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Behavioural psychological symptoms of dementia and functional connectivity changes.

A network-based study

Laura Serra^a, Michela Bruschini^a, Carlotta Di Domenico^a, Matteo Mancini^b, Giulia Bechi Gabrielli^a,
Sabrina Bonarota^a, Carlo Caltagirone^c, Mara Cercignani^{a,b}, Camillo Marra^d, Marco Bozzali^{b,e}

^aNeuroimaging Laboratory, Fondazione Santa Lucia, IRCCS, Via Ardeatina, 306, 00179, Rome, Italy.

^bBrighton & Sussex Medical School, University of Sussex – Brighton

^cDepartment of Clinical and Behavioural Neurology, Fondazione Santa Lucia, IRCCS, Via Ardeatina, 306, 00179, Rome, Italy.

^dInstitute of Neurology, Catholic University, Largo Agostino Gemelli, 8, 00168, Rome, Italy.

^eDepartment of Neuroscience "Rita Levi Montalcini", University of Torino, Via Cherasco, 15, 10126, Turin, Italy.

Running title: BPSD and functional connectivity changes in Alzheimer's disease

***Correspondence to:**

Dr. Laura Serra, Via Ardeatina 306, 00179 Rome, Italy. Telephone number: #39-06-5150 1493;

Fax number: #39-06-5150 1213; Email address: l.serra@hsantalucia.it

Abstract

Behavioural and psychological symptoms of dementia (BPSD) are commonly observed since the early stage of Alzheimer's disease (AD) associated with structural brain changes. It is conceivable that they may relate also to functional brain changes. This resting-state functional MRI (RS-fMRI) study investigated the alterations within functional brain networks of a cohort of AD patients at different clinical stages who presented with BPSD. 101 AD patients and 56 patients with amnesic mild cognitive impairment underwent a neuropsychological evaluation including the Neuropsychiatry Inventory-12 (NPI-12). All patients and 35 healthy controls (HS) underwent 3T-MRI. Factor analysis was used to extract the principal factors from NPI-12, while RS-fMRI data were processed using graph theory to investigate functional connectivity. Five factors were extracted from NPI-12. 62% of patients showed BPSD and functional brain connectivity changes in various networks compared to those without BPSD and HS. These changes contributed to account for patients' BPSD. This work opens new perspectives in terms of non-pharmacological interventions that might be designed to modulate brain connectivity and improve patients' BPSD.

Keywords: BPSD, AD, a-MCI, brain functional connectivity, MRI

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder typically characterized by behavioural symptoms in addition to a progressive cognitive decline (Bessey and Walaszek, 2019; Chakraborty et al., 2019). Behavioral disorders and psychological symptoms (Behavioural and Psychological Symptoms in Dementia; BPSD) (Tascone and Bottino, 2013), which are particularly distressing for patients' family members, are frequently observed in AD as well as in patients with amnesic cognitive impairment (a-MCI) (Köhler et al., 2016). BPSD may strongly contribute to patients' disability, and typically result in an increased need of care-giving (Feast et al, 2016).

Observational studies report an estimated prevalence of BPSD in AD patients that ranges from 25% to 80% (Mega et al., 1996). The most frequently observed BPSD include agitation (Mega et al., 1996), apathy (Marin et al., 1993; Starkstein et al., 2006; Boccardi et al., 2017), depression (Burns et al., 1990a; Frisoni et al., 1999; Boccardi et al., 2017), anxiety (Boccardi et al., 2017) and delusions (Burns et al., 1990b; Boccardi et al., 2017), while disinhibition (Teri et al., 1992), hallucinations (Hirono et al., 1998; Burns et al., 1990b), aggression (Gilley et al., 1997), wandering and disturbances in eating behaviour (Burns et al., 1990c) are less frequent (Assal and Cummings, 2002; Ropacki and Jeste, 2005). When considering the clinical evolution of AD, depression, apathy and irritability are commonly observed since the early stages of the disease (Craig et al., 2005). Conversely, psychotic symptoms and wandering are more typical of patients at a more advanced AD stage (Piccininni et al., 2005).

Specific grey (GM) and white matter (WM) abnormalities have been shown to account for the presence and severity of BPSD in patients with AD (Makovac et al., 2016). Consistent with the progression of symptoms, regional GM atrophy also spreads when moving from early (i.e., mild cognitive impairment) to more advanced stages of AD. Microstructural damage to specific WM tracts has also been described in association with BPSD severity in AD patients, thus suggesting that disconnection is a relevant contributor to AD pathophysiology (Makovac et al., 2016).

An interesting perspective to better clarify the mechanisms underlying BPSD in AD is investigating changes of functional brain organization. Resting-state functional MRI (RS-fMRI) (van den Heuvel and Hulshoff Pol, 2010) is one of the most widely used methods to investigate brain connectivity in neurological and psychiatric disorders (Raichle and Snyder, 2007; Buckner et al., 2008), whose main advantage is the need for minimal participants' compliance. RS-fMRI data can be analysed using various methodological approaches. Previous studies based on "*a priori*" selection of brain networks have shown changes in functional brain connectivity in AD patients suffering from BPSD (Balthazar et al., 2014; Munro et al., 2015; Guo et al., 2018). Balthazar and Co-workers (2014) have identified associations between brain connectivity within the salience network and agitation, irritability, aberrant motor behaviour, euphoria and disinhibition. Munro et al., (2015) who focussed their analysis on four different networks (i.e., Default Mode Network, Fronto-Parietal Control Network, Dorsal and Ventral Attention Networks) reported an inverse association between affective symptoms and connectivity within the Fronto-Parietal Control Network in patients with a-MCI. More recently, Guo et al. (2018) has described patterns of both, increased and decreased connectivity between the amygdala and frontal regions of patients with AD and depression.

