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Aging Stereotypes and Prodromal Alzheimer's Disease (AGING): Study protocol for an ongoing randomised clinical study

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Keywords:	Alzheimer disease, mild cognitive impairment, aging stereotypes, stereotype threat, diagnosis, amyloid PET

Aging Stereotypes and Prodromal Alzheimer's Disease (AGING):

Study protocol for an ongoing randomised clinical study

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Abstract

Introduction The number of older people diagnosed with amnestic Mild Cognitive Impairment (aMCI) increases in worldwide. However, some aMCI patients do not convert to Alzheimer's type of dementia, some remaining stable and others reversing back to normal. This overdiagnosis bias has been largely overlooked and hardly explained. There is ample evidence in the laboratory that negative aging stereotypes (e.g., the culturally shared belief that aging inescapably causes severe cognitive decline) contribute deteriorating cognitive performances of healthy older adults, leading them to perform below their true abilities. The present study aims to test for the first time whether such stereotypes also impair patients cognitive performances during neuropsychological examination in the memory clinic, resulting in overdiagnosis of aMCI, the prodromal state of Alzheimer's Disease (AD).

Methods and analysis The ongoing study is a 4-year randomised clinical trial comparing patients′ physiological stress and cognitive performances during neuropsychological testing in memory clinic. A total of 260 patients coming for first cognitive evaluation will be randomised to either a standard condition of tests administration assumed here to implicitly activate negative aging stereotypes, or a reduced-threat instruction condition designed to alleviate the anxiety due to these stereotypes. Both groups will be tested with the same test battery and stress biomarkers. For 30 patients diagnosed as aMCI

in each group (n=60), biomarkers of neurodegeneration and amyloidopathy will be used to distinguish between aMCI with normal and abnormal AD biomarkers. A 9 month-follow-up will be performed on all patients to identify those whose cognitive performances remain stable, impair, or improve.

Ethics and dissemination This protocol was approved by the French National Agency for Medicines and Health Products Safety and the French Ethics Committee of Sud-Est I (2017-A00946-47). Results will be published in peer-reviewed journals.

Trial registration number NCT03138018

N=290

Key words: Alzheimer disease, mild cognitive impairment, aging stereotypes, stereotype threat, memory, diagnosis, amyloid PET

Strengths and limitations of this study

- This study represents the first experiment in memory clinic examining the effects of negative aging stereotypes on patients' neuropsychological performances.
- This study provides a unique multidisciplinary approach of the diagnosis of aMCI combining neurology, neuropsychology, neuroimaging, biology, and social psychology.
- AGING project is likely to provide an efficient method to reduce age-based stereotype threat during neuropsychological testing and thus to offer new recommendations to healthcare professionals to improve aMCI diagnosis.
- A longer follow-up period may be required than originally anticipated to analyse conversion and reversion phenomena.
- An important next step, not possible in the present design, would be the analysis of how negative aging stereotypes can lead to heightened activation of emotion-regulation

brain regions, which in turn may prevent from efficient activation of task relevant brain regions in patients.

Introduction

"*Few diagnoses in modern medicine evoke deeper apprehension in patient and family than Alzheimer's disease*".[1] Such a fear of developing AD is quite understandable. AD is a progressive and fatal degenerative disorder of the brain that occurs in middle or late life and robs us our most human qualities and abilities. AD's prevalence is generally estimated to be 2% before age 65, from 2% to 4% thereafter, and around 15% at age 80.[2] If the diagnosis accuracy of AD in its middle or advanced stage is today not a real issue, the accuracy of its early diagnosis is still a big challenge.[3] As people are mainly worried about not being early diagnosed, under-diagnosis is often presented as the main problem. However, the opposite error —overdiagnosis—is also likely. Some individuals diagnosed with amnestic Mild Cognitive Impairment (aMCI) actually do not convert to AD, some patients remaining stable and others even reversing back to normal[4-7], with rates of reversion to normal varying from 4.5% to 53%.[8] The construct of MCI[9 10] designates an early stage of cognitive impairment that is supposed to precede clinically probable AD,[11-13] and patients with Subjective Cognitive Impairment (SCI) are sometimes considered as Pre-MCI (a stage that can precede AD for 15 years).

Diagnosis for aMCI relies on neuropsychological testing of memory and cognitive functions to discriminate between normal and abnormal aging cognitive decline. It is thus essential that these standardized tests and their administration are as fair as possible. Here, we argue that an important source of bias during neuropsychological testing comes from negative aging stereotypes and the related fear that severe memory decline and AD are inevitable in older people. These negative aging stereotypes may exacerbate spontaneous demand by older adults for neuropsychological testing, but also impair their performances on these tests.[14-16] A growing field of experimental research in social psychology conducted in the laboratory among healthy population has demonstrated that members of groups whose abilities are

negatively stereotyped typically underperform when the negative stereotypes are made relevant to the testing situation, a phenomenon called stereotype threat (ST). In addition to the normal anxiety associated with taking tests, the fear of confirming negative stereotypes creates an extra pressure that interferes with intellectual functioning and leads to perform below one's abilities.[17 18]

Without denying that normal aging is associated with cognitive decline, many studies demonstrate that negative aging stereotypes contribute to the differences observed in the healthy population between younger and older adults in memory tasks (for reviews see[19-21]). ST has proved to impair older adults' memory performance when ability differences between younger and older adults are highlighted[15 22 23], or when the aging stereotype is implicitly activated using priming techniques.[24-27] Likewise, simply informing older adults about the presence of younger participants (without mentioning any expected age-related differences in performance) decreased their controlled access to memory and intensified their automatic responses.[28 29] ST effects on older adults' memory performances are also readily observable and fairly easy to produce with basic instructions of the type typically used in clinics during memory testing. Simply emphasizing the memory component of the test can produce performance differences between older and younger adults.[30 31] Interestingly, age-based ST effects can be alleviated and sometimes removed when the memory component of the test is de-emphasized[31 32], when the test is presented as age-fair[29], or when older adults are exposed to positive aging stereotypes.[15 22 23]

A few studies have investigated age-based ST effects with classic clinical tests typically used for the diagnosis of predementia.[33-36] Mazerolle *et al*[36] showed that ST impaired older adults' performance on both the Mini-Mental State Examination (MMSE[37]) and the Montreal Cognitive Assessment (MoCA[38]), resulting in 40% of older adults meeting the criteria for predementia compared with 10% in reduced-ST condition. Using a complete test battery

comparable to those used in clinical setting, Fresson *et al*[34] found that performances on executive cognitive tasks were impaired under ST condition, leading to 28% of older adults meeting the clinical criteria compared with 8% in the reduced-ST condition. However, these studies were still experimental lab studies conducted among healthy older adults and relying on ST and/or reduced-ST instructions that are not compatible with the clinical context where older adults with cognitive complaints want to get a diagnosis. The present study is the first to test age-based ST effects among patients, within the clinical setting and during the neuropsychological testing for the diagnosis of aMCI. Here, we assume that negative aging stereotypes implicitly permeate the ordinary situations of neuropsychological testing in memory clinic and thus impair older adults' performance, potentially resulting in overdiagnosis of aMCI.

Methods

Aim and objectives

AGING is a multicentre two-armed randomised controlled trials that aims to compare older adults' performances on complete neuropsychological test battery (the same for all patients), under either a standard (ST condition) or a reduced-ST condition. The standard condition refers to the routine condition under which the testing is traditionally conducted in memory clinics. We assume that the standard condition of tests' administration implicitly activates negative aging stereotypes and the fear of having AD. If this assumption is correct, then cognitive performances would be lower under this standard condition compared with when ST is reduced via specific instructions. ST will be reduced by using the so-called "teaching" instructions (through a video) that consist in explaining to patients the ST phenomenon, an explanation that has proved efficient to alleviate anxiety due to negative stereotypes.[36 39]

The objectives of the study will be to:

1. Determine how many patients meet the aMCI criteria in each condition. For some of those diagnosed aMCI (in both conditions), biomarkers of neurodegeneration (MRI hippocampal volume, perfusion PET imaging, Cerebrospinal fluid levels of β-amyloid, total-tau and phospho-tau) and amyloidopathy (PET imaging) will be used to distinguish between aMCI with abnormal AD biomarkers (i.e., aMCI due to AD) and aMCI with normal AD biomarkers.

2. Examine, through the use of biomarkers of stress (heart rate variability, skin conductance, salivary biomarkers), the extent to which age-based ST induces acute physiological stress during the neuropsychological assessment.

3. Identify, through a 9-month follow-up, participants whose cognitive performances remain stable, deteriorate, or improve.

4. Identify, through a questionnaire, individual characteristics (vulnerability factors) that can make older people more or less susceptible to ST effect during neuropsychological testing (e.g., stereotypical perceptions of aging, memory complaints, anxiety about aging, subjective age).

5. Provide an efficient method to deactivate the influence of negative aging stereotypes during neuropsychological testing and provide new recommendations to improve the diagnosis accuracy of aMCI due to AD.

6. Study the brain substrate of memory evaluation under ST versus reduced-ST conditions using MR volumetry and PET perfusion.

Procedure overview

This protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (see SPIRIT checklist in online supplemental file).[40] From July 2018 to January 2021, 260 participants will be recruited from the patients coming to memory

clinic for first cognitive evaluation due to memory complaints but without dementia. Figure 1 presents the four visits planned in the study. Consistent with the traditional clinical procedure, the first visit consists of a brief screening test conducted by a neurologist, who will decide on this basis whether or not to pursue a more in-depth cognitive testing. Patients selected to pursue will be invited to participate to the study and will receive the consent form and a questionnaire (vulnerability factors to ST) to complete at home and return the day of the next visit. In the second visit, within two months following the screening, a neuropsychologist will administer a complete neuropsychological test battery, the same for all of patients, under either standard or reduced-ST instructions (random assignment based on a computer-generated allocation sequence and controlled by the central site). In addition to cognitive performances, several biomarkers of stress will be measured. The neuropsychological assessment will lead to a diagnosis of SCI, aMCI, or eventually AD. Then, 60 patients diagnosed aMCI (30 from each arm) will be assigned to a third visit during which biomarkers of neurodegeneration and amyloidopathy (MRI hippocampal volume, β-amyloid tracer) will be used to distinguish between aMCI with abnormal and normal AD biomarkers. Finally, a 9 month-follow-up (fourth visit) will test all patients under reduced-ST condition (whatever their previous condition) with the same previous neuropsychological battery and physiological stress measures to identify patients whose cognitive performances remain stable, worsen, or improve. The neuropsychology testing will be performed in five centres in France: the Public Assistance-Hospitals of Marseille (AP-HM: the central site), the University Hospital of Broca (Paris), the University Hospital of La Milétrie (Poitiers), the University Hospital of Caen Normandie (Caen), and the University Hospital of Charles Nicolle (Rouen). The neuroimagering explorations (MRI, PET) will be performed in AP-HM and Cyceron neuroimaging Centre of Caen. Risks to participating patients are minimal and mainly linked to the neuroimaging part of the research. Study staff in each hospital will

complete case report forms during each visit and notifiy the Principal Investigator and clinical investigators about any adverse events.

Figure 1 here

Inclusion criteria

To be included in the cohort, patients must be at least 50 years old, should have no psychiatric, neurological, or cardiovascular history, must not use psychotropic drugs, should not be depressive, must report cognitive complaints but should not present any sign of dementia. Inclusion in the cohort is proposed to the patients by a neurologist during the first medical examination (visit 1), based on historical background and his/her answers to the following short cognitive tests: the Questionnaire de Plainte Cognitive (QPC[41]), Geriatric Depression Scale (GDS[41]), MMSE[37], the 5-word test[43], and the Lawton Instrumental Activities of Daily Living (IADL) Scale.[44] To be included, patients should meet the following criteria: a QPC score ≥ 3; a GDS score ≤ 9; an MMSE score ≥ 24; a 5-word test score > 8; and a I.A.D.L score $= 0$.

Study arms

At the beginning of visit 2, before taking the neuropsychological test battery, patients are randomly assigned to one of two arms: standard test instructions or reduced-ST instructions. Patients, neuroimaging staff, and neurologists are all blinded to the conditions except neuropsychologists who need to know which instructions to use.

The standard test instructions refer to the instructions traditionally used in memory clinic during neuropsychological assessment. These instructions have simply been standardized to

be similar within the five centres. Patients are informed by the neuropsychologist that they are going to do a more in-depth assessment than they did in consultation with the neurologist, in order to test their memory, language, and all the functions they use in daily life. The neuropsychologist adds that the testing includes tasks of various difficulty levels so that it is normal not to be successful on all tests. Finally, all patients are encouraged to do their very best and the testing process begins. While one could intuitively consider this standard condition as a neutral condition that does not activate ST, we assume here that this condition implicitly promotes ST effects and is self-threatening for the patients.

