EFFECT OF MEMANTINE ON RESTING STATE DEFAULT MODE NETWORK ACTIVITY IN ALZHEIMER'S DISEASE

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Abstract

Background. Memantine is an approved symptomatic treatment for moderate to severe Alzheimer's Disease(AD) active on the excitotoxic effects of hyperactive glutamatergic transmission. The mechanism of the effect of memantine in AD patients is poorly known. The default mode network (DMN) is hypoactive in AD and is under glutamatergic control.

Objective. To assess the effect of memantine on the activity of the DMN in moderate to severe AD.

Methods. fMRI data of 15 moderate to severe AD patients, 7 (age 77±7, MMSE 16±4) treated with memantine and 8 with placebo (age 75±6, MMSE 13±4), were acquired at baseline (T0) and after 6 months of treatment (T6). Resting state components were extracted after spatial normalization on individual patients with independent component analysis. The consistency of the components was assessed using ICASSO and the DMN was recognized through spatial correlation with a pre-defined template. Voxel-based statistical analyses were performed to study the change of DMN activity from T0 to T6 in the two groups.

Results. At T0, the two groups showed similar DMN activity except in the precuneus, where the treated showed slightly greater activity ($p<0.05$ corrected for family wise error). The prospective comparison between T0 and T6 in the treated showed increased DMN activation mapping to the precuneus ($p<0.05$ corrected), while the prospective comparison in the untreated did not show significant changes. The treatment x time interaction term was significant at $p<0.05$ corrected.

Conclusions. The results suggest a positive effect of Memantine treatment in moderate to severe AD patients resulting in an increased resting activity in the precuneus region over 6 months. Future confirmatory analysis with adequately powered studies will be required to support the present findings.

Disclosure/Conflict of Interest

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List of abbreviations used:

fMRI: functional Magnetic Resonance Imaging

BOLD: blood oxygenation level-dependent *fMRI* signal

CONSORT: Consolidated Standards Of Reporting Trials

DMN: Default Mode Network

FWE: Family Wise Error correction for multiple comparisons

GIFT: Group ICA for fMRI Toolbox

ICA: Independent Component Analysis

ICASSO: software for investigating the reliability of ICA estimates by clustering and visualization

MMSE: Mini Mental State Examination

NMDA: *N*-methyl *D*-aspartate

SPM5: Statistical Parametric Mapping software (version 5)

TIA: Transient Ischemic Attack

Introduction

Clinical studies have shown that memantine, a non-competitive NMDA receptor antagonist, improves cognitive function in Alzheimer's Disease (AD) [1] and, if taken in conjunction with other treatments, can produce at least temporary slowing of the progression of moderate to severe Alzheimer's patients .[2] Measurable benefits were observed in several clinical trials on cognitive performance, behaviour, and daily function.[3-5]

The effect of memantine in the brain has been investigated recently in a multimodal study [6] where glucose metabolism was studied with ^{18}F–FDG positron emission tomography (PET), and total brain and hippocampal volumes were assessed with high resolution magnetic resonance (MR) imaging. After 52 weeks follow-up, the patients on memantine showed a lower rate of hippocampal atrophy (-41%) compared to placebo, together with lower rates of metabolic deterioration.

It is generally believed that normal glutamate receptor activity has a crucial role in the function of the nervous system and is the major mediator of physiological excitatory synaptic transmission in the brain. In particular, in some areas of the brain, normal NMDA receptor activity is important for learning, memory as well as for wakefulness and attention. Therefore, if NMDA receptor activity is impaired, inattention, drowsiness and even coma can result.

The mechanism of action of memantine involves the blockade of the toxic effect of excess glutamate on NMDA receptors, by restoring the physiological balance (homeostasis) of the glutamatergic system. The result is an enhancement of the physiological excitatory synaptic signal over a pathological background "noise". Treatment with memantine has been found to normalize neuronal plasticity and improve performance on behavioural tests, memory and learning in animal and human studies ([7] for a detailed review).

Recently, alternative accounts for the therapeutic effect of memantine have been proposed, according to which dopamine receptors [8] and neurons [9] might be involved. The dopamine system plays a key role in attentive circuits, it is implicated in attention-deficit/hyperactivity disorder (ADHD), [10,11] and might be involved in the development of adverse effects such as hallucinations during treatment with memantine in AD patients.[12]

In healthy persons, attention is closely related to the activity of the so called default mode resting state network (DMN), whose presence and function has been elucidated thanks to functional MRI at rest.[13] The DMN is the most stable among the networks of brain regions active when the brain is

not engaged in specific tasks, i.e. in resting wakeful state, and it is characterized by coherent low frequency oscillations of the BOLD signal at 0.01-0.1 Hz mapping to the posterior cingulate, precuneus, parietal, medial prefrontal cortex, and hippocampal formation.