One promising method to assess brain connectivity in an unbiased fashion (i.e., without an *a priori* selection of brain areas or networks) is based on network modelling and graph theory (Rubinov and Sporns, 2010), in which the brain is modelled as a network of interconnected elements and mathematically represented using graphs (Bullmore and Sporns, 2009; Mancini and Cercignani, 2018). The nodes and the edges of the graph represent brain areas and their functional coupling respectively. Using specific graph measures it is possible to characterize functional specialization (i.e., segregation) and integration of the brain as a network. In this framework, the nodes that are most critical for information processing are defined as "hubs".

Abnormal connectivity between "hubs" is believed to cause more remarkable deficits than that occurring between peripheral nodes (Rubinov and Sporns, 2010; Bullmore and Sporns, 2009).

This method allows the investigation of connectivity within the whole brain, as well as at a local level by means of dedicated graph measures.

Aim of the present resting state-fMRI study was to investigate the possible alterations of functional brain connectivity in a large cohort of patients with AD at different clinical stages who presented with BPSD. We hypothesized that peculiar patterns of disconnection may account for specific BPSD, whose identification might be relevant for non-pharmacological interventions (Koch et al., 2018). Recently, Koch and co-authors (2018) showed that modulation of functional brain connectivity through non-invasive brain stimulation (i.e., transcranial magnetic stimulation (TMS)) is able to enhance memory functions in patients with AD. TMS was indeed used to produce an increase of neural activity in specific nodes of the so-called default mode network, which is known to be selectively targeted by AD pathology. TMS has also been approved for the treatment of depression in the United States of America (for a review, see Perera et al., 2016). Against this background, we believe that identifying brain networks, whose abnormalities are related to BPSD may help design strategies for non-pharmacological interventions in AD (Fox et al., 2014; Sale et al., 2015).

2. Materials and methods

2.1 Participants

A cohort of 192 participants, 101 with a diagnosis of probable AD, 56 with a diagnosis of a-MCI, and 35 healthy elderly subjects (HS) were enrolled in the study. The diagnosis of probable AD was defined according to the clinical criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 2011; American Psychiatric Association, 2013). The diagnosis of a-MCI was defined according to current criteria (Albert et al., 2011). MCI patients could be either single or multiple domain, and did not respond to the diagnostic criteria for major cognitive disorder (American Psychiatric Association, 2013), showing a CDR (Hughes et al., 1982)

score not exceeding 0.5. As detailed below, medial temporal lobe atrophy was assessed in all subjects, and cognitively normal subjects showing the presence of significant medial temporal lobe atrophy were excluded from the study. All recruited subjects with a Hachinski score (Hachinski et al., 1975) higher than 4 were also excluded. Major systemic, past history or concomitant diagnosis of psychiatric disorders (such as major depression, schizophrenia, bipolar disorder, post-traumatic stress disorders, panic disorder, etc.), and other neurological illnesses were also carefully investigated and excluded in all participants. In addition, a trained psychologist, on the basis of dedicated clinical interviews, has carefully screened all healthy controls to exclude any evidence of psychopathological symptoms.

All subjects had to be right-handed, as assessed by the Edinburgh Handedness Inventory (Büsch et al., 2010).

Eleven out of 101 (10.9%) AD patients and 6 out of 56 (10.7%) a-MCI patients were on treatment with benzodiazepines ($\chi^2=0.0$, d.f=1, $p=0.97$); 28 out of 101 (27.7%) AD patients and 13 out of 56 (23.2%) a-MCI patients were on antidepressants ($\chi^2=0.4$, d.f=1, $p=0.54$); 7 out of 101 (6.9%) AD patients were on neuroleptics, and 42 out of 101 (41.6%) AD patients were on anticholinesterase inhibitors (AChEIs).

The study was approved by the Ethical Committee of Santa Lucia Foundation and written informed consent was obtained from all participants before study initiation. All procedures performed in this study were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2 Neuropsychological assessment

All participants underwent an extensive neuropsychological battery covering all cognitive domains, which included: a) verbal episodic long-term memory: 15-Word List (Immediate, 15-min Delayed recall and recognition) (Carlesimo et al., 1996); Short Story Test (Immediate and 20-min Delayed

recall) (Carlesimo et al., 2002); b) visuo-spatial long-term memory: Complex Rey's Figure (Immediate and 20-min Delayed recall) (Carlesimo et al., 2002); c) short-term and working memory: Digit span (forward and backward) and the Corsi Block Tapping task (forward and backward) (Monaco et al., 2013); d) executive functions: Phonological Word Fluency (Carlesimo et al., 2002) and Modified Card Sorting Test (Nocentini et al., 2002); e) language: Naming objects subtest of the BADA ('Batteria per l'Analisi dei Deficit Afasici', Italian for 'Battery for the analysis of aphasic deficits') (Miceli et al., 1991); f) Reasoning: Raven's Coloured Progressive Matrices (Carlesimo et al., 1996); g) constructional praxis: copy of simple drawings with and without landmarks (Carlesimo et al., 1996) and copy of Complex Rey's Figure (Carlesimo et al., 2002); h) general cognitive efficiency: mini mental score examination (MMSE) (Folstein et al., 1975). For the purposes of the current study neuropsychological scores were adjusted for age and education, as reported in the corresponding references.

2.3 Neuropsychiatric assessment

In order to assess the presence/absence of BPSD, AD and a-MCI patients' caregivers were required to complete the Neuropsychiatric Inventory-12 (NPI-12) (Cummings, 1997). This scale assesses the presence and severity of delusions, hallucinations, agitation/aggression, dysphoria/depression, anxiety, euphoria/elation, apathy, disinhibition, irritability/lability, aberrant motor behaviour, sleep and eating disturbances. Each item's score ranges from 0 to 12, and reflects both, ratings of severity and frequency of each behavioural symptom, with 0 corresponding to the absence of behavioural symptom and 12 corresponding to its maximum frequency and severity.

AD and a-MCI patients were divided into those who suffered from BPSD and those who did not using the output of our factor analysis. Patients with positive factor scores for one or more factors were classified in the BPSD group, while those with negative scores for all factors were classified as not having BPSDs (NoBPSD group).

