and with a radius of 6 mm. Investigators will apply principal component analysis to the time series of all voxels included in the spherical ROI. The first principal component will be considered representative of the activity of the entire ROI. The fMRI connectivity maps will be calculated by correlating the time series of each ROI with the time series of all brain voxels. The maps generated in individual space will then be non-linearly co-registered into MNI space to facilitate comparisons between different subjects. The group-level connectivity maps will be obtained by calculating the one-sample t-test between maps obtained at the individual level. The significance level will be corrected for multiple comparisons using the false discovery rate (FDR) approach (Benjamini and Hochberg, 1995).

Graph theory analysis

With the functional network generated using the AAL atlas (Tzourio-Mazoyer et al., 2002), graph theory analysis will be performed using the GRETNA software (Wang et al., 2015). Five global indices derived from the graph analysis will be analyzed and correlated with the results of the dNA scale. In this study, investigators will account for the different diagnostic groups during the analysis phase. An ANCOVA will be used to assess the interactive effect of diagnosis and FC indices on memory scores and other neuropsychological tests. A post hoc generalized linear regression model will then be applied to test the significance of the association within each diagnostic group. An additional regression analysis will be conducted to compare differences in slopes between cognitive scores (such as memory) and indices of FC and graph theory. Age, handedness, and education will be included as covariates in the ANCOVA and regression analyses.

Sample Size Calculation:

Primary objective: According to the literature, the number of participants required to validate a scale with 20 items—which has already been validated in another language (specifically in English on a sample of 815 individuals residing in the United States, Cieri et al., accepted)—is approximately 250 participants (Comrey and Lee, 1992). Other studies suggest a minimum of 200 subjects to test cross-cultural consistency and reliability (White, 2022). The dNA scale, already validated in English (Cieri et al., accepted), reports a KM O index greater than .80 (i.e., good), a specific index (Kaiser-Meyer-Olkin) for confirming the appropriateness of the sample (Kaiser, 1974). Specifically, for the CFA, this sample size calculation accounts for the need to detect a medium effect size, expressed in terms of Root Mean Square Error of Approximation (RMSEA), with an expected value of 0.05, a statistical power (1-β) of 0.80, and a significance level α of 0.05. Taking potential dropouts into account, approximately 265 subjects will be recruited to ensure the minimum sample size

required for statistical power, and this number will be verified experimentally during the validation phase using the KMO index.

Each of the 8 participating centers, depending on their recruitment capacity, will enroll approximately at least 30 participants. The majority of the final sample (i.e., 265 participants) will consist of healthy older adults (HC), while a smaller percentage will include subjects with subjective memory complaints (SMC), mild cognitive impairment (MCI), or Alzheimer's disease (AD).

Among the centers responsible for recruitment for the primary objective (validation of the dNA scale in Italian), an indicative estimate of the types of participants each center will recruit is provided below. Specifically:

- IRCCS Campus Biomedico (Rome): HC, AD, MCI, SMC (approximately 5 healthy individuals, 15 patients)
- IRCCS Centro San Giovanni di Dio Fatebenefratelli (BS): HC, SMC (approximately 20 healthy controls, 10 patients)
- IRCCS Fondazione Mondino (PV): HC, AD, MCI, SMC (approximately 10 healthy controls, 40 patients)
- IRCCS "Carlo Besta" Neurological Institute (Milan): HC, MCI (approximately 15 healthy subjects, 15 patients)
- IRCCS Neuromed (Isola Sempione): HC, AD, MCI, SMC (approximately 10 healthy subjects, 20 patients)
- IRCCS San Camillo Hospital (Venice): HC (approximately 30 healthy subjects)
- IRCCS San Raffaele Hospital (MI): HC, AD, MCI, SMC (approximately 10 healthy subjects, 20 patients)
- IRCCS San Martino (GE): HC, AD, MCI, SMC (approximately 10 healthy subjects, 25 patients)

Expected Results:

- In terms of validation and factor analysis, investigators expect results similar to those obtained from the English-language validation (Cieri et al., accepted), with a four-factor structure and no issues of multicollinearity ($r < 0.95$) or non-collinearity ($r < 0.3$). Investigators expect satisfactory results for the KMO indices (>0.70), Bartlett's sphericity test (<0.01), and Cronbach's alpha (>0.80).
- Investigators expect a correlation between greater engagement in the activities under investigation, their dynamic maintenance over time, and higher educational attainment.

- In the association of the scale with neuropsychological measures, holding diagnosis and educational attainment constant, investigators expect better results in terms of episodic memory and executive functions among subjects with higher scores on the dNA scale.
- When associating these data with fMRI measures, investigators expect, within diagnostic groups, greater activation (and segregation) in the so-called task-negative network (DN) associated with greater deactivation of so-called task-positive networks (DAN and FPCN) in subjects with higher scores on the dNA scale. Measures of segregation (within neural networks) and integration (between neural networks) will be explored, associating these measures with dNA scale scores.

Data collection and processing:

All personal and clinical information concerning the patient and/or their family unit collected during this study will be kept confidential and handled in compliance with current regulations (Good Clinical Practice standards: Ministerial Decree 15.7.1997, Legislative Decree 211/2003, Legislative Decree 200/2007) and data protection laws (Legislative Decree 196/2003 and subsequent amendments and updates). It is specified that the identity of the data collected will be known exclusively to research team members, healthcare professionals involved in the care of the participants, and physicians. The data, processed using electronic and other means, may be disseminated in a strictly anonymous form through meetings, conferences, and scientific publications; in any case, the name or any other detail capable of identifying the participant and/or their family members will not be disclosed, as the data may be presented exclusively in aggregate form or in a manner that does not make the study participants identifiable.

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