The DMN is thought to serve as a binding mechanism between internal and external attention [14] and represents the self-referential and introspective mental activity.[15] The DMN also plays a fundamental role in the transition from rest to task, acting in interplay with task-specific temporally anti-correlated networks, and underlies the ability to achieve and maintain the attentional focus.[16]

Attention has been given to the studies on DMN in the progression of Alzheimer's pathology: several studies have shown alterations in posterior cingulate and medial temporal lobe [17] and, more generally, a peculiar functional disruption believed to reflect the underlying neuropathological changes.[18,19]

Recently, the hypothesis of the involvement of the DMN in AD has been reversed. A causal role of the DMN in the pathophysiology of AD has been hypothesized, based on the observations that in AD the structural and metabolic damage largely maps to the DMN region. In an interesting work [20] it was introduced the cascade hypothesis: the functional resting activity changes and the associated levels of metabolism could antedate and cause the amyloid deposition, to finally lead to structural and functional damage.

The above evidence leads to hypothesize that the beneficial effect of memantine on the cognitive performance of AD patients might be mediated by an effect on the activity of the DMN. Aim of this study is to investigate the effect of memantine on DMN activity in AD patients by means of novel imaging tools, independent component analysis and voxel brain morphometry.

Materials and Methods

Subjects. The study population consisted of 15 patients with moderate to severe probable AD, seen at the IRCCS Centro S. Giovanni di Dio Fatebenefratelli, in Brescia, Italy. The trial (N. 2005-005859-18, protcol-code: SC05-03) has been designed following the set of the Consolidated Standards of Reporting Trials Statement (CONSORTs) recommendations, by investigating whether the treatment with memantine offers benefits compared to non-treatment in moderate-to-severe AD. [22,23] After baseline MR scan and clinical assessment, eight patients were randomized to treatment, and 7 to placebo. Treated patients received as inclusion criteria Donepezil at a dose of 5 mg once a day and then raised by 5 mg per day up to 20 mg for six months, to reach a final dose of 20mg per day. Other inclusion criteria included: diagnosis of probable AD according to NINCDS-ADRDA criteria,[24] clinical dementia rating of 2 or greater,[25] and treatment with acetylcholinesterase inhibitors (AcheI) for at least 6 months. Finally, the only drugs permitted at stable doses for at least 2 weeks before the recruitment were antidepressive, anti-inflammatory, antiipertensive, vitamine E (1200 mg/day), anticoagulants, diuretics, ipnotics. Exclusion criteria included: history of transient ischemic attack (TIA) or stroke, head trauma, alcohol or substance abuse, corticosteroid therapy, recent weight loss, or a modified Hachinski ischemic scale score greater than 4.[26]

Standardized history taking, behavioural and functional assessment, physical and neurological examination were carried out for all participants. The original case report form of the clinical assessment may be accessed at http://www.centroalzheimer.it/Public/ProtocolloMEM_T0.doc (in Italian). Moreover, a comprehensive neuropsychological battery adequate to patients' cognitive impairment severity was administered at T0 and T6 and cognitive tests for: non verbal reasoning (Raven Colored Progressive Matrices) , language comprehension (Token Test), verbal fluency (Phonemic and Semantic fluency), short and long term memory (Digit and Spatial span; Story Recall; Rey-Osterrieth complex figure recall), constructional abilities Rey-Osterrieth complex figure copy), attention and executive functions (Trail Making Test).[27] Global cognitive function was assessed with the Mini Mental State Examination (MMSE).[28] The results of cognitive assessment are reported in Table 1.

The participant or his/her primary caregiver provided written informed consent, after discussion of the participation risks and benefits. No compensation was provided. The study was approved by the local ethics committee.

fMRI scan simulation. Before the randomization procedure took place, during the screening visit at the IRCCS Centro S. Giovanni di Dio Fatebenefratelli, all the patients underwent an fMRI scan simulation. This was necessary to test the patient abilities to rest, without moving, in an "unusual" environment for the entire scan acquisition duration time (8'47"), therefore ensuring subject comfort and data quality. In fact, although the Neuropsychiatric Inventory [29] was administered to all caregivers, the simulation was useful to evidence behavioural complaints, such as agitation and anxiety, not mentioned during the NPI assessment but triggered during this "unusual" situation. Moreover, the noise produced by the scanner during the fMRI acquisition may potentially be not tolerated by patients at such advanced stage of the disease. Last, a potential unpleasant sense of claustrophobia experienced inside the scanner, causing prematurely terminated MRI acquisition also in cognitively healthy people,[30] could be accentuated in people with moderate to severe AD.