2.4 Statistical analyses

Statistical analyses were carried out in SPSS 21 (<http://www-01.ibm.com/software/it/analytics/spss/>).

Eighteen one-way ANOVAs were used to assess between group differences (BPSD, NoBPSD, HS) in neuropsychological performances. To avoid the type-I error Bonferroni's correction was applied (p value threshold $\alpha = 0.05/18 = 0.003$).

Preliminarily, twelve one-way ANOVAs were used to assess between group differences (AD, a-MCI) in NPI-12. To avoid the type-I error Bonferroni's correction was applied (p value threshold $\alpha = 0.05/12 = 0.004$). Moreover, Chi-square analyses were used to compare AD and a-MCI patients with respect to the number of BPSD shown.

An explanatory factor analysis was used to extract, in AD and in a-MCI patients, main factors from NPI-12. Factor analysis describes variability among observed correlated variables in terms of a potentially lower number of unobserved variables called factors. In the present study we hypothesized that the factors represented the common variance in the 12 subscales of NPI-12. Each NPI-12's score entered as variable of interest in factor analyses. Factor analyses were performed using the Maximum Likelihood estimation method (with eigenvalues >1 for the factors' extraction, and VARIMAX method for factors' rotation). Factors extracted from NPI-12 were then used in MRI data analyses.

2.5 MRI acquisition

All participants underwent MRI scanning at 3T. The acquisition protocol included a volumetric scan (3D Modified Driven Equilibrium Fourier Transform [MDEFT] [TR=1338 ms, TE=2.4 ms, Matrix=256x224, n. slices=176, thickness=1 mm]), and a RS-fMRI scan (T2* weighted echo planar imaging [EPI] sensitized to blood oxygenation level dependent (BOLD) contrast [TR=2080 ms, TE=30 ms, 32 axial slices parallel to AC-PC line, matrix=64x64, pixel size=3x3 mm², slice thickness=2.5 mm, flip angle:70°]). BOLD EPIs were collected during rest for 7 min and 20 s, resulting in a total of 220 volumes. During this acquisition, participants were instructed to keep their eyes closed, not to think of anything in particular, and not to fall asleep.

2.6 Medial temporal lobe atrophy

The Medial Temporal lobe Atrophy scale (MTA) (Scheltens et al., 1995) was used to assess, on volumetric images, the severity of atrophy in each subject. This scale provides a rating score from 0 to 4, with scores ≥ 1.5 (Scheltens et al., 1995) indicating significant atrophy. For each subject we averaged the scores obtained in the right and left hemispheres to obtain a single measure of medial-temporal lobe atrophy. One-way ANOVA was used to assess between-group differences.

2.7 Image Analysis for RS-fMRI

EPI images were pre-processed for resting-state fMRI using Statistical Parametric Mapping 8 (SPM8 <http://www.fil.ion.ucl.ac.uk/spm/>) and in-house Matlab scripts.

The first 4 volumes of each fMRI time series were discarded to allow for T1 equilibration effects; then, our pre-processing pipeline (previously published in Serra et al., 2018), includes head motion correction (using the standard SPM8 realignment algorithm), compensation for slice-dependent time shifts and co-registration to the corresponding MDEFT. Each MDEFT-volume was segmented using the standard SPM8 algorithm and the resulting grey matter images were used to compute each participant total grey matter volume. The white matter and CSF images were resliced in standard space and thresholded at 0.5. The mean fMRI signal was extracted for either tissue. Segmentation derived normalization parameters were used to warp the motion and slice-time corrected EPI images to Montreal Neurological Institute (MNI) coordinates. In house software was used to remove the global temporal drift using a 3rd order polynomial fit. For each dataset, motion correction was checked to ensure that the maximum absolute shift did not exceed 1.5 mm, and the maximum absolute rotation did not exceed 1°.

Finally, to minimise the risk that findings could have been affected by differing degrees of motion, we computed the global correlation (GCORR, Saad et al. 2013), the average mean displacement (root mean square or RMS of the 6 realignment parameters) and the average frame-wise displacement (FD, Power et al, 2012), and compared them between all groups using a one-way

ANOVA model. The signal in every voxel was regressed against the average white matter and CSF signals (extracted in native space, as described above), as well as against the 6 realignment parameters. EPI images were then filtered using a phase-insensitive band-pass filter (pass band 0.01–0.08 Hz) to reduce effects of low frequency drift and high frequency physiological noise. Finally, images were smoothed using a 8 mm³ FWHM 3D Gaussian Kernel.

2.8 Construction of connectivity matrices.

For each participant, the whole brain was parcellated into 116 regions of interests (ROIs) using the automated anatomical labelling (AAL) atlas. For each ROI, the related mean time course was computed averaging the fMRI time series across all the voxels within the region. The correlation values between all pairs of ROIs mean signals were then arranged in a matrix, in order to represent the functional connectivity by means of nodes (i.e. the ROIs) and edges (i.e. the correlations). The matrices were analysed using 2 complementary approaches: 1) graph measures (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010), which characterise the network describing its properties in terms of topological organization, including the brain efficiency; 2) Network-based statistics (NBS) (Zalesky et al., 2010), which identifies significantly different subnetworks in group comparisons. To test the bias potentially caused by movement we looked at the edge-wise correlation between functional connectivity and FD, and at the correlation between mean functional connectivity and Euclidean distance between nodes. These metrics were computed in Matlab.