The reduced-ST instructions are designed to reduce ST by using the so-called "teaching instructions", which consist in explaining to patients the ST phenomenon to alleviate anxiety due to negative aging stereotypes. In order to standardize and optimize the effectiveness of these instructions, we developed a 4-min video presenting either a female or male patient during part of her/his interview in memory clinic. For identification purpose, the gender of the ficticious patient is matched to the participants' gender, resulting in two videos. The video shows a patient coming to memory clinic for a first neuropsychological assessment because of worries about his/her memory. Several scenes are enacted and represent the usual steps in the circuit of a MCI/AD diagnosis. The patient meets first a neurologist and then a neuropsychologist who both give the"teaching instructions" to reduce ST: "*In our societies, we are all exposed in the media to sensational and often fearful information about AD that inevitably induce a lot of stress. This stress can lead to experience difficulties during the testing, which can impair peformances. The tests themselves can be stressful. It is thus normal to make some mistakes due to stress and these mistakes are not necessarily the sign of AD*". We definitely chose not to use a video in the standard (control) condition to be the most similar as possible to the classic testing practices during neuropsychological assessment in memory clinic.

Measures and analyses

Sample size

The number of participants in the study was determined a priori[45] on the basis of the type of statistical analysis (multiple regression), the desired power (0.80), the alpha threshold (0.05), the number of predictors (10 in the main analysis), and the anticipated effect size of the ST phenomenon (moderate size effect, see meta-analysis[21]). Soper's sample size calculator[46] indicated that 250 participants were required, and we added an additional 10-member safety margin ($N = 260$) to get at least 30 participants in each arm meeting aMCI criteria for visit 3. Patients are recruited continuously until the desired sample size is achieved but before January 2021 to ensure a last 9-month follow-up on September 2021. Since February 2019, two strategies have been implemented to increase patients' recruitment: a new hospital was recruited (Broca hospital) and communication on AGING protocol has been improved (e.g., poster displays in hospitals and general practices, call for participation to AGING on hospitals website).

Vulnerability factors to ST effects

In addition to demographic information (sex, birth date, years of education, socioeconomic status, native language, living at home, etc), a questionnaire captures participants' reports of memory complaint (IPQ-M[47]), stereotypic perceptions of aging and AD[26 48], and self-categorization as older versus younger.[36] Participants complete this questionnaire at home and bring it back to the memory clinic in visit 2. These variables will be used as potential moderators of ST effects on cognitive and stress outcomes.

Neuropsychological test battery (Visit #2 and 3)

The same neuropsychological test battery (Table 1) is used for all patients, whatever the condition to which they are randomly assigned (standard vs reduced-ST condition). All the tests are commonly used in memory clinic, except for the second and last ones that assess visuo-spatial attentional flexibility and prospective memory respectively.

Table 1 Neuropsychological test battery included in the study for visits #2 and 4

	Tests	Duration (min)	Domain assessed	Authors
1	The episodic 16-item free and cued reminding test (FCSRT), with parallel list for the 9-month follow-up (visit #4)	20	Episodic memory	Van der Linden *et al*[49]
2	Visuo-Spatial Focused Attention task	5	Visuo-spatial attentional flexibility	Herrera *et al*[50]
3	Part A and B of the Trail Making Test	5	Flexibility	Reitan[51]
4	Stroop task	10	Inhibition	Seo *et al*[52]
5	N-back task	5	Working memory	Adapted from Perlstein *et al*[53]
6	Category and lexical fluency task	5	Verbal Fluency (language)	Cardebat *et al*[54]
7	The Rey-Osterrieth Complex Figure (copy and delayed -3 mn-recall) for visit #2 and The Taylor Figure for visit # 4	5-7	Visuo-construction and memory	Osterrieth[55], Rey[56], Taylor[57]
8	The Boston Naming test	5	Visual confrontation naming skills (language)	Kaplan *et al*[58]
9	MemPro test	15	Prospective memory	Adapted from Gonneaud *et al*[59]

To be categorized aMCI or non-amnestic MCI (naMCI), participants have to meet the corresponding criteria of the revised NINCDS-ADRDA standards.[60 61] Our MCI

categorization is based on the FCSRT (either total recall lower or equal to 40/48; Sarazin & Dubois[62] or scores lower than pathological thresholds of Van der Linden[49]). The z-scores obtained for the other cognitive function tests are used to complete the diagnosis, to perform between-group comparisons (standard vs reduced-ST conditions), and to perform within-subject comparisons thanks to the 9-month follow-up. These additional tests will permit to distinguish between aMCI single and multiple domains. For the naMCI patients (if any), the diagnosis will be established at the end of the study. The raw scores are used for the tests without normative data (Visuo-Spatial Focused Attention task and MemPro tests). Participants reporting memory complaints but having normal cognitive performances will be categorized as SCI.

Analysis of neuropsychological performances

Multiple regression analyzes will be performed on the scores obtained in different tests of the neuropsychological battery to determine whether performance is worse in patients placed in standard condition compared to those of the reduced-ST condition.

Physiological stress measures

We use the heart rate variability (HRV), cutaneous conductance, and biomarkers of stress to measure physiological stress (Table 2) during neuropsychological testing in the visit 2 under either standard or reduced-ST condition, and during the follow-up visit. These three measures of physiological stress will be used as potential mediators of the ST effect on cognitive performances and as complementary indication of any potential progression of the disease.

Table 2 Materiel of physiological stress measures and characteristic

Measure	Materiel	Characteristics
Heart rate variability (HRV)	Thin elasticized heart rate transmitter belt placed on the chest	Detect heart rates between the range of 25 - 240 beat per minute and respiratory rate within a 3-70 breaths

		per minute range
Cutaneous conductance	Wristwatch placed on the wrist at the same time the thin of HRV	Measure the skin conductance with micro siemens, 64Hz frequency and the movements by a triaxial accelerometer.
Stress biomarkers	Salivette®. A synthetic cotton swab is removed from the Salivette® and placed in the mouth of the participant to chew for about 1 minute	The Salivette® is stored at -80°C and then sent to laboratory for analyses.

Analysis of heart rate variability

Heart rate variability (HRV) data will be first examined according to Task Force recommendations.[63] Premature atrial and ventricular beats and the subsequent intervals will be automatically discarded and visually checked. We will explore HR data simultaneously in time and frequency domains. We will analyze, in the time domain, R-R intervals, standard deviation of R-R intervals (SDNN), square root of the mean squared difference of successive R-R intervals (rMSSD), number of adjacent N-N differing by more than 50 milliseconds divided by the total number of N-N intervals (pNN50). The rMSSD and pNN50 are associated with high-frequency power (HF) and parasympathetic activity. In the spectral domain, we will analyze low-frequency power (LF; 0.04-0.15 Hz), an index of both sympathetic and parasympathetic activity, and HF (0.15-0.4 Hz), representing the most efferent vagal (parasympathetic) activity to the sinus node. The LF/HF ratio (i.e. the sympathovagal balance) will also be calculated.

Analysis of stress biomarkers

Biomarkers of stress consist of cortisol, dehydroepiandrosterone (DHEA) and its sulfated stable form (DHEAS) from the HPA axis, and Immunoglolulin A (IgA). We will assay on saliva samples at two points time (on arrival at the clinic and before leaving). For biomarkers of stress (cortisol, etc), sensitivity, intra- and inter-assay coefficients of variation are respectively less than 0.05 ng/ml, 8% and 10%, for all biomarkers.[64]

Neurodegeneration and amyloidopathy biomarkers

For 60 patients diagnosed aMCI after the neuropsychological examination (30 from the standard condition and 30 from the reduced-ST condition), neuroimaging biomarkers will be used to identify those with a high likelihood of AD etiology: those showing positive AD biomarkers of both neuronal injury (from MRI: hippocampal atrophy) and β-amyloid deposition (from Florbetaben PET), as recommended by Dubois *et al.*[65] Early Florbetaben PET acquisitions will be conducted to evaluate brain perfusion.[66]

Analysis of MRI data

Half of the patients will undergo an MRI session at the Cyceron center (Caen, France) with a Philips (Eindhoven, The Netherlands) Achieva 3.0 T scanner. A high-resolution T1-weighted anatomical image will be acquired using a 3D fast field echo sequence (sagittal; repetition time, 20 ms; echo time, 4.6 ms; flip angle, 10°; 180 slices; slice thickness, 1 mm; field of view, 256 x 256 mm2; matrix, 256 x 256). For the other half, MRI will be performed in the imaging center of Marseille with a Siemens (Erlangen, Germany) 3T scanner Magnetom Skyra syngo~~x~~ MR D13. The high resolutionT1 weighted images will be obtained with the 3D magnetization prepared rapid gradient echo (MPRAGE) sequence (sagittal; repetition time 2300ms; echo time 2,98 ms; flip angle 9°; inversion time 900 ms; 176 slices; slice thickness 1 mm; field of view 256; matrix 256x256). In both centers, participants will be given earplugs and head will be stabilized with foam pads to minimize head motions. Visual analyses will be performed by 2 experts independently respecting anonymity with blinding about clinical data. In case of discrepancies between the two experts, a third analysis will be performed by another expert and the final result will be obtained by consensus. Particular attention will be paid to the amygdalo-hippocampal complex as a marker of neurodegeneration. A whole-brain voxel-based analysis will be also conducted on SPM12

(https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) after cortical segmentation, spatial normalization and smoothing, to study correlation between the memory performance evaluated in the standard versus reduced-ST condition and the grey-matter density.

Analysis of PET data, Amyloid load (Florbetaben ^{18}F)

We will measure the amyloid load using Florbetaben (18F) (NEURACEQ®, Piramal Imaging Limited), a radiotracer with high sensitivity for β-amyloid deposition and suitable for our laboratory setup due to his physical half-life (110 minutes). Florbetaben (18F) has been purchased from Piramal Imaging Limited (United Kingdom). Subjects will be examined using whole body PET / CT scanners, ie a Dual-Gemini (Philips Medical Systems) and a Discovery 710 (General Electric) in Caen and Marseille, respectively. A low dose CT scan will be performed first (scanning range 600 mm, increment 5 mm, cutting thickness 3.2 mm, step 1.5, 75 seconds per rotation, matrix 512x512, 120 KV, 80 mAs), followed by a cerebral scan lasting 20 minutes, starting 90 minutes after the injection of a dose of 300 MBq of Florbetaben (18F). Minimum dose of 240 MBq, maximum dose of 360 MBq, dose recommended 300MBq. An additional 5 min acquisition will be added 1 min after injection to evaluate cerebral perfusion.

Visual analyses will be first performed by 2 experts independently respecting anonymity with blinding about clinical data. In case of discrepancies between the two experts, a third analysis will be performed by another expert and the final result will be obtained by consensus. In order to validate the results obtained by the visual analyses, the molecular imaging experts will assess a semi quantitative index, SUVr (standard uptake value ratio[67]). Standardized uptake value (SUV) will be obtained for ROIs. We will use the regional-to-cerebellum Standard Uptake Value Ratios (SUVr) for inter-subject comparison as cerebellum is reported to be a region free of fibrillar amyloid plaques in the AD brain considering whole brain and regions of interest (ROIs). Each regional SUVr value will be expressed as the mean

over the region of interest (ROI). To evaluate the fixation of Florbetaben (18F), 13 ROIs will be used, values being averaged on the ROI: precuneus, anterior cingulate, posterior cingulate, frontal, temporal, parietal, occipital, hippocampus, oval centrum semi, anterior putamen, posterior putamen, the caudate nucleus and pons, as defined in the atlas MNI-AAL. We will use neocortical SUVr Florbetaben (18F) values to distinguish amyloid-positive patients from amyloid-negative patients, using a threshold value of Florbetaben (18F) SUVr between 1.4 and 1.5, in agreement with previous studies using Florbetaben (18F).[68 69] We will reconstruct all PET sinograms with a 3-D iterative algorithm, with corrections for randomness, dispersion, photonic attenuation and decomposition, imaging with an isotropic voxel of 2 × 2 × 2 mm 3 and a spatial resolution of approximately 2-3mm wide at mid-height of the central field of view. A whole-brain voxel-based analysis will be also conducted on SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) after spatial normalization and smoothing to study correlation between the memory performance evaluated under standard or reduced-ST condition and the brain perfusion and also the Aβ burden.

Analysis of CSF data

CSF data from current practice will be integrated into AGING data. The diagnosis of aMCI due to AD in the CSF is based on the crossing of lowered β-Amyloid peptide (1-42 and 1-40) and increased Tau and Phospho-Tau protein curves.

Data management and monitoring

A study staff is reponsable for data entry and range checks for data values. All data are stored on a secure server provided by one of the Universities. Missing data will be handled based on the "missing at random" hypothesis (e.g., multiple imputation, longitudinal mixed effects models) as recommended for missing data in clinical trials involving patients with potential neurodegenerative disease.[70] Due to the minimal risk nature of the study, the data are

internally monitored (AP-HM, which is independent from the funder). Reports on study progress and milestones are submitted every year to the funder.

Patient and Public Involvement

Patients and the public were not involved in the development of this study protocol. However, alleviating the anxiety patients can experience due to negative aging stereotypes during neuropsychology testing and thereby contributing to improve the diagnosis accuracy of MCI was a major motivator for the AGING consoritum to develop and conduct this study. The findings will be disseminated to the participants and the community in general through newsletters and conferences.