The patients were asked to lie down on a bed. A semi-cylinder panel, simulating the limited space inside the scanner, was placed in order to cover their entire body from the head to the knees. A loud white noise, miming the scanner noise, was sent binaurally to the patients through headphones for the entire duration of the simulation (9 minutes totally). Twelve out of twenty-eight patients that underwent the simulation were not enrolled in the study due to evident behavioral problems during the simulation time. The simulation failed only in one patient out of the sixteen successfully screened. This patient complained dizziness after the first real scan and has been excluded from the study.

MR acquisition. Resting state functional MRI were acquired at baseline (T0), and six months later (T6), on a 3.0 T Siemens Allegra scanner at the Neuroradiology Unit of the Ospedale Maggiore Borgo Trento, Verona, Italy, with a standard head coil. Scans were acquired with the following acquisition protocol: TR= 2610 ms, TE= 30 ms, flip angle= 90°, gap= 10%, voxel=3x3x3 mm, acquisition matrix=64x64, total number of slices=36, acquisition time 8'47" and anteroposterior phase-encoding direction. Resting state sequence was acquired after the localizer and during the acquisition the patients were lying down in the scanner with closed eyes. No cognitive or motor tasks were performed during the session.

Image Processing. Data were preprocessed with SPM5 (Statistical Parametric Mapping, version 5; http://www.fil.ion.ucl.ac.uk/spm/software/spm5). After motion correction performed with a 6 parameter spatial transformation and using the first image of each subject's series as the reference for the subsequent scan realignment, each subject's fMRI was spatially normalized to the SPM

echo-planar template through a non linear transformation and smoothed with a 5x5x5-mm full width at half-maximum Gaussian Kernel.

Functional MRI images were then divided in the four groups, placebo and memantine at T0 and T6, in order to apply separately independent component analysis (ICA) using GIFT.[31] The ICA result consists of a set of components extracted from the fMRI dataset representing the different temporally coherent, maximally independent hemodynamic sources related to the BOLD signal. Briefly, the GIFT approach is based on a preliminary Principal component analysis (PCA) data reduction step performed on the whole dataset in order to reduce the computational load. The complexity of each subject functional MR series is reduced through a PCA and a subsequent reduction step is operated on the temporal concatenation of the reduced series. The result consists of about 40 components. ICA is then performed with the Infomax algorithm on the final set and the resulting estimated mixing matrix is used to back-reconstruct spatial maps and time courses from the global results for each individual subject and for each component. Component intensities are scaled to Z-scores so as to enhance the reliability of the following voxel by voxel comparisons.

Since the Infomax algorithm is an iterative process, the ICASSO tool provided by GIFT was used to assess the consistency of the components resulting from the analysis. The process consists of 20 distinct computational runs of the ICA on the same dataset where the components are recomputed each time and the results are compared across runs. For each component the “centroid” (i.e. the most stable result) is determined and his consistency is expressed through a stability index ranging from 0 to 1.[32] This operation ensures the robustness of the findings across the groups, in order to improve the reliability of the further direct statistical comparisons.

Finally, the DMNs computed in the four groups were recognized through both visual inspection and spatial correlation with an a priori template created using Wfu-Pickatlas,[33] a tool available as an SPM toolbox and including posterior cingulate cortex, precuneus, medial prefrontal cortex and the medial, lateral and inferior parietal cortex.

Statistical Analysis. Statistical analyses were carried out using the SPM5 General linear model (GLM) on the 4 DMN sets. The significance was declared using the restrictive threshold for the p-value of 0.05 corrected for the family wise error (FWE). Initially, for both groups placebo and memantine, the global DMNs spatial extension at T0 was assessed through the one sample t-test computed on the two distinct sets of DMNs obtained with ICA (Figure 1) and the two resulting maps were used as spatial mask for the successive comparisons.

