Title: A non-invasive, multimodal approach to restore functional networks and cognition in Alzheimer's disease and frontotemporal dementia

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OVERALL SUMMARY

Non-invasive and effective treatments are much needed for the cure of dementia. Available treatments, however, are mainly symptomatic and their mechanism of action is largely unrelated to disease pathophysiology. Intermediate phenotypes can bridge the gap between pathology and clinical symptoms. Targeting these biomarkers might represent a biologically-sound and effective treatment approach. Increasing evidence suggests that the disruption of large-scale networks might provide the missing link between pathology and clinical symptoms in several neurodegenerative dementias. This pilot project will test a non-invasive approach to restore large-scale networks connectivity in two neurodegenerative disorders in which pathology has been clearly linked to large-scale network disruption, i.e. Alzheimer`s disease (AD) and behavioual frontotemporal dementia (bvFTD). The effect of network stimulation on the clinical symptoms, as well as on brain connectivity, will be tested.

A. Background and State of Art

Resting-state fMRI studies have shown that Alzheimer`s disease (AD) is associated with the progressive degeneration of the default mode network (DMN), and behavioural frontotemporal dementia (bvFTD) with salience network (SN) degeneration (Pievani et al, 2011a). These 2 diseases also feature anti-correlated patterns (DMN connectivity is reduced and SN is hyper-activated in AD, while the opposite is seen in bvFTD; Zhou et al, 2010). Although DMN and SN disruption offer an attractive target for AD and bvFTD treatment, no study has investigated the efficacy of recalibrating these networks. Repetitive transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation are non-invasive techniques capable to restore brain function through direct stimulation of disrupted networks or indirect suppression of hyper-activated networks (Cotelli et al, 2011; Naeser etal, 2005). In this pilot project we will test the efficacy of DMN and SN recalibration in AD and bvFTD through tDCS.

B. Hypothesis and Specific Aims

a. Hypothesis and significante

Neurodegenerative disorders disrupt large-scale neuronal networks. Modulating disease-specific networks might provide an effective approach to restore network function and improve clinical symptoms.

b. Specific Aims

1) To assess the clinical/cognitive effect of disease-specific networks stimulation in AD (DMN stimulation) and bvFTD (SN stimulation);
2) To assess the clinical/cognitive effect of anti-correlated networks suppression in AD (SN suppression) and bvFTD (DMN suppression);
3) To investigate correlations between tDCS-related network changes and biomarker changes in AD and bvFTD.

c. Experimental design

<u>Aim 1</u>

Population study

Twenty patients (10 mild AD and 10 mild bvFTD) will be recruited at the IRCCS-Fatebenefratelli of Brescia. Inclusion criteria will be a diagnosis of AD or bvFTD according to the current clinical criteria (Albert et al., 2011; Rascovsky et al., 2011) and ability to provide written informed consent. Exclusion criteria will be moderate/severe dementia (MMSE<18) and/or the presence of any medical or psychiatric illness that could interfere in completing assessments.

Paradigm 1 - direct stimulation of disconnected networks
The DMN is anchored to `posterior` regions and the SN is anchored to `anterior` regions (Zhou et al., 2010). DMN connectivity will be stimulated in AD patients through anodal tDCS of the posterior DMN; SN connectivity will be stimulated in bvFTD patients through anodal tDCS of the anterior SN.

Clinical assessment
Each patient will undergo a clinical and neuropsychological evaluation to assess symptoms and cognition at baseline (before tDCS session, T0) and follow-up (after the tDCS sessions, T1; and 6 months after tDCS treatment, T2). Patients will also undergo biological samples collection (CSF at T0, blood samples at T0, T1, T2) to assess the effect of tDCS on inflammatory measures (optional sub-study).

Outcome measures
We will test whether the stimulation of AD- and bvFTD-disrupted networks improves patients clinical condition.