Ethics and dissemination

This protocol was approved on July 2017 and the manuscript details the protocol on the latest version approved on April 2019. All changes to the study were decided by the consortium and reviewed by French National Agency for Medicines and Health Products Safety and the French Ethics Committee of Sud-Est I. Written informed consent of the patient is requested prior to the inclusion. People with direct access to the data will take all necessary precautions to maintain confidentiality. All data collected during the study are rendered anonymous. Only inclusion number is registered.

The results will be disseminated via peer-reviewed publications, conferences, and clinical networks targeting researchers, policy makers, clinicians and caregivers.

Conclusion

The evolution of AD is progressive, results in dependence and need of institutionalization, and is yet untreatable. Ethical questions are raised by the overdiagnosis of aMCI, whose

consequences can be extremely damaging not only for the patient but also for the family and the society.[71] Without denying that aging is associated with cognitive decline and neurodegenerative diseases for many people, we suggest to pay special attention to the influence of psychosocial factors largely overlooked regarding neuropsychological testing within memory clinics. The AGING project has potential important theoretical and practical implications for improving neuropsychological testing to the benefit of many older people who otherwise may be wrongly classified as aMCI.

N=4071 words

Trial status

The recruitment phase started in July 2018 for AP-HM, in September 2018 for the University Hospital of La Milétrie, in October 2018 for the University Hospital of Caen Normandie, in November 2018 for the University Hospital of Charles Nicolle, and will start in July 2019 for the University Hospital of Broca (Paris). Twenty-eight patients have been recruited till now. The estimated end date for this study is in September 2021.

Abbreviations

HF: high frequency; HR: heart rate; HRV: heart rate variability; LF low frequency; RR: distance between two consecutive R-waves on electrocardiogram.

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Competing interests

The authors declare that they have no competing interests.

Ethics approval

French National Agency for Medicines and Health Products Safety and the French Ethics Committee of Sud-Est I (2017-A00946-47)

Data sharing statement

Within 6 months of the end of the final year of funding, the investigators will create a complete, cleaned, de-identified copy of the final data set and a plan for conducting the outcomes analyses outlined in the study protocol will be made available upon reasonable request.

Reference

1. Selkoe DJ. Preventing Alzheimer's Disease. *Science* 2012;337:1488-92.
2. INSERM, Blum D, Buée L, *et al*. Alzheimer (maladie d'): Une maladie neurodégénérative complexe mais de mieux en mieux comprise. 2019. Retrieved from https://www.inserm.fr/information-en-sante/dossiers-information/alzheimer-maladie
3. Tian T, Zhang B, Jia Y, *et al*. Promise and Challenge: The Lens Model as a Biomarker for Early Diagnosis of Alzheimer's Disease. *Dis Markers* 2014;2014:1-5.
4. Ewers M, Walsh C, Trojanowski JQ, *et al*. Prediction of conversion from mild cognitive impairment to Alzheimer's disease dementia based upon biomarkers and neuropsychological test performance. *Neurobiol Aging* 2012;33:1203-1214.
5. Han JW, Kim TH, Lee SB, *et al*. Predictive validity and diagnostic stability of mild cognitive impairment subtypes. *Alzheimers Dement* 2012;8:553-9.
6. Morris JC, Storandt M, Miller JP, *et al*. Mild Cognitive Impairment Represents Early-Stage Alzheimer Disease. *Arch Neurol* 2001; 2019];58(3).

7. Solfrizzi V, Panza F, Colacicco AM, *et al*. Vascular risk factors, incidence of MCI, and rates of progression to dementia. *Neurology* 2004;63:1882-91.

8. Sachdev PS, Lipnicki DM, Crawford J, *et al*. Factors Predicting Reversion from Mild Cognitive Impairment to Normal Cognitive Functioning: A Population-Based Study. Ginsberg SD, éditeur. *PLoS ONE* 2013;8:e59649.

9. Flicker C, Ferris SH, Reisberg B. Mild cognitive impairment in the elderly: Predictors of dementia. *Neurology* 1991;41:1006-1006.

10. Petersen RC, Smith GE, Waring SC, *et al*. Aging, memory, and mild cognitive impairment. *International psychogeriatrics* 1997;*9*:65-69.

11. Petersen RC, Smith GE, Waring SC, *et al*. Mild Cognitive Impairment: Clinical Characterization and Outcome. *Arch Neurol* 1999;56:303.

12. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med* 2004;256:183-94

13. Winblad B, Palmer K, Kivipelto M, *et al*. Mild cognitive impairment - beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment *J Intern Med* 2004;256:240-6.

14. Hess TM, Hinson JT. Age-related variation in the influences of aging stereotypes on memory in adulthood. *Psychology and aging* 2006;21(3):621.

15. Hess TM, Hinson JT, Hodges EA. Moderators of and Mechanisms Underlying Stereotype Threat Effects on Older Adults' Memory Performance. *Exp Aging Res* 2009;35:153-77.

16. Kit KA, Tuokko HA, Mateer CA. A Review of the Stereotype Threat Literature and Its Application in a Neurological Population. *Neuropsychol Rev* 2008;18:132-48.

17. Steele CM. A threat in the air: How stereotypes shape intellectual identity and performance. *Am Psychol* 1997;52:613-29.

18. Steele CM, Aronson J. Stereotype threat and the intellectual test performance of African Americans. *J Pers Soc Psychol* 1995;69:797-811.

19. Barber SJ. An Examination of Age-Based Stereotype Threat About Cognitive Decline: Implications for Stereotype-Threat Research and Theory Development. *Perspect Psychol Sci* 2017;12:62-90.

20. Chasteen AL, Kang SK, Remedios JD. *Aging and stereotype threat: Development, process and interventions.* In Inzlicht M, Schmader T. Stereotype ThreatTheory, Process, and Application. *Oxford University Press*; 2011 ;202-216.

21. Lamont RA, Swift HJ, Abrams D. A review and meta-analysis of age-based stereotype threat: Negative stereotypes, not facts, do the damage. *Psychol Aging* 2015;30:180-93.

22. Hess TM, Auman C, Colcombe SJ, *et al*. The Impact of Stereotype Threat on Age Differences in Memory Performance. *J Gerontol B Psychol Sci Soc Sci* 2003;58:3-11.

23. Hess TM, Emery L, Queen TL. Task Demands Moderate Stereotype Threat Effects on Memory Performance. *J Gerontol B Psychol Sci Soc Sci* 2009;64B:482-6.

24. Hess TM, Hinson JT, Statham JA. Explicit and Implicit Stereotype Activation Effects on Memory: Do Age and Awareness Moderate the Impact of Priming? *Psychol Aging* 2004;19:495-505.

25. Levy B. Improving memory in old age through implicit self-stereotyping. *J Pers Soc Psychol* 1996;71:1092-107.

26. Levy BR, Zonderman AB, Slade MD, *et al*. Memory Shaped by Age Stereotypes over Time. *J Gerontol B Psychol Sci Soc Sci* 2012;67:432-6.

27. Stein R, Blanchard-Fields F, Hertzog C. The Effects of Age-Stereotype Priming on the Memory Performance of Older Adults. *Exp Aging Res* 2002;28:169-81.

28. Mazerolle M, Régner I, Morisset P, *et al*. Stereotype Threat Strengthens Automatic Recall and Undermines Controlled Processes in Older Adults. *Psychol Sci* 2012;23:723-7.

29. Mazerolle M, Régner I, Rigalleau F, *et al*. Stereotype Threat Alters the Subjective Experience of Memory. *Exp Psychol* 2015;62:395-402.

30. Chasteen AL, Bhattacharyya S, Horhota M, *et al*. How Feelings of Stereotype Threat Influence Older Adults' Memory Performance. *Exp Aging Res* 2005;31:235-60.

31. Desrichard O, Köpetz C. A threat in the elder: the impact of task-instructions, self-efficacy and performance expectations on memory performance in the elderly. *Eur J Soc Psychol* 2005;35:537-52.

32. Kang SK, Chasteen AL. The Moderating Role of Age-Group Identification and Perceived Threat on Stereotype Threat among Older Adults. *Int J Aging Hum Dev* 2009;69:201-20.

33. Barber SJ, Mather M, Gatz M. How Stereotype Threat Affects Healthy Older Adults' Performance on Clinical Assessments of Cognitive Decline: The Key Role of Regulatory Fit. *J Gerontol B Psychol Sci Soc Sci* 2015;70:891-900.

34. Fresson M, Dardenne B, Geurten M, *et al*. The effect of stereotype threat on older people's clinical cognitive outcomes: investigating the moderating role of dementia worry. *Clin Neuropsychol* 2017;31:1306-28.

35. Haslam C, Morton TA, Haslam SA, *et al*. "When the age is in, the wit is out": Age-related self-categorization and deficit expectations reduce performance on clinical tests used in dementia assessment. *Psychol Aging* 2012;27:778-84.

36. Mazerolle M, Régner I, Barber SJ, *et al*. Negative Aging Stereotypes Impair Performance on Brief Cognitive Tests Used to Screen for Predementia. *J Gerontol B Psychol Sci Soc Sci* 2016;gbw083.

37. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". *J Psychiatr Res* 1975;12:189-98.

38. Nasreddine ZS, Phillips NA, Bédirian V, *et al*.. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society* 2005; *53*(4):695-699.

39. Johns M, Schmader T, Martens A. Knowing Is Half the Battle: Teaching Stereotype Threat as a Means of Improving Women's Math Performance. *Psychol Sci* 2005;16:175-9.

40. Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158(3):200-207.

41. Thomas Antérion C, Ribas C, Honoré-Masson S, *et al*. Le questionnaire de plainte cognitive (QPC): un outil de recherche de plainte suspecte d'évoquer une maladie d'Alzheimer. *L'année Gérontologique* 2003;*17* :56-65.

42. Yesavage JA, Brink TL, Rose TL, *et al*. Development and validation of a geriatric depression screening scale: a preliminary report. *J Psychiatr Res* 1982;*17*:37-49.

43. Dubois B, Touchon J, Portet F, *et al*. "Les 5 mots", épreuve simple et sensible pour le diagnostic de la maladie d'Alzheimer. Presse Méd 2002;31:1696-99.

44. Lawton MP, Brody EM. Assessment of Older People: Self-Maintaining and Instrumental Activities of Daily Living. *Gerontologist* 1969;9:179-86.

45. Tabachnick BG, Fidell LS. Using multivariate statistics. 5th ed. Boston: Pearson/Allyn & Bacon; 2007. 980 p.

46. Soper, D. (2006–2013). Statistics calculators Version 3.0. Retrieved from tiny.cc/sopercalc

47. Hurt CS, Burns A, Brown RG, *et al*. Perceptions of subjective memory complaint in older adults: the Illness Perception Questionnaire – Memory (IPQ-M). *Int Psychogeriatr* 2010;22:750-60.

48. Rust TB, Kwong See ST. Beliefs about Aging and Alzheimer's Disease in Three Domains. *Can J Aging* 2010;29:567-75.

49. Van der Linden M, Coyette F, Poitrenaud J, *et al*. L'épreuve de rappel libre/rappel indicé à 16 items (RL/RI-16). In M. Van der Linden (ed.), L'évaluation des troubles de la mémoire. Présentation de quatre tests de mémoire épisodique (avec leur étalonnage). Marseille: Solal 2004;25-47

50. Herrera C, Chambon C, Michel BF, *et al*. Positive effects of computer-based cognitive training in adults with mild cognitive impairment. Neuropsychologia 2012;50:1871-81.

51. Reitan RM. Validity of the Trail Making Test as an Indicator of Organic Brain Damage. *Percept Mot Skills* 1958;8:271-6.

52. Seo EH, Lee DY, Choo H, *et al*. Normative study of the Stroop Color and Word Test in an educationally diverse elderly population. *Int J Geriatr Psychiatry* 2008;23:1020-7.

53. Perlstein WM, Dixit NK, Carter CS, *et al*. Prefrontal cortex dysfunction mediates deficits in working memory and prepotent responding in schizophrenia. *Biol Psychiatry* 2003;53:25-38.

54. Cardebat D, Doyon B, Puel M, *et al*. Évocation lexicale et sémantique chez des sujets normaux : performances et dynamiques de production en fonction du sexe, de l'âge, et du niveau d'étude. *Acta Neurol Belg* 1990;90, 207–217.

55. Osterrieth PA. Le test de copie d'une figure complex: Contribution à l'étude de la perception et de la mémoire [The test of copying a complex figure: A contribution to the study of perception and memory]. *Arch Psychol* 1944;28:1021–1034.

56. Rey A. (1941). L'examen psychologique dans les cas d'encephalopathie traumatique [Psychological examination of traumatic encephalopathy]. *Arch Psychol* 1941;28:286–340.