The comparisons at T0 and T6 between placebo and memantine groups was performed with a two sample t-test, restricting the analysis to the mask provided by the conjunction of the DMN activation areas previously computed. Both contrasts were used to compute the voxels of greater activation in placebo compared to memantine and vice versa. Paired t-test was used to study within group activation differences for the T6 images compared to the baseline and finally the overall effect was assessed through the SPM flexible factorial model, with treatment (placebo, memantine) and time (T0, T6) factors of interest and introducing the time x treatment interaction term.

Results.

The two groups were similar at baseline for all the considered sociodemographic and cognitive features. The clinical changes measured at T6 on the same neuropsychological battery were not significantly different from baseline (Table 1).

The ICA algorithm found 42 and 41 components respectively for Placebo and Memantine Groups at T0, and 51 and 45 at T6. The 4 components identified as DMN via spatial correlation with the pre-defined DMN template showed good consistency under ICASSO multiple runs test. The results gave compact and isolated clusters for the components centroids (i.e. stable results across runs) with stability indexes greater than 0.95.

At baseline, the Memantine group showed clusters of greater activation compared to the Placebo in the precuneus and cuneus, assessed after correction for multiple comparison, while the opposite comparison did not show significant results after the correction (Figure 2).

On longitudinal analysis, the paired t-test on the Memantine group showed greater activation at T6 as compared to the baseline in the right precuneus (23 voxels, $p<0.05$ FWE corrected – Supplementary Figure 1). On the other hand, the Placebo group did not show any statistically significant difference in the longitudinal comparison. The treatment effect was confirmed by the significant time x treatment interaction, mapping to the precuneus and calcarine cortex (Figure 3). The increased activation of the Memantine group in the follow-up assessment was finally appreciated in the direct between-group comparison at T6 (Figure 4). Again, the Memantine subjects showed increased activation as compared to the Placebo mapping to a significant cluster in right precuneus (25 voxels, $p<0.05$ FWE corrected).

Discussion

In this study we tested the effect of memantine on DMN activity in patients with moderate to severe AD. The analysis was performed in two separated steps: 1) independent component analysis, to extract the networks of interest from the functional dataset, and 2) voxel based morphometry, to assess the areas of significant changes of activations in the longitudinal setting.

The main result was an enhancement of the resting activity after six months of treatment, mapping primarily to the precuneus and posterior cingulate cortex. This is, to our knowledge, the first study assessing the effectiveness of a drug developed for AD on resting state network. These results should be interpreted in view of the neurochemical systems believed to underlie the DMN, and the known neurochemical effects of memantine in the brain.

The energetic balance (antiglutamatergic) hypothesis.

AD is characterized by an energetic imbalance due to a gap between decreased energy availability and raised energy demand. Increased energy requirements, associated with energy failure, cause abnormal accumulations of glutamate,[35,36] either by impairment of uptake (into neurons and especially astrocytes) mediated via glutamate transporters or by reversal of the direction of transport .[37] The consequent augmentation of extracellular glutamate overstimulates NMDA receptors to finally lead to an extended excitotoxicity.[38]

Since memantine could normalize NMDA receptor activity by blocking excessively-opened NMDA channels,[39] it may appear counterintuitive that it causes partial restoration of the normal energetic balance and improves the symptomatology of Alzheimer's disease (AD).
The outlined scenario is plausible in view of human studies indicating that the glutamate-glutamine cycle accounts for the largest part (60 to 80%) of metabolic consumption in resting human cerebral cortex.[40, 42] Moreover, a linear 1:1 relationship between glutamate-glutamine cycle and neuronal glucose oxidation was shown in animal studies.[43] These result suggest that the majority of cortical energy production supports synaptic glutamatergic neuronal activity.

If, as it is believed, BOLD signal in fMRI reflects the neuronal metabolic activity, it is reasonable to assume that the glutamate-glutamine cycle accounts for a large proportion of the BOLD fMRI signal.[44, 46] Any interference with the glutamate cycle might thus affect both glucose oxidation and metabolic activity. This was suggested by a study on a group of AD patients where a measure of neuronal integrity (N-acetyl aspartate) was significantly correlated with the markers of glutamate

neurotransmission and glucose oxidation.[47] The authors hypothesized that the reduced glutamate neurotransmission may affect the overall rate of glucose oxidation via impaired glia-neurons energetic interaction, thus contributing to cognitive impairment in AD. Further studies have shown a link between metabolic deficits and altered glutamatergic concentration in AD. Both phenomena seem to be caused by the the beta amyloid deposition, and in vitro experiments in astrocytes have shown an amyloid-mediated dependency.[48]

The above findings indicate that DMN activity might be an indirect measure of glutamate-mediated metabolic activity. The effect of memantine on the regulation of the glutamatergic levels has an impact on the brain metabolic activity, causing increased oxygen consumption and the subsequent change detected through the BOLD signal. This hypothesis is in agreement with previous works [6], which indicate a widespread increased glucose metabolism on the patients treated with memantine for 52 weeks.