PRIMARY efficacy parameters will be:
- change in scores at T1 compared with T0 for clinical and behavioural scores in AD and bvFTD patients

SECONDARY outcome measures will be:
- change in scores at T1 compared with T0 for cognitive performance in AD and bvFTD patients
- change in scores at T2 compared with T0 for clinical, behavioural, and cognitive performance in AD and bvFTD patients

<u>Aim2</u>
DMN and SN feature a divergent pattern of connectivity in AD and bvFTD patients (Zhou et al., 2010). These anti-correlated networks offer an alternative approach to recalibrate network connectivity: low-frequency/cathodal stimulation can indeed be used to inhibit hyper-activated networks and improve clinical conditions (Naeser et al., 2005; Martin et al., 2004). We will therefore test whether inhibition of AD- and bvFTD-hyper-activated networks improves clinical condition. The experimental design of this task is analogous to that of AIM1, the key difference being the administration of cathodal tDCS to suppress hyper-activated networks.

Population study and clinical assessment
Ten mild AD and 10 mild bvFTD patients will be recruited and assessed following the procedures and inclusion/exclusion criteria described in AIM1. Patients will be evaluated at baseline (T0), after tDCS sessions (T1), and after 6 months (T2).

Paradigm 2 – suppression of hyper-activated networks
Cathodal tDCS will be applied (i) to the posterior DMN in bvFTD patients to inhibit DMN connectivity, and (ii) to the anterior SN in AD patients to inhibit SN connectivity.

Outcome measures
We will test whether the suppression of AD and bvFTD anti-correlated networks improves clinical condition.

PRIMARY and SECONDARY efficacy parameters will be the same as in AIM1.

Aim3

To elucidate the mechanisms underlying network recalibration, in vivo biomarkers of structural and functional networks connectivity will be collected off-line. Each subjects will undergo an MRI scan on a 3T scanner at the UO3. The following sequences will be collected:

- resting-state functional MRI (rs-fMRI), to assess DMN and SN functional connectivity
- arterial spin labelling (ASL), to measure DMN and SN brain perfusion
- diffusion tensor imaging (DTI), to measure DMN and SN structural connectivity
- structural MRI, for the routine assessment of cortical and hippocampal atrophy

Scan will be collected at baseline (T0, prior to tDCS experiment), after completion of the tDCS sessions (T1), and after 6 months (T2). The subjects included in this analysis will be those recruited for AIM1 and AIM2 (20 AD and 20 bvFTD).

Outcome measures

We will identify (surrogate) imaging biomarkers of DMN and SN recalibration in AD and bvFTD.

Surrogate primary outcome measures will be:

- change at T1 compared with T0 for rs-fMRI DMN and SN connectivity
- change at T1 compared with T0 for ASL DMN and SN connectivity

Surrogate secondary outcome measures will be:

- change at T1 compared with T0 for DMN and SN structural connectivity
- change at T2 compared with T0 for rs-fMRI, ASL, and DTI connectivity biomarkers

Control group

For baseline comparison of clinical, cognitive, and imaging variables, a group of 20 cognitively healthy controls of comparable age will be recruited. Controls will undergo the same clinical, neuropsychological and MRI assessment of patients at baseline.

d. Preliminary data

In a recent review, we observed that AD and bvFTD target distinct large-scale brain networks (Pievani et al., Lancet Neurol 2011). Using modern imaging techniques, such as rs-fMRI and diffusion tensor imaging (DTI), we showed that structural and functional connectivity is disrupted along specific networks in AD and FTD (Pievani et al., Hum Brain Map 2010; Pievani et al., Alzheimer's Dementia 2014). Our lab previously used rs-fMRI to investigate the effect of pharmacological interventions on DMN connectivity in AD patients (Lorenzi et al., Drugs Aging 2011). We previously used brain stimulation to modulate cognition in AD and FTD (Manenti et al., Brain Stimulation 2012; Behav Brain Res 2011; Cotelli et al., Eur J Neurol. 2012; Neurocase 2012), showing that the beneficial effects of brain stimulation in AD are still present 12 weeks after intervention (Cotelli et al., JNNP 2011). Previous experience also includes the combination of fMRI and brain stimulation techniques to modulate neural networks underlying memory (Manenti et al., Brain Topogr 2010).