57. Taylor LB. Localisation of Cerebral Lesions by Psychological Testing. *Neurosurgery* 1969;16:269-87.

58. Kaplan E, Goodglass H, Weintraub S. The Boston Naming Test. Philadelphia: Lea & Febiger. 1983

59. Gonneaud J, Rauchs G, Groussard M, *et al*. How do we process event-based and time-based intentions in the brain? an fMRI study of prospective memory in healthy individuals: Neuroimaging of Prospective Memory. *Hum Brain Mapp* 2014;35:3066-82.

60. Albert MS, DeKosky ST, Dickson D, *et al*. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:270-9.

61. 2012 Alzheimer's disease facts and figures. *Alzheimers Dement* 2012;8:131-68.

62. Sarazin M, Berr C, De Rotrou J, *et al*. Amnestic syndrome of the medial temporal type identifies prodromal AD: A longitudinal study. *Neurology* 2007;69:1859-67.

63. Electrophysiology TF of the ES. Heart Rate Variability: Standards of Measurement, Physiological Interpretation, and Clinical Use. *Circulation* 1996;93(5):1043-65.

64. Lac G, Dutheil F, Brousse G, *et al*. Saliva DHEAS changes in patients suffering from psychopathological disorders arising from bullying at work. *Brain Cognition* 2012;80:277-81.

65. Dubois B, Hampel H, Feldman HH, *et al*. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement* 2016;12:292-323.

66. Daerr S, Brendel M, Zach C, *et al*. Evaluation of early-phase [18 F]-florbetaben PET acquisition in clinical routine cases. *Neuroimage Clin* 2017;14:77-86.

67. Wong JT, Gallo DA. Activating Aging Stereotypes Increases Source Recollection Confusions in Older Adults: Effect at Encoding but Not Retrieval. *J Gerontol B Psychol Sci Soc Sci* 2019;74:633-41.

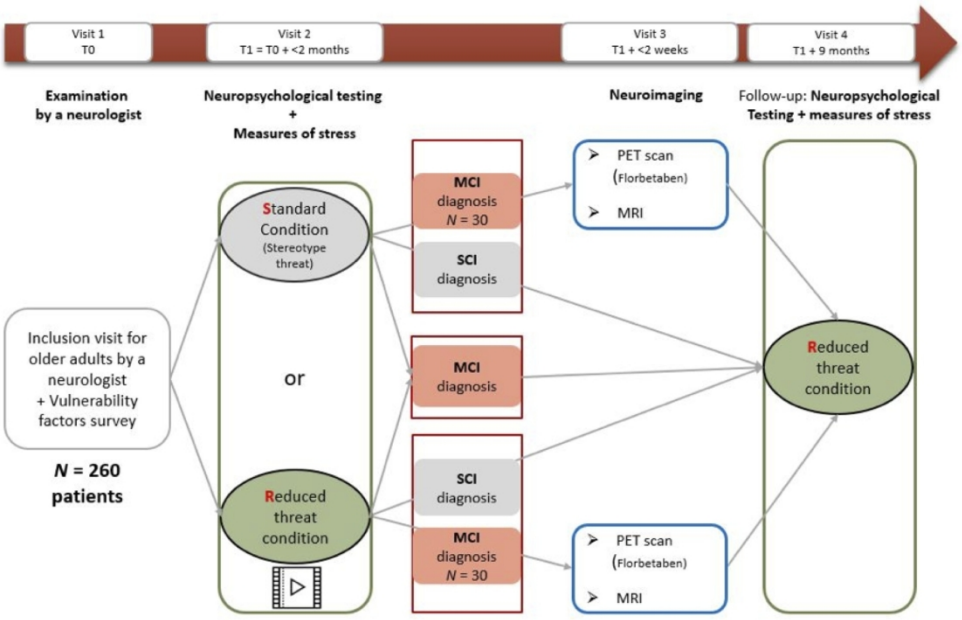
68. Sabri O, Sabbagh MN, Seibyl J, *et al*. Florbetaben PET imaging to detect amyloid beta plaques in Alzheimer's disease: Phase 3 study. *Alzheimers Dement* 2015;11:964-74.

69. Seibyl J, Catafau AM, Barthel H, *et al*. Impact of Training Method on the Robustness of the Visual Assessment of 18F-Florbetaben PET Scans: Results from a Phase-3 Study. *J Nucl Med* 2016;57:900-6.

70. Coley N, Gardette V, Cantet C, *et al*. How should we deal with missing data in clinical trials involving Alzheimer's disease patients? *Curr Alzheimer Res* 2011;8(4):421-33.

71. Merckelbach H, Jelicic M, Jonker C. Planting a misdiagnosis of Alzheimer's disease in a person's mind. *Acta Neuropsychiatr* 2012;24:60-2.

Figure 1 Flow chart of the main steps for patients. MCI: Mild Cognitive Impairment, MRI : Magnetic Resonance Imaging, PET: Positron Emission Tomography, CSF: CerebroSpinal Fluid. As a routine procedure, CSF puncture will be proposed to aMCI patients (this possibility is included in the consent form).



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Aging Stereotypes and Prodromal Alzheimer's Disease (AGING): Study protocol for an ongoing randomised clinical study

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Aging Stereotypes and Prodromal Alzheimer's Disease (AGING):

Study protocol for an ongoing randomised clinical study

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Abstract

Introduction The number of older people diagnosed with amnestic mild cognitive impairment (aMCI), the prodromal state of Alzheimer's disease (AD), is increasing worldwide. However, some patients with aMCI never convert to the AD type of dementia, with some remaining stable and others reverting to normal. This overdiagnosis bias has been largely overlooked and gone unexplained. There is ample evidence in the laboratory that negative aging stereotypes (e.g., the culturally shared belief that aging inescapably causes severe cognitive decline) contribute to the deteriorating cognitive performances of healthy older adults, leading them to perform below their true abilities. The study described here is intended to test for the first time whether such stereotypes also impair patients' cognitive performances during neuropsychological examinations in memory clinics, resulting in overdiagnosis of aMCI.

Methods and analysis The ongoing study is a 4-year randomised clinical trial comparing patients' physiological stress and cognitive performances during neuropsychological testing in memory clinics. A total of 260 patients attending their first cognitive evaluation will be randomised to either a standard condition of test administration, assumed here to implicitly activate negative aging stereotypes, or a reduced-threat instruction

condition designed to alleviate the anxiety arising from these stereotypes. Both groups will be tested with the same test battery and stress biomarkers. For 30 patients diagnosed with aMCI in each group (n = 60), biomarkers of neurodegeneration and amyloidopathy will be used to distinguish between aMCI with normal versus abnormal AD biomarkers. A 9-month follow-up will be performed on all patients to identify those whose cognitive performances remain stable, deteriorate, or improve.

Ethics and dissemination This protocol has been approved by the French National Agency for Medicines and Health Products Safety and the Sud-Est I French Ethics Committee (2017-A00946-47). Results will be published in peer-reviewed journals.

Trial registration number NCT03138018

Key words: Alzheimer disease, mild cognitive impairment, aging stereotypes, stereotype threat, memory, diagnosis, amyloid PET

Strengths and limitations of this study

- This study represents the first experiment in memory clinics to examine the effects of negative aging stereotypes on patients' neuropsychological performances.
- It applies a unique multidisciplinary approach to the diagnosis of aMCI, combining neurology, neuropsychology, neuroimaging, biology, and social psychology.
- This approach will yield a comprehensive picture of the sociopsychological factors liable to bias the diagnosis of aMCI.
- A longer follow-up period may be required than originally anticipated, to analyse conversion and reversion.

- An important next step, not possible with the present design, will be the analysis of how negative aging stereotypes may prevent efficient activation of the task-relevant brain regions in patients.

Introduction

"Few diagnoses in modern medicine evoke deeper apprehension in patient and family than Alzheimer's disease".[1] This fear of developing Alzheimer's disease (AD) is quite understandable. It is a progressive and fatal degenerative disorder of the brain that occurs in middle or later life and robs us of our most human qualities and abilities. Its prevalence is generally estimated to be 2% before age 65 years, 2-4% thereafter, and around 15% at age 80 years.[2] Whereas the accuracy of AD diagnosis in its middle or advanced stage is no longer a real issue, achieving accurate early diagnosis is still a major challenge.[3] As people are mainly worried about not being diagnosed at an early stage, underdiagnosis is often presented as the main problem. However, the opposite error — overdiagnosis — is also likely. Some individuals diagnosed with amnestic mild cognitive impairment (aMCI) never actually convert to AD, with some patients remaining stable and others even reverting to normal[4-7] (4.5-53% reversion to normal rate).[8] The MCI construct[9 10] designates an early stage of cognitive impairment that is supposed to precede clinically probable AD,[11-13] and patients with subjective cognitive impairment (SCI) are sometimes considered to be pre-MCI, a stage that can precede AD by up to 15 years.

The diagnosis of aMCI relies on the neuropsychological testing of memory and cognitive functions to discriminate between normal and abnormal cognitive decline in aging. It is thus essential that these standardised tests and their administration are as fair as possible. Here, we argue that an important source of bias during neuropsychological testing comes from negative aging stereotypes and the related fear that severe memory decline and AD are

inevitable in older people. These negative aging stereotypes may exacerbate spontaneous demands by older adults for neuropsychological testing, but also impair their performances on these tests.[14-16] In the field of social psychology, increasing experimental research conducted in the laboratory among healthy populations has demonstrated that members of groups whose abilities are negatively stereotyped typically underperform when the negative stereotypes are made relevant to the testing situation - a phenomenon called stereotype threat (ST). In addition to the normal anxiety associated with taking tests, the fear of confirming negative stereotypes creates extra pressure, which interferes with intellectual functioning and leads individuals to perform below their abilities.[17 18]

While not denying that normal aging is associated with cognitive decline, many studies have demonstrated that negative aging stereotypes contribute to the differences observed in healthy populations between younger and older adults on memory tasks (for reviews, see[19-21]). ST has been proven to impair older adults' memory performance when differences in abilities between younger and older adults are highlighted[15 22 23], or when the aging stereotype is implicitly activated using priming techniques.[24-27] Likewise, merely informing older adults of the presence of younger participants (without mentioning any expected age-related differences in performance) is enough to decrease their controlled access to memory and intensify their automatic responses.[28 29] ST effects on older adults' memory performances are readily observable and fairly easy to induce with basic instructions of the type typically used in clinics during memory testing. Simply emphasizing the memory component of the test can generate performance differences between older and younger adults.[30 31] Interestingly, age-based ST effects can be alleviated and sometimes removed when the memory component of the test is de-emphasised[31 32], when the test is presented as age-fair[29], or when older adults are exposed to positive aging stereotypes.[15 22 23]

Several studies have investigated age-based ST effects with classic clinical tests that are

typically used for the diagnosis of predementia.[33-36] Mazerolle et al.[36] found that ST impaired older adults' performances on both the Mini-Mental State Examination (MMSE[37]) and the Montreal Cognitive Assessment[38], resulting in 40% of older adults meeting the criteria for predementia, compared with 10% in a reduced-ST condition. Using a comprehensive test battery comparable to those used in clinical settings, Fresson et al.[34] found that performances on executive cognitive tasks were impaired in an ST condition, leading to 28% of older adults meeting the clinical criteria, compared with 8% in a reduced-ST condition. A recent meta-analysis[21] revealed that age-based ST effects are robust, with significant effect sizes for older adults' memory (d = .21, 95% CI [.020, .385]) and cognitive performance (d = .68, 95% CI [.399, .845]) that can be even larger when the threat is more subtly induced by the situation (d = .52, 95% CI [.248, .717]). Nonetheless, these were experimental laboratory studies conducted among healthy older adults and relying on ST and/or reduced-ST instructions that do not correspond to the clinical context in which older adults with cognitive complaints seek a diagnosis. The present study is therefore the first to test age-based ST effects among patients in a clinical setting and during neuropsychological testing for aMCI. It is based on the assumption that negative aging stereotypes implicitly permeate neuropsychological testing in memory clinics and thus impair older adults' performance, potentially resulting in the overdiagnosis of aMCI.

Methods

Aim and objectives

AGING is a multicentre two-armed randomised controlled trial designed to compare older adults' performances on a comprehensive neuropsychological test battery (the same for all patients) under either a standard (ST condition) or reduced-ST condition. The standard condition replicates the one in which testing is routinely conducted in memory clinics. It tests

the assumption that routine tests administration implicitly activates negative aging stereotypes and the fear of having AD. If this assumption is correct, cognitive performances will be poorer in this standard condition than in a condition where ST is reduced via so-called *teaching* instructions, where a video gives participants an explanation of ST that has been shown to efficiently alleviate anxiety arising from negative stereotypes.[36 39]

Primary objective

The study's primary objective is to test whether fewer patients meet the aMCI criteria in the reduced-ST condition than in the standard condition, by comparing their cognitive performances. For some of those diagnosed with aMCI in each condition, biomarkers of neurodegeneration (MRI hippocampal volume, perfusion PET imaging, cerebrospinal fluid (CSF) levels of β-amyloid, total-tau and phospho-tau) and amyloidopathy (PET imaging) will be used to improve diagnostic accuracy, by distinguishing between aMCI with abnormal AD biomarkers (i.e., aMCI due to AD) and aMCI with normal AD biomarkers.