2.9 Graph measures

We used Brain Connectivity Toolbox (Rubinov and Sporns, 2010) and Matlab custom scripts to explore global and local topological properties of each participant's brain. Correlation matrices were thresholded to obtain undirected binary connectivity matrices using fixed-density multi-threshold approach (van Wijk et al., 2010), thus obtaining several connectivity matrices for each subject in a specific density range, where the density is the ratio between the actual number of

connections and the maximum possible number of connections (van Wijk et al., 2010). This approach has the advantage of avoiding connection density bias (De Vico Fallani et al., 2014), but still requires the choice of the density range. As in previous studies (Bullmore and Bassett, 2011; Serra et al., 2017), the range was selected in order to (1) avoid disconnected nodes (i.e., brain regions functionally disconnected) and (2) preserve small-world organization. In this way, we were able to avoid the cases of threshold values being too high, potentially leading to fragmented networks and disconnected nodes, or too low, leading to densely connected networks with random topology (Bassett et al., 2008). Graph theory and its application to brain networks has already been widely described in the literature (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010; Mancini and Cercignani, 2018), together with a full description of all indices that can be derived from it. As global measures, *mean clustering coefficient* (a measure of segregation), *characteristic path length* (a measure of integration), *assortativity* (an index of how the high-degree nodes are linked together), *modularity* (measuring the tendency of the network to form distinct sub-networks) and *small-worldness* were calculated (Bullmore and Sporns, 2009). With respect to local measures, we considered *betweenness centrality* (fraction of all the shortest paths passing through a given node), *degree* (number of connections), and *nodal efficiency* (a measure of integration inversely related to the path length).

For each global and local measure, and at each density value we analysed differences between groups. Then, in order to obtain scalar values for each metric, the area under the curve (AUC) of density distribution was calculated.

Two-sample t-tests were performed in order to evaluate significant differences between-groups (BPSD group vs. HS group, NoBPSD group vs. HS group, and BPSD patients vs. NoBPSD patients). Considering that a proportion of AD and a-MCI patients were on psychoactive medication (i.e., benzodiazepines, antidepressants and neuroleptics) that might modulate brain connectivity and

potentially mask some BPSD related changes, we run additional analyses by individuals on medication.

2.10 Network-based statistics (NBS)

To detect between-group differences in functional subnetworks, a series of two-sample t-tests were performed in NBS (10000 permutation-tests and a p-value=0.005 were set). We compared the BPSD group vs. HS, the NoBPSD group vs. HS, and the BPSD group vs. the NoBPSD group. Finally, in order to investigate potential changes at a subnetwork-level associated with different behavioural symptoms, post-hoc analyses were performed by grouping patients according to the factor analysis's results. Specifically, t-tests were performed between patients grouped by factors extracted vs. NoBPSD patients. In all analyses, clinical status (AD or a-MCI), grey matter volumes, MMSE score, age, gender, and years of formal education were used as covariates of no interest. As for the graph theory measures, we run additional NBS analyses including only those patients who were on no psychoactive medication.

3. Results

3.1 Demographical and clinical characteristics

Ninety-eight patients (67 AD and 31 a-MCI) showed the presence of neuropsychiatric symptoms (BPSD group), while 59 patients (34 AD and 25 a-MCI) did not (NoBPSD group). There were no significant differences in the AD/a-MCI distribution between BPSD and NoBPSD groups ($\chi^2=1.8$, d.f.=1, $p=0.17$). There were no significant differences between groups (BPSD, NoBPSD, HS) in age ($F_{2,189}=2.6$, $p=0.07$). There were significant differences in the years of formal education between both group of patients (BPSD and NoBPSD) compared to HS ($F_{2,189}=7.0$, $p=0.001$), and in gender distribution between the BPSD group and HS ($\chi^2=3.9$, d.f.=1, $p=0.04$). As expected, general cognitive efficiency was significantly different across groups (BPSD, NoBPSD and HS) ($F_{2,189}=35.2$, $p<0.001$), with the BPSD group reporting worse MMSE scores than the NoBPSD

group and HS. Moreover, both patient groups showed a significantly higher MTA score than HS ($F_{2,189}= 71.8, p<0.001$). No significant difference of the age at onset was observed between BPSD and NoBPSD patients ($F_{1,155}= 3.3, p=0.131$). All these data are summarised in Table 1, panel A.

3.2 Neuropsychological assessment

As expected, all patients reported a significantly worse performance than HS in most administered tests. Conversely, there were no significant differences in cognitive functioning between BPSD and NoBPSD patients (See table 2).

3.3 Neuropsychiatric assessment

In the BPSD group, patients with AD and MCI did not differ for severity scores (measured as frequency by severity) on NPI-12 subscales, with the only exception of the Aberrant Motor Behaviour subscale, in which AD patients reported significantly higher scores (Figure 1).

3.3.1 Factor analysis

As reported in Table 3, from the initial 12 NPI subscales entered in the analysis five factors were extracted (55.18% of variance). The Communalities (Table 3) showed that the extracted factors explained almost the global variance of scores obtained on the Euphoria, Depression, Irritability, Eating disorders and Hallucinations subscales (correlation coefficients between NPI-12 subscales and extracted factors are summarized as Rotated Factor matrix in Table 3). The NPI-12 subscales with a saturation threshold ≥ 0.35 (Overall and Klett, 1972) were included in 5 factors as follows: Hallucination, Disinhibition and Delusions in Factor 1; Irritability and Agitation in Factor 2; Depression and Apathy in Factor 3; Euphoria and Aberrant Motor Behaviour in Factor 4; Eating and sleep disorders in Factor 5. Factor 1 included 31 patients, Factor 2 included 51 patients, Factor 3 included 57, Factor 4 included 16 patients, Factor 5 included 34 patients.

3.4 Functional connectivity results

Twenty-seven participants were excluded from the fMRI analyses as their motion parameters exceeded the thresholds defined in the methods, leaving a total of 169 participants included in MRI analyses (please see Table 1, panel B for demographical and clinical characteristics). The GCORR, mean RMS and mean FD of the remaining participants did not differ between groups (GCORR, p -value=0.618; FD, p -value=0.171; mean displacement, p -value=0.121). The results of the analysis of the connectivity matrices sensitivity to motion and node distance are shown in the supplementary Material. Figure S1 panel A shows the correlation between connectivity matrices and FD. Visual inspection suggests that more connections were correlated with FD for the AD and MCI groups than for healthy controls, as expected. When we compared BPSD and No BPSD, however, the results were very similar. Figure S1 panel B and S2 show the average functional connectivity as a function of the Euclidean distance between nodes – confirming the trend was similar for all groups.