e. Methodologies and Statistical Analyses

Clinical assessment

Severity of dementia will be assessed with the Clinical Dementia Rating (CDR) scale (Hughes et al., 1982), behavioural symptoms with the neuropsychiatric inventory (NPI) scale (Cummings et al., 1994). The neuropsychological assessment will include the MMSE and specific tests for each cognitive domain (Rey`s word list immediate and delayed recall, Rey figure delayed recall, logical memory test immediate and delayed recall, digit span test backward and forward for memory; Rey figure copy for visuospatial abilities, letter and category fluency for language, token test for comprehension, and Trail

Making Test for executive functions; Reading the mind in the Eyes Test, 60 Ekman faces Test for emotions recognition).

tDCS experiment
Patients will receive 25-minute treatment sessions five consecutive days a week for two weeks. tDCS will be delivered (anodal or cathodal at 2 mA) by a battery-driven stimulator through two pairs of saline-soaked sponge electrodes, placed over the left and right inferior parietal cortex (for the DMN), and the left and right prefrontal cortex (for SN). The reference electrodes will be fixed on the contralateral supraorbital area.

MRI sequences
- echo planar imaging (EPI) sequence for rs-fMRI analysis (TR/TE=2700/30ms, flip angle=85°, matrix size=64x64, FOV=192x192mm, 40 axial slices, slice thickness 3 mm, 200 volumes);
- single post-label delay (PLD) pseudo-continuous ASL (pCASL) for ASL analysis (128x128x25mm3; TR/TE=4000/16ms; 1650ms label duration; 1525ms PLD; 160 averages);
- spin echo echo-planar imaging (SE-EPI) for DTI analysis (TR/TE=9300/87ms, slice thickness 2 mm, no gap, matrix size=128x128, FOV=256x256mm), with 5 b0 images (b factor = 0 s/mm2), and 30 gradient directions (b=700 s/mm2);
- 3D MPRAGE for cortical thickness and hippocampal atrophy measurement (TR/TE/IT=2300/3/900ms, slice thickness 1 mm, matrix size=240x240, FOV=256x256mm).

MRI analyses
- Resting-state connectivity in the DMN and SN will be analyzed at T0, T1, and T2 with Independent Component Analysis (ICA), according to procedures previously described (Agosta et al., 2012).

- Brain perfusion will be quantified on pCASL through the application of a modified monocompartment model to obtain whole brain CBF maps (Buxton et al., 1998). Brain perfusion will be measured in the DMN and SN through a regions-of-interest analysis. In addition, a data driven ICA analysis will be applied for the automated extraction of these networks.

- White matter (WM) tracts connectivity will be assessed on DTI scans using previously described procedures (Pievani et al., 2010). Briefly, WM tracts connecting DMN and SN nodes (anterior and posterior cingulum, parahippocampal gryrs, SLF) will be reconstructed and biomarkers of fractional anisotropy, mean diffusivity, axial and radial diffusivity will be assessed.

- Cortical thickness will be measured on structural MRI with FreeSurfer (Fischl and Dale, 2000), hippocampal atrophy with manual tracing according to previously described procedures (Pievani et al., 2011b).

Biological samples (optional sub-study)
Levels of Abeta42, total tau, and phospho-tau proteins will be determined at baseline by commercially available enzyme linked immunosorbent assay (Innogenetics, Belgium). CSF levels will be defined as normal/abnormal according to the normative values of Sjogren and colleagues (Sjogren et al., Clin Chem 2001). DNA extraction and APOE genotyping will be carried out on blood samples according to procedures previously described (Pievani et al., 2011b). DNA extraction will be carried out on blood cells through miRNA Blood PaxGene kit to measure changes in peripheral inflammatory markers (a panel of pro-inflammatory (IL-6, IL-1beta, TNF-alpha) and anti-inflammatory (IL-10) cytokines) following intervention, using gene expression analysis in Real Time PCR.