Secondary objectives

1. Examine, through the use of biomarkers of stress (heart rate variability (HRV), skin conductance, salivary biomarkers), the extent to which age-based ST induces acute physiological stress during the neuropsychological assessment.

2. Conduct a 9-month follow-up to identify participants whose cognitive performances remain stable, deteriorate, or improve.

3. Administer a questionnaire, to pinpoint the individual characteristics (vulnerability factors) that can make older people more or less susceptible to ST bias during neuropsychological testing (e.g., stereotypical perceptions of aging, memory complaints, anxiety about aging, subjective age).

4. Develop an efficient method for deactivating the influence of negative aging stereotypes during neuropsychological testing and provide new recommendations to improve the diagnosis accuracy of aMCI due to AD.

5. Study the brain substrate of memory evaluation under ST versus reduced-ST conditions using MR volumetry and PET perfusion.

Procedure overview

This protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (see SPIRIT checklist in online supplemental file).[40] Over a period lasting from July 2018 to December 2020, the aim is to recruit 260 participants among individuals with memory complaints but without probable AD attending a memory clinic for their first cognitive assessment. Figure 1 indicates the four visits planned in the study. Consistent with the traditional clinical procedure, the first visit consists of a brief screening test conducted by a neurologist, who decide on this basis whether or not to pursue more in-depth cognitive testing. Patients selected for this in-depth testing are invited to take part in the study and receive the consent form and a questionnaire (ST vulnerability factors) to complete at home and bring on their next visit. During this second visit, within two months of the screening, a neuropsychologist administers a comprehensive neuropsychological test battery - the same for all of patients - under either standard or reduced-ST instructions (random assignment based on a computer-generated allocation sequence and controlled by the central site). In addition to cognitive performances, several biomarkers of stress are measured. The neuropsychological assessment leads to a diagnosis of SCI, aMCI, or possibly AD. A proportion of the patients diagnosed with aMCI (n = 60; 30 from each arm) are assigned to a third visit, during which biomarkers of neurodegeneration and amyloidopathy (MRI hippocampal volume, β-amyloid tracer) are used to distinguish between aMCI with abnormal versus normal AD biomarkers. Finally, at 9-month follow-up (fourth visit), all

patients are tested in a reduced-ST condition (whatever their previous testing condition) with the same neuropsychological battery as before and physiological stress measures to identify patients whose cognitive performances have remained stable, worsened, or improved. The neuropsychology testing is performed in four centres in France: Marseille Public Hospitals (central site), La Milétrie University Hospital in Poitiers, Caen University Hospital, and Charles Nicolle University Hospital in Rouen. The neuroimaging explorations (MRI, PET) are performed in Marseille (Hospital) and Caen (Cyceron neuroimaging centre). Risks to participating patients are minimal and linked mainly to the neuroimaging part of the research. Study staff in each hospital complete case report forms during each visit and notify the Principal Investigator and clinical investigators of any adverse events.

Figure 1 here

Inclusion and noninclusion criteria

Inclusion in the cohort is offered to patients by a neurologist during the first medical examination (Visit 1), based on their background and their responses to the following short cognitive tests: Questionnaire de Plainte Cognitive (QPC[41]), Geriatric Depression Scale (GDS[42]), MMSE,[37] 5-word test,[43] and Lawton Instrumental Activities of Daily Living (IADL) Scale.[44] The patients targeted by the study are SCI (cognitive complaints without cognitive decline) and/or MCI patients (cognitive complaints with cognitive decline but without AD dementia). To be included, patients must be at least 50 years old, report cognitive complaints, and meet the following criteria: QPC score ≥ 3, GDS score ≤ 9, MMSE score ≥ 24, 5-word test score > 8, and IADL score $= 0$. Patients presenting signs of probable AD according to the NINCDS-ADRDA criteria are not enrolled in AGING. Other noninclusion criteria include

prior or current traumatic brain injury, neurological or cardiovascular disorders, psychiatric disorder (schizophrenia, bipolar disorder, major depression), use of psychotropic medication, and alcohol abuse.

Study arms

At the beginning of Visit 2, before taking the neuropsychological test battery, patients are randomly assigned to one of two arms: standard test instructions or reduced-ST instructions. The randomisation is computer generated, based on an Excel file (created by an independent coworker), and is performed using permuted blocks (size = 4). Within each hospital, randomisation is performed according to the patients' age, sex, and socio-economic status, in order to ensure that the groups are balanced in terms of size and patient characteristics that are of potential importance for investigating susceptibility to age-based ST effects. Patients, neuroimaging staff, and neurologists are all blinded to the conditions, except for the neuropsychologists, who need to know which instructions to use. To ensure the blinding of the diagnosis, neuropsychologists who were in charge of the neuropsychological evaluation will not participate in the diagnosis decision-making.

The standard test instructions replicate the instructions traditionally used in memory clinics during neuropsychological assessments. These instructions have simply been standardised across the four centres. Patients are informed by the neuropsychologist that they are about to undergo a more in-depth assessment than they did during the consultation with the neurologist, in order to test their memory, language, and all the functions they use in daily life. The neuropsychologist emphasises that the testing includes tasks of varying difficulty, so it is normal not to be successful on all the tests. Finally, all the patients are encouraged to do their very best and the testing process then begins. While one might intuitively consider this standard condition to be a neutral condition that does not activate ST, we assume that it implicitly promotes ST effects and is self-threatening for patients.

The reduced-ST instructions are designed to reduce ST by using so-called *teaching instructions*, which consist in explaining ST to patients in order to alleviate any anxiety arising from negative aging stereotypes. In order to standardise and optimise the effectiveness of these instructions, we have developed a 4-min video showing a female or male patient during an interview in a memory clinic. For identification purposes, the gender of the fictitious patient is matched to that of each participant. The video shows a patient coming to a memory clinic for an initial neuropsychological assessment because of memory concerns. Several scenes are enacted and represent the usual steps in the circuit of an MCI/AD diagnosis. The patient meets a neurologist first, then a neuropsychologist, who both give the following teaching instructions to reduce ST: "In our societies, we are all exposed in the media to sensational and often fearful information about AD that inevitably induces a lot of stress. This stress can lead people to experience difficulties during the testing, which can impair performances. The tests themselves can be stressful. It is thus normal to make some mistakes due to stress, but these mistakes are not necessarily signs of AD". We chose not to use a video in the standard (control) condition, in order to stay as close as possible to classic testing practices during neuropsychological assessments in memory clinics.

Measures

Sample size

The number of participants needed in the study was determined[45] on the basis of the anticipated effect size of the ST. Lamont, Swift, and Abrams[21] conducted a meta-analysis of age-based ST and found an effect size of $d = .52$ (95% CI [.248, .717], corresponding to $f^2 = .15$), when they manipulated ST as we do here. However, as ours is the first study of age-based ST in a clinical setting, we decided to use a lower effect size ($f^2 = .07$) to determine our target sample size, in order to have sufficient power to accurately detect a probably smaller

effect in an ecologically valid field experiment. Using this smaller effect size, with the error rate set at 0.05, and power set at 0.80, a power analysis[46] indicated that a sample of 250 participants would be sufficient to detect the critical effects of the conditions and their potential interactions with several moderators in a multiple regression analysis (11 predictors: condition, age, physiological stress, 4 vulnerability factors and the interaction terms between condition and these vulnerability factors). We added 10 participants as a safety margin (N = 260), in order to ensure that at least 30 participants in each arm meet aMCI criteria for Visit 3. Patients will be continuously recruited until the desired sample size is achieved, but not beyond December 2020, to allow sufficient time for the 9-month follow-up before September 2021. Since February 2019, strategies have been implemented to increase recruitment: communication on the AGING protocol has been improved (e.g., poster displays in hospitals and general practices, call for participation in AGING on hospital websites), and appointment scheduling and reminders via post, email, and/or telephone are being used to retain patients already enrolled in the trial.

Vulnerability factors to ST effects

In addition to demographic information (sex, date of birth, years of education, socioeconomic status, native language, etc.), a questionnaire captures participants' reports of memory complaints (IPQ-M[47]), their stereotypical perceptions of aging and AD[26 48], and their self-categorisation as older versus younger.[36] Participants complete this questionnaire at home and bring it back to the memory clinic at Visit 2. These variables will be tested as potential moderators of ST effects on cognitive and stress outcomes.

Neuropsychological test battery (visits 2 and 3)

The same neuropsychological test battery (Table 1) is used for all patients, whatever the condition to which they are randomly assigned (standard vs. reduced-ST condition). All the tests are commonly used in memory clinics, except for the second one assessing visuospatial attentional flexibility and the last one assessing prospective memory.

Table 1 Neuropsychological test battery used in the study for Visit 2 and 4

	Test	Duration (min)	Domain assessed	Authors
1	16-item Free and Cued Selective Reminding Test (FCSRT), with parallel list for 9-month follow-up (Visit 4)	20	Episodic memory	Van der Linden et al.[49]
2	Visuospatial focused attention task	5	Visuospatial attentional flexibility	Herrera et al.[50]
3	Parts A and B of the Trail Making Test	5	Flexibility	Reitan[51]
4	Stroop task	10	Inhibition	Seo et al.[52]
5	*N*-back task	5	Working memory	Adapted from Perlstein et al.[53]
6	Category and lexical fluency task	5	Verbal fluency (language)	Cardebat et al.[54]
7	Rey-Osterrieth Complex Figure (copy and delayed 3-min recall) for Visit 2, and Taylor figure for Visit 4	5-7	Visuoconstruction and memory	Osterrieth[55], Rey[56], Taylor[57]
8	Boston Naming test	5	Visual confrontation naming skills (language)	Kaplan et al.[58]
9	MemPro test	15	Prospective memory	Adapted from Gonneaud et al.[59]

To be categorised as having aMCI or nonamnestic MCI (naMCI), participants have to meet the corresponding criteria of the revised NINCDS-ADRDA standards.[60 61] Our MCI

categorisation is based on the FCSRT (either total recall below or equal to 40/48, Sarazin & Dubois,[62] or scores below Van der Linden's pathological thresholds[49]). The *z* scores obtained for the other cognitive function tests are used to complete the diagnosis, conduct between-group comparisons (standard vs. reduced-ST conditions), and perform within-participants comparisons based on the 9-month follow-up. These additional tests enable us to distinguish between aMCI affecting single versus multiple domains. For the patients with naMCI (if any), the diagnosis will be established at the end of the study. The raw scores are used for the tests without normative data (i.e., visuospatial focused attention and MemPro). Participants who report memory complaints but have normal cognitive performances are categorised as having SCI.

Physiological stress measures

We use HRV, skin conductance, and biomarkers of stress to measure physiological stress (Table 2) during the neuropsychological testing at Visit 2 under either standard or reduced-ST conditions, and during the follow-up visit. These three measures of physiological stress will be tested as potential mediators of the ST effect on cognitive performances and as complementary indicators of any potential progression of the disease.

Table 2 Characteristics of devices used to measure physiological stress

Measure	Device	Characteristics
Heart rate variability (HRV)	Thin elasticated heart rate transmitter belt placed around the chest	Can detect heart rates of 25-240 beats per minute and respiratory rates of 3-70 breaths per minute
Skin conductance	Wristwatch placed around the wrist at the same time as the thin heart rate transmitter belt	Measures skin conductance in micro siemens, 64 Hz frequency, and movements with a triaxial accelerometer
Stress biomarkers	Salivette®. A synthetic cotton swab is removed from the Salivette® and placed in the mouth of the participant to chew	The Salivette® is stored at -80 °C and then sent to a laboratory for analysis

for about 1 minute

Analysis of heart rate variability

HRV data will first be examined according to Task Force recommendations.[63] Premature atrial and ventricular beats and the subsequent intervals will be automatically discarded and visually checked. We will simultaneously explore HR data in time and frequency domains. In the time domain, we will analyse R-R intervals, the standard deviation of R-R intervals (SDNN), the square root of the mean squared difference of successive R-R intervals (rMSSD), and the number of adjacent N-N differing by more than 50 milliseconds, divided by the total number of N-N intervals (pNN50). The rMSSD and pNN50 are associated with high-frequency power (HF) and parasympathetic activity. In the spectral domain, we will analyse low-frequency power (LF; 0.04-0.15 Hz), an index of both sympathetic and parasympathetic activity, and HF (0.15-0.4 Hz), representing the most efferent vagal (parasympathetic) activity to the sinus node. The LF/HF ratio (i.e., sympathovagal balance) will also be calculated.