3.4.1 Graph measures

Both BPSD and NoBPSD patients compared to HS showed significant reductions in global topological measures, such as normalised clustering coefficient and smallworldness. There were no differences between BPSD and NoBPSD patients. Significant between-group changes were also observed in local topological measures (Figure 2). Patients with BPSD compared to HS showed reduced betweenness centrality, nodal degree and nodal efficiency in the left inferior/superior frontal gyrus, in the posterior cingulate cortex bilaterally, in the right anterior cingulate gyrus, and in the right supramarginal gyrus. In contrast, BPSD patients showed an increased nodal degree and nodal efficiency in the cerebellum bilaterally, in the left posterior cingulate, in the inferior occipital gyrus bilaterally, in the right inferior frontal gyrus, and in the superior frontal gyrus bilaterally. NoBPSD patients compared to HS showed decreased local measures of connectivity in the left inferior/superior frontal gyri, in the lingual gyrus, in the right anterior cingulate, in the right

supramarginal gyrus, in the left putamen and in the pallidum bilaterally. Conversely, they showed an increased nodal degree in the left middle temporal gyrus, in the right calcarine cortex, and in the occipital gyrus bilaterally.

When BPSD patients were directly compared with those with NoBPSD, the former group showed reduced betweenness centrality and nodal degree in the left posterior cingulate cortex, in the precuneus, in the cuneus bilaterally, in the right angular gyrus, in the right occipital gyrus, and in the right calcarine cortex. BPSD compared to NoBPSD patients showed also some areas of increased connectivity, which included the right cerebellum, the right putamen, the bilateral pallidum, the left amygdala and the bilateral superior frontal gyrus.

When removing from the analysis all patients on psychoactive medication (46 from the BPSD group and 8 from the NoBPSD group) (see Table 1) all results remained substantially unchanged (Figure 4).

3.5 Network-based statistics

NBS analyses revealed widespread disconnected subnetworks when comparing patients and controls (See Figure 3, panel A). Briefly, the NoBPSD group compared to HS showed reduced connectivity in a subnetwork involving the frontal, occipital and cerebellar nodes; patients with BPSD compared to HS showed a larger disrupted subnetwork involving fronto-striatal-temporo-parietal nodes. Patients with BPSD compared to those with NoBPSD showed disconnection in a subnetwork involving mainly temporal and cerebellar nodes.

More interestingly, post-hoc NBS analyses (Figure 3, panel B and Table 4) revealed reduced connectivity in various brain subnetworks of BPSD patients as grouped by extracted factors compared to NoBPSD patients. BPSD patients with large factor 1 scores (i.e., Delusions, Hallucinations and Disinhibition) showed reduced connectivity in the right cingulum, hippocampus and insula, and in the bilateral supramarginal gyrus. Patients with large factor 5 scores (i.e., Sleep and Eating disorders) showed reduced connectivity in the right middle and superior frontal gyrus

and in the cerebellum (vermis and lobule 3). Patients with large factor 2 scores (Agitation and Irritability) revealed reduced connectivity in the posterior cingulate cortex and olfactory cortex. Patients with large factor 4 scores (Euphoria and Aberrant motor behaviour) revealed reduced connectivity in the medial orbito-frontal cortex and fusiform gyrus.

When removing from the NBS analyses all patients on psychoactive medication, the results remained substantially unchanged (Figure 4). Indeed, more widespread changes (i.e., involving larger portions of network) were observed, compared to the analysis including all participants. **In order to establish whether these results were biased by individual motion, we repeated all these analyses including FD as a covariate. Most results remained substantially unchanged (please see Supplementary Figure S3).**

4. Discussion

In this study, we used a network-based approach to investigate functional connectivity changes in a large cohort of patients with AD and a-MCI. They were classified according to the presence and severity of BPSD, which resulted being independent from their level of cognitive decline. On cognitive testing, BPSD and NoBPSD patients reported a similar performance. Additionally, the level of BPSD severity was independent from patients' diagnosis of AD and MCI, thus further suggesting that the level of cognitive decline is not strictly associated to patients' behavioural disorders.

When considering the factor analysis, five main factors were extracted that explained the majority of variance of NPI-12 symptoms. NPI-12 symptoms are known to be extremely heterogeneous across studies due to an intrinsic limitation of this assessment. Reassuringly, when grouping the symptoms in factors, data tend to be more homogeneous as demonstrated by our current results that are highly consistent with those reported in previous independent studies (Hollingworth et al., 2006; Garre-Olmo et al, 2010).

A previous study investigating a large cohort of AD patients (Hollingworth et al., 2006) found that NPI-12 symptoms are grouped in four principal components: behavioural dyscontrol (including Euphoria, Disinhibition, Aberrant Motor Behaviour, Sleep and Eating disorders), psychosis (including Delusions and Hallucinations), mood (including Depression, Anxiety and Apathy), and agitation (including Aggression and Irritability). Consistently, in a longitudinal study Garre-Olmo et al. (2010) found that some NPI symptoms are more stable than others. In particular, over a 24 months observation period, Delusions and Hallucinations factored in a psychotic dimension; Depression, Apathy, Anxiety, Agitation factored in an emotional dimension; Euphoria, Irritability, Disinhibition and Aberrant Motor Behaviours, factored in a behaviour dimension. This study used NPI-10 and did not consider Sleep and Eating disorders (Garre-Olmo et al., 2010). Garre-Olmo and co-workers (Garre-Olmo et al., 2010) hypothesised the existence of “more stable” and “more variable” symptoms in AD, with the former characterizing the *nuclear syndrome* (including psychotic and emotional dimensions) and the latter characterizing a *satellite syndrome* (including the behaviour dimensions).