Statistical analysis

Sample size for this pilot study (n=10 subjects for each experimental group) has been determined on the basis of previous tDCS (Cotelli et al., 2011) and rs-fMRI studies (Lorenzi et al., 2011; Pievani et al., Alzheimer's Dementia 2014) of the Consortium.

Paired t-tests will be used to compare pre-treatment (T0) *versus* post-treatment (T1, and T2) variables in each treatment group (AD and bvFTD).

Correlations between clinical/cognitive changes and imaging biomarkers will be assessed with Pearson`s correlation coefficient.

Two-sample t-tests will be used for cross-sectional comparisons of clinical/cognitive scores between paradigm 1 and 2 (i.e. network stimulation *versus* network inhibition) in the experimental groups and to evaluate differences in clinical, cognitive, and neuroimaging measures between the experimental groups and controls at baseline.

f. Expected outcomes, possible problems and solutions.
The main expected outcomes of the study will be:

- clinical improvement in AD and bvFTD patients following network recalibration
- identification of surrogate biomarkers of network recalibration

Possible problems:

- Few patients recruited: Low risk. The IRCCS-FBF is a leading centre in the study of AD and related disorders, with hundreds of outpatients seen every year.
- High drop out: Medium risk. Patients will undergo an intensive research program, which might lead to a high drop out rate. To overcome this problem, we will enlarge the patient recruitment pool to neighbouring clinical centers in Brescia and elsewhere.

C. Significance and innovation
This will be the first intervention targeting networks clearly linked to AD and bvFTD pathophysiology. The majority of stimulation studies for AD patients target task-specific networks, e.g. the dorsolateral prefrontal cortex (DLPFC) (NCT01746498 trial; Cotelli et al., 2011). Conversely, the present project will target disease-specific networks, and we expect that this approach will be more effective to modify the disease course.

Secondly, no previous study has attempted to recalibrate bvFTD networks. This study will be the first to a treatment option specific for these patients.

The main innovations of this pilot project will be:
- innovative disease-modifying treatments for AD and bvFTD patients (AIM1)
- potentially minimally invasive treatments (AIM2)
- better understanding of the neuronal mechanisms underlying brain damage and repair (AIM3)

D. Description of complementary and synergy research team
The project will bring together three research groups sharing a common and solid expertise in the study of brain connectivity in patients with dementia. In addition, these groups show complementary expertise in their own field: UO1 (Lab. of Epidemiology, Neuroimaging and Telemedicine, IRCCS Fatebenefratelli, Brescia) has a well documented expertise in the assessment of disease biomarkers with imaging techniques; UO2 (Neuropsychology Lab, IRCCS Fatebenefratelli, Brescia) has wide experience in the application of tDCS for the treatment of AD; and UO3 (Neuroradiology Unit, Azienda Ospedaliera Universitaria Integrata Verona) in the development of methods for the study of brain connectivity. The track record of collaboration between these research lab is well-documented (Frisoni

et al., 2007, 2008; Canu et al., 2011; Lorenzi et al., 2011) and will be further amplified through the integration of these levels of expertise.

E. Bibliography

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F. Timeline/Deliverables/Payable Milestones

Months 1-29: Implementation of the clinical study (baseline assessment, T0)
Months 1-29: tDCS sessions
Months 2-30: Implementation of the longitudinal study (T1)
Months 7-36: Implementation of the longitudinal study (T2)

G. Milestone Mo 18

M1: Successful implementation of the clinical study (First 2 patients completing T0 assessment; Month 1)
M2: Successful implementation of paradigm 1 (First patient completing tDCS sessions; Month 1)
M3: Successful implementation of paradigm 2 (First patient completing tDCS sessions; Month 1)
M4: Successful implementation of the longitudinal study (First patient completing T1 assessment; Month 2)
M5: Successful implementation of the longitudinal study (First patient completing T2 assessment; Month 8)

H. Milestone Mo 36

M1: Recruitment of targeted patient group size (Last patient completing T0 assessment; Month 29)
M2: Completion of the clinical study (Last patient completing T2 assessment; Month 36)
M3: Project validation (Report of statistical analysis; Month 36)