Analysis of stress biomarkers

Biomarkers of stress include cortisol, dehydroepiandrosterone (DHEA) and its sulphated stable form (DHEAS) from the HPA axis, and immunoglobulin A (IgA). We perform assays of saliva samples taken at two time points (on arrival at the clinic and before leaving). For biomarkers of stress (cortisol, etc.), sensitivity and intra- and inter-assay coefficients of variation are below 0.05 ng/ml, 8%, and 10%, for all biomarkers.[64]

Neurodegeneration and amyloidopathy biomarkers

For the patients diagnosed with aMCI after the neuropsychological examination (30 in the standard condition and 30 in the reduced-ST condition), neuroimaging biomarkers are used to identify those with a high likelihood of AD aetiology: those showing positive AD biomarkers of both neuronal injury (MRI: hippocampal atrophy) and β-amyloid deposition (florbetaben

PET), as recommended by Dubois et al.[65] Early florbetaben PET acquisitions are conducted to evaluate brain perfusion.[66] The hospitals involved in the present study routinely use the CSF biomarkers beta-amyloid and tau, owing to their high diagnostic accuracy in detecting early or even prodromal AD.[4] A lumbar puncture is therefore offered as a routine procedure to all patients categorised as having aMCI (i.e., at risk of AD conversion) on the basis of their neuropsychological performances at Visit 2. It is notified in the consent form that some patients will be asked for their consent to use their CSF data within the AGING protocol (see Appendix I in online supplemental file).

Analysis of MRI data

Half the patients will undergo an MRI session at the Cyceron centre (Caen, France) with a Philips (Eindhoven, The Netherlands) Achieva 3.0 T scanner. A high-resolution T1-weighted anatomical image is acquired using a 3D fast field echo sequence (sagittal; 20 ms repetition time; 4.6 ms echo time; 10° flip angle; 180 slices; 1 mm slice thickness; 256 x 256 mm2 field of view; 256 x 256 matrix). The other half will undergo an MRI session at the imaging centre in Marseille with a Siemens (Erlangen, Germany) MAGNETOM Skyra syngo~~x~~ MR D13 3T scanner. The high-resolutionT1-weighted images are obtained with the 3D magnetisation prepared rapid gradient echo (MPRAGE) sequence (sagittal; 2300ms repetition time; 2.98 ms echo time; 9° flip angle; 900 ms inversion time; 176 slices; 1 mm slice thickness; 256 x 256 mm2 field of view; 256 x 256 matrix). In both centres, participants are given earplugs and their head is stabilised with foam pads to minimise head motion. Visual analyses will be independently performed by two experts who will be blinded to the clinical data and the experimental conditions. In the case of discrepancies between the two experts, an analysis will be performed by a third expert and the final result will be reached by consensus. Particular attention will be paid to the amygdala-hippocampal complex as a marker of neurodegeneration. A whole-brain voxel-based analysis will be also conducted on

SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) after cortical segmentation, spatial normalization and smoothing, to study correlations between memory performances in the standard versus reduced-ST condition and grey-matter density.

Analysis of PET data: Amyloid load (florbetaben ^{18}F)

Amyloid load is measured using florbetaben (18F) (NEURACEQ®; Piramal Imaging Limited, Cambridge, UK), a radiotracer with high sensitivity for β-amyloid deposition and a physical half-life (110 minutes) that makes it suitable for our laboratory setup. Participants are examined using whole-body PET/CT scanners: Dual-Gemini (Philips Medical Systems) in Caen and Discovery 710 (General Electric) in Marseille. A low-dose CT scan is performed first (600 mm scanning range, 5 mm increment, 3.2 mm cutting thickness; 75 seconds per rotation, 512 x 512 matrix, 120 kV, 80 mAs), followed by a brain scan lasting 20 minutes that starts 90 minutes after injection of a dose of 300 MBq of florbetaben (18F) (minimum dose 240 MBq, maximum dose 360 MBq, recommended dose 300 MBq). An additional 5-min acquisition takes place 1 min after injection to assess brain perfusion.

Visual analyses will first be independently performed by two experts blinded to the clinical data and experimental conditions. In the case of discrepancies between the two experts, an analysis will be performed by a third expert and the final result will be reached by consensus. In order to validate the results of the visual analyses, the molecular imaging experts will assess the standard uptake value ratio (SUVr)[67], a semiquantitative index. A standardised uptake value (SUV) will be obtained for each region of interest (ROI). As the cerebellum is reported to be free of fibrillar amyloid plaques in the AD brain, we will use the region-to-cerebellum SUV ratios (SUVrs) for the between-participants comparison, considering both the whole brain and ROIs. Each regional SUVr value will be expressed as the mean over the ROI. To evaluate florbetaben (18F) uptake, we will use 13 ROIs, with values averaged for each one (precuneus, anterior cingulate, posterior cingulate, frontal,

temporal, parietal, occipital, hippocampus, centrum semiovale, anterior putamen, posterior putamen, caudate nucleus and pons, as defined in the MNI-AAL atlas). We will use neocortical SUVr florbetaben (18F) values to distinguish amyloid-positive patients from amyloid-negative patients, using a threshold florbetaben (18F) SUVr value of between 1.4 and 1.5, in agreement with previous studies using florbetaben (18F).[68 69] We will reconstruct all PET sinograms with a 3-D iterative algorithm, with corrections for randomness, dispersion, photonic attenuation and decomposition, imaging with an isotropic voxel of 2 × 2 × 2 mm3 and a spatial resolution of approximately 2-3mm wide at mid-height of the central field of view. A whole-brain voxel-based analysis will be also conducted on SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) after spatial normalisation and smoothing, to study correlations between memory performances in the standard or reduced-ST condition and brain perfusion and Aβ burden.

Analysis of CSF data

CSF data collected in routine practice will be integrated into AGING data.

The CSF-based diagnosis of aMCI due to AD relies on a combination of lowered β-amyloid peptide (1-42 and 1-40) and increased tau and phospho-tau protein curves.

Statistical analyses

Analyses will be conducted in a blinded way, with codes assigned to conditions (A vs. B) and diagnostic categories (numbers). The characteristics of all the demographic, clinical and biological data that are collected, both at baseline and at each visit, will be reported using descriptive statistics. The continuous data will be summarised using mean, standard deviation, median and range values, and the normality assumption will be systematically checked. The categorical data will be presented in frequency tables (*n*, %). Missing data will be handled according to the *missing at random* hypothesis (e.g., multiple imputation, longitudinal mixed-

effects models), as recommended for missing data in clinical trials involving patients with potential neurodegenerative disease.[70]

To achieve the primary objective (testing whether fewer patients meet the aMCI criteria in a reduced-ST condition than in the standard condition), analyses will first be conducted on the whole sample, using multiple linear regression on the FCSRT scores and logistic regression analysis on the diagnostic categories, with both analyses controlling for demographic variables. These analyses will then be rerun on the subsample of 60 patients with a potentially refined diagnosis based on the combination of neurodegeneration and amyloidopathy biomarker data.

To achieve the secondary objectives, we will run a mediation analysis to estimate whether the standard (threatening) condition induces acute physiological stress during the neuropsychological assessment (compared with the reduced-ST condition) that impairs cognitive performances. Mixed models will be performed to compare patients' cognitive performances on the neuropsychological assessment between Visits 2 and 4, in order to identify patients whose scores remain stable, deteriorate, or improve. Finally, moderation and conditional process analyses will be performed on cognitive performances to identify whether some individual characteristics (vulnerability factors: stereotypical perceptions of aging, memory complaints, anxiety about aging, subjective age) can moderate patients' susceptibility to age-based ST effects.

Data management and monitoring

Study staff are responsible for data entry and range checks for data values. All data are stored on a secure server provided by one of the universities. Given the minimal risk nature of the study, the data are internally monitored (Marseille Public Hospitals, which is independent of the funder). Reports on study progress and milestones are submitted to the funder each year.

Patient and Public Involvement

Patients and members of the public were not involved in the development of this study protocol. However, alleviating the anxiety that patients can experience due to negative aging stereotypes during neuropsychology testing and thereby helping to improve the accuracy of MCI diagnosis was a major motivation for the AGING consortium to develop and conduct this study. The findings will therefore be disseminated to participants and to the community at large through newsletters and conferences.

Ethics and dissemination

This protocol was originally approved in July 2017 and the present study details the protocol contained in the latest version approved in April 2019. All changes to the study were decided by the consortium and reviewed by the French National Agency for Medicines and Health Products Safety and the Sud-Est I French Ethics Committee. Written informed consent is required from each patient prior to inclusion. Those people who have direct access to the data take all necessary precautions to maintain confidentiality. All data collected during the study are rendered anonymous. Only the inclusion number is registered.

The results will be disseminated via peer-reviewed publications, conferences, and clinical networks targeting researchers, policy makers, clinicians and caregivers.

Conclusion

AD is a progressive disease that results in dependence and a need for institutionalisation, and is so far untreatable. Ethical questions are raised by the overdiagnosis of aMCI, as its consequences can be extremely damaging - not only for the patient, but also for the family and society.[71] Without denying that aging is associated with cognitive decline and

neurodegenerative diseases for many people, we suggest that special attention should be paid to the influence of psychosocial factors that have been largely overlooked, regarding neuropsychological testing in memory clinics. The AGING project has potentially important theoretical and practical implications for improving neuropsychological testing, to avoid many older people from being wrongly classified as having aMCI.

Trial status

The recruitment phase began in July 2018 for Marseille Public Hospitals, September 2018 for La Milétrie University Hospital, October 2018 for Caen University Hospital, and November 2018 for Charles Nicolle University Hospital. Twenty-eight patients had been recruited at the time of the study. The estimated end date for this study is September 2021.

Abbreviations

aMCI: amnestic mild cognitive impairment; AD: Alzheimer's disease; HF: high frequency; HR: heart rate; HRV: heart rate variability; LF low frequency; R-R: distance between two consecutive R-waves on electrocardiogram; SCI: subjective cognitive impairment; ST: stereotype threat; MMSE: Mini-Mental State Examination; CSF: cerebrospinal fluid; GDS: Geriatric Depression Scale; QPC: questionnaire de plainte cognitive [cognitive complaint questionnaire]; IADL: Lawton Instrumental Activities of Daily Living; IPQ-M: Illness Perception Questionnaire – Memory; FCSRT: 16-item Free and Cued Selective Reminding Test; DHEA: dehydroepiandrosterone; DHEAS: sulphated stable form of DHEA; Ig A: immunoglobulin A; SDNN: standard deviation of R-R intervals; rMSSD: square root of the mean squared difference of successive R-R intervals; pNN50: number of adjacent N-N differing by more than 50 milliseconds divided by the total number of N-N intervals; SUV: standardised uptake value; SUVr: standard uptake value ratio.

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Competing interests

The authors declare that they have no competing interests.

Ethics approval

French National Agency for Medicines and Health Products Safety and Sud-Est I French Ethics Committee (2017-A00946-47).

Data sharing statement

Within 6 months of the end of the final year of funding, the investigators will create a complete, cleaned, de-identified copy of the final dataset, and a plan for conducting the outcome analyses outlined in the study protocol will be made available upon reasonable request.

References

1. Selkoe DJ. Preventing Alzheimer's disease. *Science* 2012;337:1488-92.
2. INSERM, Blum D, Buée L, et al. Alzheimer (maladie d'): une maladie neurodégénérative complexe mais de mieux en mieux comprise. 2019. Retrieved from https://www.inserm.fr/information-en-sante/dossiers-information/alzheimer-maladie
3. Tian T, Zhang B, Jia Y, et al. Promise and challenge: the lens model as a biomarker for early diagnosis of Alzheimer's disease. *Dis Markers* 2014;2014:1-5.

4. Ewers M, Walsh C, Trojanowski JQ, et al. Prediction of conversion from mild cognitive impairment to Alzheimer's disease dementia based upon biomarkers and neuropsychological test performance. *Neurobiol Aging* 2012;33:1203-1214.

5. Han JW, Kim TH, Lee SB, et al. Predictive validity and diagnostic stability of mild cognitive impairment subtypes. *Alzheimers Dement* 2012;8:553-9.

6. Morris JC, Storandt M, Miller JP, et al. Mild cognitive impairment represents early-stage Alzheimer disease. *Arch Neurol* 2001;58(3):397-405.

7. Solfrizzi V, Panza F, Colacicco AM, et al. Vascular risk factors, incidence of MCI, and rates of progression to dementia. *Neurology* 2004;63:1882-91.

8. Sachdev PS, Lipnicki DM, Crawford J, et al. Factors predicting reversion from mild cognitive impairment to normal cognitive functioning: a population-based study. *PLoS ONE* 2013;8:e59649.

9. Flicker C, Ferris SH, Reisberg B. Mild cognitive impairment in the elderly: predictors of dementia. *Neurology* 1991;41:1006.

10. Petersen RC, Smith GE, Waring SC, et al. Aging, memory, and mild cognitive impairment. *International Psychogeriatrics* 1997;9:65-9.

11. Petersen RC, Smith GE, Waring SC, et al. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 1999;56:303.

12. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med* 2004;256:183-94.

13. Winblad B, Palmer K, Kivipelto M, et al. Mild cognitive impairment - beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment *J Intern Med* 2004;256:240-6.

14. Hess TM, Hinson JT. Age-related variation in the influences of aging stereotypes on memory in adulthood. *Psychology and Aging* 2006;21(3):621.

15. Hess TM, Hinson JT, Hodges EA. Moderators of and mechanisms underlying stereotype threat effects on older adults' memory performance. *Exp Aging Res* 2009;35:153-77.