Against this background, our factor analysis appears consistent with previous studies and possibly provides a reliable picture of the behavioural disorders occurring to AD patients.

The connectivity analysis suggests that some topological alterations are specifically associated with the presence of BPSDs, while other ones are related with cognitive decline. Specifically, reduced connectivity in the inferior and superior frontal gyrus, in the anterior and the posterior cingulate cortex, was detected in both patient groups (BPSD and NoBPSD) compared to HS. This suggests that this pattern of disconnection may be related to the level of patient cognitive decline more than to the presence and severity of BPSD. Conversely, an increase of functional connectivity in the cerebellum and, at a lesser extent, in the superior frontal gyrus seems to be more strictly associated to the presence and severity of BPSD. This same finding was indeed obtained when comparing BPSD patients with either HS or patients with NoBPSD, and when looking at both topological organization changes and subnetwork-level alterations. An explanation for this finding lies in the

role that the cerebellum is known to play in emotion processing as previously described in various neurological and psychiatric disorders (Phillips et al., 2015). We have recently reported (Toniolo et al., 2018) that grey matter atrophy is remarkably present in the cerebellum of AD patients and correlates with their cognitive deficits (Toniolo et al., 2018). The cortico-cerebellar projections, involving mainly the frontal regions, are well known (Bernard et al., 2013) to sub-serve both cognitive and affective functions.

In terms of types of BPSD, the involvement of the fronto-cerebellar subnetwork was mainly associated with patients' sleep and eating disorders. We speculate that the neurobiological substrate of BPSD involves large subnetworks with the engagement of direct and indirect cortico-subcortical connections. In this view, sleeping and eating disorders might be at least partially caused by an abnormal circuiting between the cerebellum, frontal areas and their projections to the basal ganglia (Percheron et al., 1996; Sakai et al., 1996).

In the current study we also identified abnormal connectivity within a large subnetwork comprising the hippocampus, frontal areas, cingulum, insula and supramarginal gyrus, which was associated with the presence and severity of delusions, hallucinations and disinhibition. We previously reported a relationship between hippocampal atrophy and delusions in AD (Serra et al., 2010). We hypothesised a common pathological substrate between delusions (in terms of misidentification of person, places, objects, in association with delusional memories and content-related confabulations as assessed by NPI-12) and episodic memory dysfunctions (Serra et al., 2010). Moreover, in the same study, we reported an inverse association between grey matter volumes in the cingulate gyrus and patients' disinhibition (Serra et al., 2010). The cingulate cortex is a key structure in a network that connects the orbito-frontal to temporal areas (Rolls, 2019). This subnetwork is involved in higher-order behaviours such as control of initiative, spontaneity, impulsiveness, social and sexual behaviours. Disinhibition is due to the loss of these control mechanisms, and functional disconnection between nodes of this subnetwork contributes to understand the pathophysiology of these symptoms.

Remarkably all results have remained substantially unchanged when removing from both analyses (graph theory measures and NBS) patients on medication with psychoactive treatments (benzodiazepines, antidepressants and neuroleptics). Additionally, in some cases, between-group changes in functional connectivity increased after their removal. We argue that psychoactive treatments tend to normalise functional brain connectivity thus masking the neurobiological correlates of BPSD. These pharmacological effects on fMRI signal have been reported in previous literature (Dandash et al., 2018; Gudayol-Ferré et al., 2015).

One important limitation of the present study is the lack of evidence of underlying AD-related pathology in our patients, as required by the most recent research criteria (Jack et al., 2018).

Although all our patients showed clinical/cognitive and hippocampal features suggestive for underlying neurodegenerative pathology, none of them were tested for amyloid/tau biomarkers.

This is particularly important for the participants with MCI, as it is known that this population is heterogeneous, usually including individuals who will remain stable, or convert to other forms of dementia. Nevertheless, we can confirm that 22 out of 56 participants have already been followed up for 2 years or more. Of these 22, 9 (40%) have already converted to probable AD, while the remaining 13 have remained stable. None converted to other forms of cognitive decline. This proportion of AD conversions, which is larger than in other observational studies (Gillis et al., 2019), confirms that the recruitment criteria used for this study increase the probability of including patients with AD-type dementia. A typical limitation of this type of study is that functional connectivity is affected by motion, and patients with AD are more likely to move during their scan than healthy controls. Although we compared indices of motion between groups, showing no significant differences, when analyzing the residual correlation between connectivity matrices and motion, this more commonly observed in patients with AD and MCI than in healthy controls. Nevertheless, no differences were observed between the BPSD and NoBPSD groups, which were used for most of the results presented in this work, suggesting that our results are not the mere consequence of motion.

In conclusion, this study confirms that BPSD are clinical features of AD that occur also in patients with a-MCI. We highlighted here that changes of functional connectivity in different brain subnetworks, together with brain atrophy, account for the pathophysiological mechanisms underlying the psychological and behavioural symptoms observed in AD patients. This observation might be valuable when designing non-pharmacological interventions to modulate brain connectivity (e.g. using TMS, TDCS, etc.) to possibly improve specific BPSD. Indeed, TMS efficacy has been recently proven modulate the cognitive symptoms of AD (Koch et al., 2018). Moreover, TMS has been approved in the United States of America for the treatment of depression (Perera et al., 2016). Identifying critical nodes of the AD brains associated with specific BPSD might represent the first step for designing protocols of network modulation to improve patients' symptoms (Fox et al., 2014; Sale et al., 2015).

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Conflict of interests

None of the Authors has any conflict of interest to disclose

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Figure legends

Figure 1. Behavioural and Psychological Symptoms in Dementia observed in patients with a-MCI and AD

The figure shows the Frequency x Severity in a-MCI (in red) and AD (in blue) patients, as assessed by NPI-12.