16. Kit KA, Tuokko HA, Mateer CA. A review of the stereotype threat literature and its application in a neurological population. *Neuropsychol Rev* 2008;18:132-48.

17. Steele CM. A threat in the air: how stereotypes shape intellectual identity and performance. *Am Psychol* 1997;52:613-29.

18. Steele CM, Aronson J. Stereotype threat and the intellectual test performance of African Americans. *J Pers Soc Psychol* 1995;69:797-811.

19. Barber SJ. An examination of age-based stereotype threat about cognitive decline: implications for stereotype-threat research and theory development. *Perspect Psychol Sci* 2017;12:62-90.

20. Chasteen AL, Kang SK, Remedios JD. Aging and stereotype threat: development, process and interventions. In Inzlicht M, Schmader T. *Stereotype threat theory, process, and application*. Oxford University Press:2011;202-16.

21. Lamont RA, Swift HJ, Abrams D. A review and meta-analysis of age-based stereotype threat: negative stereotypes, not facts, do the damage. *Psychol Aging* 2015;30:180-93.

22. Hess TM, Auman C, Colcombe SJ, et al. The impact of stereotype threat on age differences in memory performance. *J Gerontol B Psychol Sci Soc Sci* 2003;58:3-11.

23. Hess TM, Emery L, Queen TL. Task demands moderate stereotype threat effects on memory performance. *J Gerontol B Psychol Sci Soc Sci* 2009;64B:482-6.

24. Hess TM, Hinson JT, Statham JA. Explicit and implicit stereotype activation effects on memory: do age and awareness moderate the impact of priming? *Psychol Aging* 2004;19:495-505.

25. Levy B. Improving memory in old age through implicit self-stereotyping. *J Pers Soc Psychol* 1996;71:1092-107.

26. Levy BR, Zonderman AB, Slade MD, et al. Memory shaped by age stereotypes over time. *J Gerontol B Psychol Sci Soc Sci* 2012;67:432-6.

27. Stein R, Blanchard-Fields F, Hertzog C. The effects of age-stereotype priming on the memory performance of older adults. *Exp Aging Res* 2002;28:169-81.

28. Mazerolle M, Régner I, Morisset P, et al. Stereotype threat strengthens automatic recall and undermines controlled processes in older adults. *Psychol Sci* 2012;23:723-7.

29. Mazerolle M, Régner I, Rigalleau F, et al. Stereotype threat alters the subjective experience of memory. *Exp Psychol* 2015;62:395-402.

30. Chasteen AL, Bhattacharyya S, Horhota M, et al. How Feelings of Stereotype Threat Influence Older Adults' Memory Performance. *Exp Aging Res* 2005;31:235-60.

31. Desrichard O, Köpetz C. A threat in the elder: the impact of task-instructions, self-efficacy and performance expectations on memory performance in the elderly. *Eur J Soc Psychol* 2005;35:537-52.

32. Kang SK, Chasteen AL. The moderating role of age-group identification and perceived threat on stereotype threat among older adults. *Int J Aging Hum Dev* 2009;69:201-20.

33. Barber SJ, Mather M, Gatz M. How stereotype threat affects healthy older adults' performance on clinical assessments of cognitive decline: the key role of regulatory fit. *J Gerontol B Psychol Sci Soc Sci* 2015;70:891-900.

34. Fresson M, Dardenne B, Geurten M, et al. The effect of stereotype threat on older people's clinical cognitive outcomes: investigating the moderating role of dementia worry. *Clin Neuropsychol* 2017;31:1306-28.

35. Haslam C, Morton TA, Haslam SA, et al. "When the age is in, the wit is out": age-related self-categorization and deficit expectations reduce performance on clinical tests used in dementia assessment. *Psychol Aging* 2012;27:778-84.

36. Mazerolle M, Régner I, Barber SJ, et al. Negative aging stereotypes impair performance on rief cognitive tests used to screen for predementia. *J Gerontol B Psychol Sci Soc Sci* 2016;gbw083.

37. Folstein MF, Folstein SE, McHugh PR. Mini-mental state. *J Psychiatr Res* 1975;12:189-98.

38. Nasreddine ZS, Phillips NA, Bédirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society* 2005; *53*(4):695-9.

39. Johns M, Schmader T, Martens A. Knowing is half the battle: teaching stereotype threat as a means of improving women's math performance. *Psychol Sci* 2005;16:175-9.

40. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 Statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158(3):200-7.

41. Thomas Antérion C, Ribas C, Honoré-Masson S, et al. Le questionnaire de plainte cognitive (QPC): un outil de recherche de plainte suspecte d'évoquer une maladie d'Alzheimer. *L'Année Gérontologique* 2003;17:56-65.

42. Yesavage JA, Brink TL, Rose TL, et al. Development and validation of a geriatric depression screening scale: a preliminary report. *J Psychiatr Res* 1982;17:37-49.

43. Dubois B, Touchon J, Portet F, et al. "Les 5 mots", épreuve simple et sensible pour le diagnostic de la maladie d'Alzheimer. *Presse Méd* 2002;31:1696-99.

44. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist* 1969;9:179-86.

45. Tabachnick BG, Fidell LS. *Using multivariate statistics. 5th ed*. Boston: Pearson/Allyn & Bacon;2007.

46. Soper, D. (2006–2013). Statistics calculators Version 3.0. Retrieved from tiny.cc/sopercalc

47. Hurt CS, Burns A, Brown RG, et al. Perceptions of subjective memory complaint in older adults: the Illness Perception Questionnaire – Memory (IPQ-M). *Int Psychogeriatr* 2010;22:750-60.

48. Rust TB, Kwong See ST. Beliefs about aging and Alzheimer's fisease in three domains. *Can J Aging* 2010;29:567-75.

49. Van der Linden M, Coyette F, Poitrenaud J, et al. L'épreuve de rappel libre/rappel indicé à 16 items (RL/RI-16). In M. Van der Linden (ed.), *L'évaluation des troubles de la mémoire. Présentation de quatre tests de mémoire épisodique (avec leur étalonnage).* Marseille: Solal 2004;25-47.

50. Herrera C, Chambon C, Michel BF, et al. Positive effects of computer-based cognitive training in adults with mild cognitive impairment. *Neuropsychologia* 2012;50:1871-81.

51. Reitan RM. Validity of the Trail Making Test as an indicator of organic brain damage. *Percept Mot Skills* 1958;8:271-6.

52. Seo EH, Lee DY, Choo H, et al. Normative study of the Stroop Color and Word Test in an educationally diverse elderly population. *Int J Geriatr Psychiatry* 2008;23:1020-7.

53. Perlstein WM, Dixit NK, Carter CS, et al. Prefrontal cortex dysfunction mediates deficits in working memory and prepotent responding in schizophrenia. *Biol Psychiatry* 2003;53:25-38.

54. Cardebat D, Doyon B, Puel M, et al. Évocation lexicale et sémantique chez des sujets normaux: performances et dynamiques de production en fonction du sexe, de l'âge, et du niveau d'étude. *Acta Neurol Belg* 1990;90, 207–17.

55. Osterrieth PA. Le test de copie d'une figure complex: contribution à l'étude de la perception et de la mémoire [The test of copying a complex figure: A contribution to the study of perception and memory]. *Arch Psychol* 1944;28:1021–34.

56. Rey A. (1941). L'examen psychologique dans les cas d'encephalopathie traumatique [Psychological examination of traumatic encephalopathy]. *Arch Psychol* 1941;28:286–340.

57. Taylor LB. Localisation of cerebral lesions by psychological testing. *Neurosurgery* 1969;16:269-87.

58. Kaplan E, Goodglass H, Weintraub S. The Boston Naming Test. Lea & Febiger, 1983.

59. Gonneaud J, Rauchs G, Groussard M, et al. How do we process event-based and time-based intentions in the brain? An fMRI study of prospective memory in healthy individuals: neuroimaging of prospective memory. *Hum Brain Mapp* 2014;35:3066-82.

60. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:270-9.

61. 2012 Alzheimer's disease facts and figures. *Alzheimers Dement* 2012;8:131-68.

62. Sarazin M, Berr C, De Rotrou J, et al. Amnestic syndrome of the medial temporal type identifies prodromal AD: a longitudinal study. *Neurology* 2007;69:1859-67.

63. Electrophysiology TF of the ES. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation* 1996;93(5):1043-65.

64. Lac G, Dutheil F, Brousse G, et al. Saliva DHEAS changes in patients suffering from psychopathological disorders arising from bullying at work. *Brain Cognition* 2012;80:277-81.

65. Dubois B, Hampel H, Feldman HH, et al. Preclinical Alzheimer's disease: definition, natural history, and diagnostic criteria. *Alzheimers Dement* 2016;12:292-323.

66. Daerr S, Brendel M, Zach C, et al. Evaluation of early-phase [18 F]-florbetaben PET acquisition in clinical routine cases. *Neuroimage Clin* 2017;14:77-86.

67. Wong JT, Gallo DA. Activating aging stereotypes increases source recollection confusions in older adults: effect at encoding but not retrieval. *J Gerontol B Psychol Sci Soc Sci* 2019;74:633-41.

68. Sabri O, Sabbagh MN, Seibyl J, et al. Florbetaben PET imaging to detect amyloid beta plaques in Alzheimer's disease: phase 3 study. *Alzheimers Dement* 2015;11:964-74.

69. Seibyl J, Catafau AM, Barthel H, et al. Impact of training method on the robustness of the visual assessment of 18F-florbetaben PET scans: results from a phase-3 study. *J Nucl Med* 2016;57:900-6.

70. Coley N, Gardette V, Cantet C, et al. How should we deal with missing data in clinical trials involving Alzheimer's disease patients? *Curr Alzheimer Res* 2011;8(4):421-33.

71. Merckelbach H, Jelicic M, Jonker C. Planting a misdiagnosis of Alzheimer's disease in a person's mind. *Acta Neuropsychiatr* 2012;24:60-2.

Figure 1 Flow chart of the main steps for patients. MCI: mild cognitive impairment; MRI : magnetic resonance imaging; PET: positron emission tomography; CSF: cerebrospinal fluid. As a routine procedure, aMCI patients will be offered a lumbar puncture (included in the consent form).

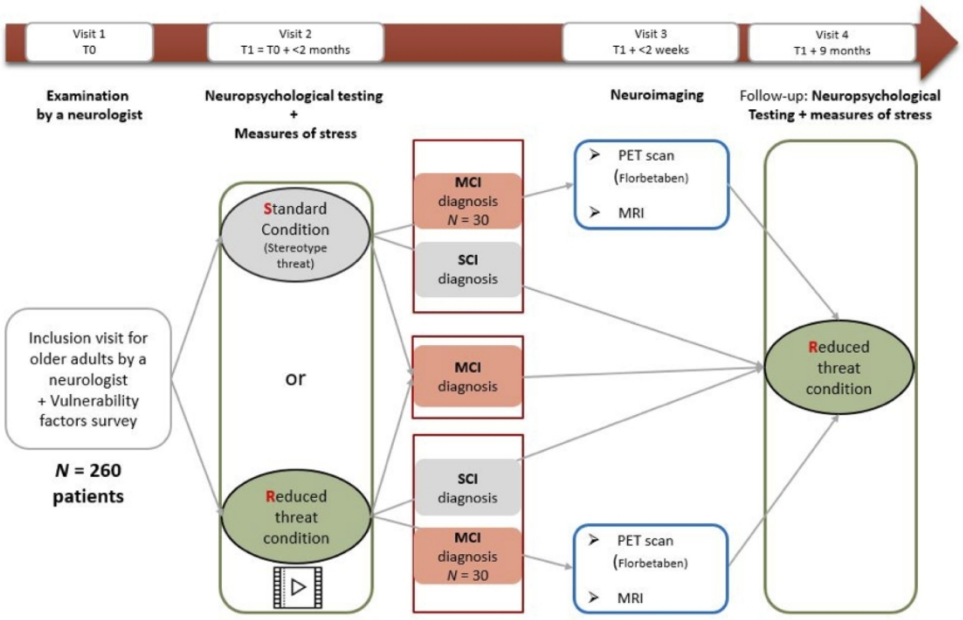

Figure 1 Flow chart of the main steps for patients. MCI: Mild Cognitive Impairment, MRI : Magnetic Resonance Imaging, PET: Positron Emission Tomography, CSF: CerebroSpinal Fluid. As a routine procedure, CSF puncture will be proposed to aMCI patients (this possibility is included in the consent form).

332x212mm (150 x 150 DPI)

Appendix I Participant information sheet / Consent form

Participant information sheet

Neuropsychological Assessment and Aging

Project sponsor: Assistance Publique Hôpitaux de MARSEILLE (AP-HM) 80 Rue BROCHIER, 13354 MARSEILLE Cedex 5.

Principal Investigator: Bernard François MICHEL (MD), Department of Behavioral Neurology, 270 Boulevard de SAINTE-MARGUERITE, BP 29, 13274 MARSEILLE cedex 9.