* AD vs a-MCI $p \leq 0.004$ Bonferroni corrected;

Abbreviations: a-MCI= amnesic Mild Cognitive Impairment; Ab. Motor Behv= Aberrant Motor Behaviour AD= Alzheimer's Disease; NPI-12= Neuropsychiatric Inventory-12;

See text for further details

Figure 2. Abnormal topological properties obtained by the graph analysis in healthy subject, patients with BPSD and NoBPSD.

Patients with BPSD and with NoBPSD compared each other and with healthy subjects, showed both decreased and increased of functional connectivity in centrality measures (Betweenness centrality, nodal degree and nodal efficiency). The brain network was visualized using the BrainNet Viewer (<http://www.nitric.org/projects/bnv/>) (Xia et al., 2013). See text for further details. Abbreviations: BPSD=Behavioural and Psychological Symptoms in Dementia; HS= Healthy Subjects; L=Left; NoBPSD= No Behavioural and Psychological Symptoms in Dementia.

Figure 3. Reduced functional connectivity between patients and controls obtained by network-based analysis.

Network based analyses revealed widespread disconnected brain networks between patients and controls (Panel A). In particular, NoBPSD group compared to HS showed reduced connectivity in a frontal, occipital and cerebellar nodes; patients with BPSD compared to HS showed a larger disrupted network involving fronto-striatal-temporo-parietal nodes. Finally, patients with BPSD

compared to those NoBPSD showed disconnection in a network involving mainly temporal and cerebellar nodes. Panel B shows post-hoc NBS analyses between BPSD compared to NoBPSD patients grouped by factors extracted. Patients with symptoms grouped in F1 (delusions, hallucinations and disinhibition) showed reduced FC in the right cingulum, the right hippocampus the insula and bilateral supramarginal gyrus; patients with symptoms grouped in F5 (sleep and eating disorders) showed reduced FC in the right middle frontal, the right superior frontal gyrus and cerebellum (vermis and lobule 3). The brain network was visualized using the BrainNet Viewer (<http://www.nitric.org/projects/bnv/>) (Xia et al., 2013). See text for further details.

Abbreviations: BPSD=Behavioural and Psychological Symptoms in Dementia; HS= Healthy Subjects; F1= Factor 1; F5= Factor 5; NoBPSD= No Behavioural and Psychological Symptoms in Dementia.

Figure 4. Reduced functional connectivity between patients on no psychoactive medication and controls obtained by graph and network-based analyses.

When considering patients on no psychoactive medication compared to healthy controls and among them, both graph analyses (in the left box) and the network-based statistics analyses (in the right box) substantially remained unchanged. Additionally, an enhancement of changes in the network-based analyses was observed between groups. The brain network was visualized using the BrainNet Viewer (<http://www.nitric.org/projects/bnv/>) (Xia et al., 2013). See text for further details.

Abbreviations: BPSD=Behavioural and Psychological Symptoms in Dementia; HS= Healthy Subjects; F1= Factor 1; F5= Factor 5; NoBPSD= No Behavioural and Psychological Symptoms in Dementia.

Table 1. Principal demographic and clinical characteristics of the participants.

A) Whole sample	BPSD	NoBPSD	HS
N=192	N=98	N=59	N=35
Mean (SD) Age [years]	72.7 (6.6)	74.3 (7.6)	71.1 (5.1)
Mean (SD) Education [years]	9.4 (4.4)*	10.1 (4.4)*	12.6 (3.6)
Gender (M/F)	37/61*	30/29	20/15
Mean (SD) MMSE score	21.8 (4.6)*	23.4 (3.5)*	28.3 (1.9)
Mean (SD) MTA score	2.7 (0.9)*	2.5 (0.9)*	0.7 (0.5)
AD/a-MCI	67/31	34/25	-
Age at onset	72.4 (7.6)	70.6 (7.1)	-
Patients on Psychoactive medication ^o	46 (46.7%)	8 (13.5%)	-
B) Participants with fMRI data N=169	BPSD	NoBPSD	HS
N=81	N=53	N=35	
Mean (SD) Age [years]	72.6 (6.6)	74.0 (7.7)	71.1 (5.1)
Mean (SD) Education [years]	9.7 (4.3)*	9.4 (4.3)*	12.6 (3.6)
Gender (M/F)	33/48	24/29	20/15
Mean (SD) MMSE score	21.6 (4.6)*	23.2 (3.7*)	28.3 (1.9)
Mean (SD) MTA score	2.6 (0.8)*	2.5 (0.8)*	0.7 (0.5)
AD/a-MCI	57/24	33/20	-
Age at onset	71.9 (7.8)	70.4 (7.2)	-
Patients on Psychoactive medication ^o	38 (46.9%)	6 (11.3%)	-

Tukey's post-hoc comparisons: * Patients (BPSD or NoBPSD) vs. HS p-value<0.05. °

Benzodiazepines/Antidepressant/Neuroleptics

Abbreviations: AD= AD=Alzheimer's Disease; a-MCI=amnesic Mild Cognitive Impairment;

BPSD= Behavioral and Psychological Signs in Dementia; NoBPSD=No Behavioral and

Psychological Signs in Dementia; HS= Healthy Subjects; MMSE= Mini Mental State Examination;

MTA= Medial Temporal lobe Atrophy scale.

See text for further details.

Table 2. Performance of BPSD, NoBPSD and HS groups on neuropsychological tests.