Protocol Number: AGING – 2017-A00946-47

Protocol version: 24/01/2018

1- Introduction

You are invited by [insert Neurologist name] to participate in a study on "Neuropsychological Assessment and Aging", which aims to improve the conditions of memory disorders assessment. Before your decision, it is important that you read this information sheet carefully: it will help you understand the rationale for this research, its progress, your role and the expected benefits. Your participation is completely voluntary. You are free to accept or refuse to participate. If you agree, you are free to change your mind at any time without having to provide any justification and your decision will not affect your routine treatment, your relationship with those treating you or your relationship with [insert Institution].

2- Rationale of the study

This study, which is sponsored by AP-HM, is led by Bernard François MICHEL (MD) of the Department of Behavioral Neurology (Hospital SAINTE-MARGUERITE, AP-HM) in collaboration with researchers from the CNRS, INSERM, and physicians from several hospitals in FRANCE, obtained funding from the National Research Agency (ANR). Its goal is to improve the conditions of neuropsychological assessment.

3- Overview of the procedure

This research plans to include 260 participants (at least 50 years old who come to the memory clinic for an initial assessment). It is conducted in 5 Hospitals in France. Your participation in this study will last about 10 months. Without counting the visit of today, you will have to return 2 or 3 times to the hospital, with a delay of 9 to 10 months between the first and the last visit. Participation in this research does not imply any additional financial cost for you. Any costs of transportation, meals, and accommodation will be covered by the center where you will take the tests (especially if they generate too many constraints compared to the routine care). The inclusion period in this study will run for 4 years.

4- Who can participate?

➢ Visit 1

Anyone who is at least 50 years old, coming to the hospital in memory consultation for a first evaluation (for example recommended by primary care physician) because of memory complaint. Cannot participate people with the following characteristics: Chronic ethylic addiction, psychiatric disorders (schizophrenia, bipolar disorder, major depression), traumatic brain injury, developmental pathologies, abuse of psychotropic drugs (if modified dosage in the last 3 months).

5- What does participation involve?

➢ Visit 2

If you agree to participate, the next visit (approximate duration: 2 hours) will be a consultation with the neuropsychologist, with whom you will take the memory tests usually proposed, and you will complete a questionnaire about your opinions and your perceptions about yourself and aging in general. Aging itself only leads to a slowing down in neuropsychological test responses, but these effects are well known and limited. The neuropsychological tests will be accompanied by physiological measurements that will be obtained thanks to a belt, a wristwatch, and a salivary gum (which looks like a chewing gum). You will need to wear the belt and wristwatch during the neuropsychological tests. The salivary gum should be chewed for a few seconds. Two salivary gums will be proposed to you: one before the beginning of the neuropsychological tests, the other after the tests.

➢ Visit 3

Depending on the diagnosis, after Visit 2, only certain participants will be invited to take two additional neuroimaging tests (MRI and PET described below) to complete the assessment during a third visit (length of visit: about 4h). The medical team responsible for monitoring the study, specific to each center, will meet to decide on the usefulness, for each participant, to carry out these neuroimaging examinations in order to reach the scientific objectives of the study (the decision criteria are indeed exclusively scientific). Magnetic Resonance Imaging (MRI) of the brain is a painless biological examination that allows to visualize the brain. At the time of the visit, the absence of contraindication to the MRI will be checked by the physician (claustrophobia, metallic material in the body). The only inconveniences related to this examination come from the constraint of remaining stay lying down, without moving and the noise caused by the operation of the MRI. No adverse biological effects are known to date. The duration of this examination is approximately one hour. Positron Emission Tomography (PET), which is associated with radiotracers such as ^{18}Fflorbetaben, is an imaging technique that can be used to visualize the presence of amyloid plaques in patients' brains. The duration of this examination is about 2 hours. You will lie on your back and an infusion will be placed. The dose of radiation delivered is controlled by the hospital: it is limited by legislation. The maximum dose absorbed by the whole body during a PET scan is about 7 milliSieverts, which is usual for an imaging and risk-free examination as reported by different clinical studies. To ensure the validity of the study, the results of the neuroimaging examinations performed during visit 3 will be communicated to you at the end of visit 4. The results of the neuropsychology done on visit 2 will be communicated to you after visit 2.

➢ **Visit 4**

Finally, a last follow-up visit (approximately 2 hours) with the neuropsychologist will be offered 9 to 10 months later, during which you will take similar tests to those of visit 2 to evaluate your memory. At the end of this visit, patients who have had a lumbar puncture as part of routine care, will be asked for their agreement to use their results for the present study. Lumbar puncture is a relatively invasive examination that is performed in routine medical practice as part of the diagnosis and follow-up of patients with mild cognitive impairment.

6- What are the costs?

As a patient, you are in a situation of usual care and there will be no additional financial cost to you to be in this study: the neuroimaging examinations and the physiological measurements are funded by the ANR (whose management is provided by the sponsor), and neuropsychological examinations and Lumbar Puncture are part of a routine care consultation. Similarly, travel expenses for visits 1 (first cognitive assessment), 2 (neuropsychological assessment) and 4 (9-month follow-up: neuropsychological assessment) will be covered by a regular consultation. Depending on the centers (including CAEN), the visit 3 (neuroimaging) may lead to travel or accommodation expenses that will be funded by the sponsor up to compensation (travel, accommodation, meals).

7- What are the expected benefits?

Societal benefits: improved test conditions for neuropsychological assessments at hospital.

8- What are the possible risks and disadvantages of taking part?

Participation in the study requires some availability to go several times to the hospital (but most of visits will be scheduled as part of your usual care).

9- What are your rights as a participant in this research?

You can refuse to participate in this research without having to provide any justification and without any consequence on the continuation of your treatment. Likewise, you can withdraw at any time from the trial without justification, and without consequence on the continuation of your treatment or on the quality of the care which will be provided to you. To participate in this study you must be affiliated to a social security scheme. This research falls under the application of the Public Health Code. It is subject to the new regulatory system that applies to research "involving the human person", namely Law No. 2012-300 of 5 March 2012 on research involving the human person (called law JARDE) as amended by the order n° 2016-800 of June 16, 2016, and its decrees of application. This information is available on the Legifrance website (www.legifrance.gouv.fr). The coordinating investigator of this study is the Dr [insert Neurologist name]. The promoter of this test is the AP-HM, 80 rue BROCHIER 13005 MARSEILLE, with a SHAM insurance contract (Contract No. 145.166). This study has been originally approved by the Committee for the Protection of South-East Persons I (date 19/07/2017) and the authorization of the National Agency for the Safety of Drugs and Health Products (date 12/06/2017).

10- Confidentiality of the data

Your medical and socio-demographic data that are necessary for this research, will be the subject of a computerized processing in accordance with the law n° 2004-801 of August 6th, 2004 relative to the protection of persons and the processing of personal data, and modifying the law n° 78-17 of January 6th, 1978 on data processing, computers files and freedoms. These data will be anonymized and identified by a number and your initials. In no case will these data be identifiable. They will remain strictly confidential and can only be consulted by the medical team, the persons duly mandated by the promoter and possibly by the representatives of the Competent Authorities. In accordance with the provisions of the law on data processing, computers files and freedoms (Law No. 78-17 of January 6, 1978 amended by Law No. 2004-801 of August 6, 2004), you have the right to access and rectification of your personal data and the right of objection to the transmission of such data, protected by professional secrecy, likely to be used and processed in the context of this research. You can also access directly or through a doctor of your choice to all of your medical data (Article L 1111-7 of the Public Health Code). In case of any injuries or complications resulting from your participation in the study, you can have access to compensation according to the terms of Articles L. 1121-10 and L.1142-3 of the Public Health Code.

11- If you decide to participate

This document belongs to you and we invite you to discuss it with your doctor and / or your relatives. Participation in this study requires the signature of the form below named "Consent Form". You will need to have read and understood this information sheet and you will need to sign the document called "Free and Informed Consent". Your consent does not relieve the sponsor and the doctors of their responsibilities, you also retain all rights guaranteed by law. The Physician who takes care of you may at any time decide to interrupt your participation if he has new elements calling into question the conduct of this study. You can also decide to interrupt your participation at any time during the study, without any justification. You have the right to get information held by investigators about your health. To make your decision concerning your participation or not in this project, you have a reflection period of at least 1 week (minimum delay between the first visit this day, and the second visit corresponding to the neuropsychological assessment).

12- If you decide to not participate

You can refuse to participate without having to justify your decision. This will not affect your care or your relationship with the healthcare team.

To get more information
To request access to your data
To obtain the overall results of the study

You can contact Bernard François MICHEL (MD), Department of Behavioral Neurology, 270 Boulevard of SAINTE-MARGUERITE, BP 29, 13274 MARSEILLE cedex 9
Tel: 04 91 74 46 75

Free and Informed Consent

Neuropsychological Assessment and Aging

Project sponsor: Assistance Publique des Hôpitaux de MARSEILLE (AP-HM) 80 Rue BROCHIER, 13354 MARSEILLE Cedex 5.

Principal Investigator: Bernard François MICHEL (MD), Department of Behavioral Neurology, 270 Boulevard de SAINTE-MARGUERITE, BP 29, 13274 MARSEILLE cedex 9.

I, the undersigned, ... (first and last name), born on .... / .... / .... declares:

1- I freely agree to participate in the research involving the interventional category 1 human person entitled "Neuropsychological Assessment and Aging", without this relieving the research organizers from their responsibilities;
2- I understand that I have a period of reflection between the moment the information was given to me and the moment of the signature of this document;
3- I have been informed that I have the right to withdraw my consent to participate in the study at any time, without any justification and without changing my relationship with the nursing staff or my care;
4- I have been informed that I retain all my rights guaranteed by the law (The law n ° 2012-300 of March 5th, 2012 relating to the research involving the human person (known as law JARDE) / Public Health Code, title II of the book first relative research involving the human person);
5- I have been informed that this research was approved by the Committee of Protection of the People SOUTHEAST I on 19/07/2017 and the National Agency for the Safety of Drugs and health products on 12/06/2017;
6- I have been informed of the purpose, process, advantages and disadvantages of this research, and have been informed that it will be conducted in accordance with the Good Clinical Practices defined in the Official Bulletin published by the Ministry of Social Affairs and Social Affairs. 'Employment;
7- I was able to ask all the questions I wanted and received adapted answers that I clearly understood, and I have noted that I could complete this information throughout the study with the Pr / Dr [insert Neurologist name];
8- I have been informed that the Sponsor of this study is represented by the AP-HM (DRCI 80, rue BROCHIER, 13354 Marseille Cedex 05), and has subscribed a contract of insurance "Civil Liability" in accordance with the law in force with SHAM (contract No. 145.166);
9- I have been informed of the anonymous use of the data concerning me, collected as part of this research by computerized treatment. The presentation of the results of the study will not allow my direct or indirect identification;
10- I have been informed that these data can only be consulted by the investigators of the study and the promoter or by persons mandated by the sponsor and bound by professional secrecy, or by persons mandated by the administrative, health and judicial authorities;

11- I have been informed that I could if I wish to access these data, to check them and to request modifications if necessary, according to the law in force (guaranteed by the articles 39 and 40 of the law n ° 78-17 of January 6th, 1978 on data processing, computers files and freedoms, and subsequent laws including Law No. 2004-801 of August 6, 2004);
12- I have noted that any new information occurring during the study, likely to call into question my participation, will be communicated to me as soon as possible;
13- I have understood that the sponsor or the investigator can decide at any moment to interrupt the study;
14- I am affiliated to a social security scheme;
15- I have been informed that the overall results of the study may be communicated to me in accordance with article L1122-1 of the Public Health Code;
16- I have understood that it is possible for me to join the Pr / Dr .... for any further information with the following phone number .....
17- I have understood that if I agree to participate in this research, I must sign this document;
18- Do not allow my identification.

Having had enough time for reflection before making my decision:

☐ **I freely and voluntarily agree to participate in the research AGING under the conditions specified above.**

☐ **I accept two additional neuroimaging examinations (MRI and PET) to complete my assessment during a third visit, in case I am selected for this phase of the study.**

Date……. **Date…….**

Name and Signature of the patient **Name and Signature of the Investigator**

Done in duplicate (one for the investigating physician and one for the patient)

Reporting checklist for protocol of a clinical trial.

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	1, 3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	#3	Date and version identifier	20
Funding	#4	Sources and types of financial, material, and other support	23
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 21-23
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	2, 22
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	19, 22
Roles and responsibilities:	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint	22

committees		adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6
Objectives	#7	Specific objectives or hypotheses	7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	N/A

		change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12-18
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8, Figure 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	12
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or	10

		assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8, 10
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8, 9, 10
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10, 16, 17, 18
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Appendix 1
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-17
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	12
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management	19

		procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18-19
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	19
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	18
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	18, 22
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18
Ethics and dissemination			
Research ethics	#24	Plans for seeking research ethics committee /	3, 20

approval		institutional review board (REC / IRB) approval	
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	20
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8, 16, 20
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Legend of Figure 1
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	23
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	23
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	19

Appendices

Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A