Domain	Test	BPSD	NoBPSD	HS
Verbal episodic long-term memory				
	15-Rey's words List:			
	Immediate recall (cut-off ≥ 28.5)	26.7 (8.3)*	27.7 (8.8)	44.7 (8.6)
	Delayed recall (cut-off ≥ 4.6)	3.1 (2.7)*	3.7 (2.8)	9.5 (2.3)
	Recognition: Hit rates	9.7 (3.9)*	8.4 (3.7)*	13.4 (1.6)
	Short Story:			
	Immediate recall (cut-off ≥ 3.3)	3.1 (2.2)*	3.2 (2.3)*	5.6 (1.6)
	Delayed recall (cut-off ≥ 2.8)	1.7 (2.3)*	2.6 (2.7)*	5.5 (1.5)
Visuo-spatial episodic long-term memory				
	Rey's Complex Figure			
	Immediate recall (cut-off $\geq$)	7.5 (5.6)*	8.5 (6.4)*	16.2 (6.7)
	Delayed recall (cut-off $\geq$)	6.3 (6.0)*	7.9 (6.5)*	16.4 (5.7)
Verbal short-term and working memory				
	Digit span forward (cut-off ≥ 3.7)	5.0 (1.0)	4.8 (1.0)*	5.6 (1.1)
	Digit span backward	3.0 (1.5)*	3.1 (1.7)*	4.2 (0.7)
Visuo-spatial short-term and working memory				
	Corsi span forward (cut-off ≥ 3.5)	4.1 (1.0)*	3.9 (1.6)*	5.1 (0.8)
	Corsi span backward	3.2 (1.6)	3.2 (1.8)	4.6 (0.9)
Executive functions				
	Phonological Word Fluency (cut-off ≥ 17.3)	25.1 (9.6)*	24.5 (10.0)*	36.4 (10.4)

Modified Card Sorting test				
	Criteria	2.8 (2.1)*	2.9 (1.8)*	5.7 (0.6)
Language				
	Naming of objects (cut-off ≥ 22)	25.3 (7.5)	26.7 (3.5)	28.5 (2.1)
Reasoning				
	Raven's Coloured Progressive	23.6 (7.7)*	25.1 (6.8)*	31.6 (3.5)
	Matrices (cut-off ≥ 18.9)			
Constructional praxis				
	Copy of drawings (cut-off ≥ 7.1)	8.4 (3.1)*	7.8 (3.4)*	10.7 (1.1)
	Copy of drawings with landmarks	59.8 (15.8)	58.2 (20.0)	69.4 (0.6)
	(cut-off ≥ 61.8)			
	Rey's Complex Figure			
	Copy (cut-off ≥ 23.7)	24.4 (11.0)	25.5 (11.1)	32.3 (3.6)

Tukey's post-hoc comparisons: * Patients (BPSD or NoBPSD) vs HS $p < 0.003$ Bonferroni corrected.

Abbreviations: BPSD= Behavioral and Psychological Signs in Dementia; NoBPSD=No Behavioral and Psychological Signs in Dementia; HS= Healthy Subjects.

Table 3. Results of factor analysis in BPSD group.

Total variance explained									
Factor	Initial Eigenvalues			Extraction Sum of Squares			Rotate Extraction Sum of Squares Loadings*		
	Loadings#			Loadings#			Loadings#		
	Total	% of	% Cumulative	Total	% of	%	Total	% of	% Cumulative
	Variance			Variance		Cumulative	Variance		
							e		
1	3.33	27.80	27.80	1.29	10.76	10.76	1.55	12.98	12.98
2	1.59	13.30	41.10	2.07	17.28	28.05	1.54	12.88	25.86
3	1.21	10.10	51.20	1.70	14.22	42.27	1.27	10.64	36.51
4	1.16	9.66	60.90	0.91	7.58	49.85	1.18	9.85	46.37
5	1.10	9.00	69.90	0.63	5.32	55.18	1.05	8.81	55.18
6	0.87	7.25	77.10						
7	0.66	5.51	82.60						
8	0.58	4.83	87.40						
9	0.48	4.04	91.50						
10	0.41	3.44	94.90						

11	0.37	3.07	98.00
12	0.25	2.00	100

Communalities and rotated factor matrix

Communalities#			Rotated Factor Matrix*				
	Initial	Extraction	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
		n					
Delusions	0.28	0.30	0.48	0.16	0.20	0.01	0.03
Hallucinatio	0.47	0.72	0.82	-0.07	0.07	0.17	0.12
on							
Agitation	0.41	0.43	0.11	0.63	0.15	-0.03	0.06
Depression	0.33	0.93	0.12	0.10	0.95	0.06	-0.07
Anxiety	0.22	0.15	0.03	0.08	0.32	-0.08	0.17
Euphoria	0.24	0.99	0.08	-0.02	-0.05	0.99	0.09
Apathy	0.33	0.33	0.12	0.32	0.42	0.09	0.17
Disinhibiti	0.53	0.62	0.63	0.39	-0.05	0.05	0.26
on							
Irritability	0.54	0.85	0.11	0.89	0.14	0.07	0.14

Ab. Mot	0.30	0.27	0.32	0.09	0.07	0.36	0.15
Beh.							
Sleep	0.16	0.19	0.03	0.03	0.08	0.11	0.40
Eating dis.	0.41	0.84	0.30	0.23	0.06	0.03	0.83

Extraction method: Maximum Likelihood; *Rotation method: Varimax with Kaiser Normalization; in bold the variables included in each factor according the saturation threshold $\geq$ 0.35 (Overall & Klett, 1972).

Table 4. Between groups difference of functional connectivity into pairwise brain regions

Groups	Factor	Pairwise brain regions	t-values*
BPSD<NoBPSD*	F1	R Insula ↔ R Cingulum (middle part)	3.64
		L Precentral gyrus ↔ R Hippocampus	3.58
		L Supramarginal gyrus ↔ R Supramarginal gyrus	3.81
BPSD<NoBPSD*	F2	R Cingulum posterior ↔ Olfactory cortex	3.83
BPSD<NoBPSD*	F4	L Orbito-frontal cortex (medial part) ↔ R Fusiform gyrus	3.44
BPSD<NoBPSD*	F5	R Middle Frontal gyrus ↔ Cerebellum (lobule 3)	3.69
		R Superior Frontal gyrus ↔ Cerebellum (Vermis 3)	3.99
		R Superior Frontal gyrus ↔ Cerebellum (Vermis 4-5)	4.00

*Two-sample t test

Figure 1.

Figure 2

Figure 3

Figure 4