The above scenario helps to interpret the topographic location of the effect we found in the present study (cuneus and posterior cingulate cortex), where early metabolic impairment in AD is earliest. [49] A recent study investigated in these areas the longitudinal changes of the DMN in mild to moderate AD [50], and showed significantly decreased activation over 4 years. Although these regions are not the only areas in DMN to be rich of NMDA receptors, it appears indeed reasonable that an effective benefits of memantine treatment could be detected mainly in those regions known to be more affected by metabolic and functional impairment and where an improvement of the energetic balance can be more clearly appreciated.

The dopaminergic hypothesis.

An alternative explanation comes from results of clinical studies, which suggest that the dopaminergic effect of memantine might be responsible for the increased DMN activity. It has been already reported that memantine enhances dopaminergic transmission via sigma receptors activation [51] or by blocking potassium channels in dopamine (DA) neurons.[9] A protective effect of memantine on DA function has been also suggested by a recent study on simian immunodeficiency virus infected macaques [52] where the prevention of the DA deficit onset following from memantine treatment was reported. This result supported the hypothesis that DA loss in SIV-infected macaques may be due to NMDA receptor activation. Furthermore, an agonistic effect of memantine on DA D2High receptors has been reported.[8] The importance of DA in regulating DMN activity and, generally, resting network integrity has been suggested in.[53] These authors

found an impairment of the network efficiency after pharmacological blockade of DA D2 receptors, which might be due to the role of this neurotransmitter in modulating the frequency, phase, and spatial coherence of endogenous oscillations in the basal ganglia and cortex.

Alternative hypotheses.

It is not certainly necessary that memantine should contemporary act on both the glutamatergic and dopaminergic systems to affect DMN activity. In theory, the exclusive change of dopaminergic function might affect, in turn, the glutamatergic system. For instance, using transgenic mice, it has been demonstrated that alterations in DRD4 expression can alter the Glutamatergic neurotransmission.[54] Moreover, an alteration of the balance between the D1 and D2 receptors could underlie the behavioural effects induced by the stimulation of the glutamatergic system.[55, 56]

Alternatively, since a role of GABA on the DMN BOLD activity has been described,[57] an effect of memantine on GABAA receptors [58] could account for changes in DMN activity. We believe that an effect of memantine on Abeta metabolism is unlikely in a 6-months trial.[59]

Caveats and limitations.

The present study shows a lack of correspondence between the neurochemical effects that we detected through the increased functional activity, and the improvement on the cognitive function assessed by the neuropsychological battery. From one side, this result suggests that the increased DMN activity could be a sub-threshold biomarker of drug efficacy, similarly to the role of the hippocampal atrophy in recent findings on drug trials. By the other hand, the high variability of the neuropsychological scores (Table 1) evidences a loss of sensitivity of the cognitive assessment at the advanced stages of the Alzheimer's disease. This fact could impair the ability to detect the possible subtle changes taking place in the brain which can still be captured by the more sensitive imaging analysis.

The small group size, although understandable in view of the difficulty to have severely impaired patients lying in an MR scanner, and the homogeneity of the group, composed prevalently by women with low education, suggest that the results need to be confirmed in adequately powered studies. As possible consequence, we found at the baseline a cluster of significantly greater activation in the precuneus in the memantine group compared to the placebo group. This cannot

exclude that the precuneus in the subjects treated with memantine was relatively less compromised prior to any experimental manipulation.

Although the ICA followed by the ICASSO procedure aims to provide results as much robust as possible, a certain variability due to the algorithmic approximations could affect the related statistical analysis. In order to confirm the above findings, a separated and additional analysis was replied on the whole dataset using the constrained ICA algorithm.[34] This ICA approach, available on GIFT software, uses apriori information provided by a spatial mask, in order to drive the ICA result and computes, among the observed mixture, the closest component to the reference provided. In our analysis the spatial information was provided by the DMN template and the resulting components computed for the four groups were used to replicate the statistical analysis. The components obtained with the constrained ICA approach were similar to those obtained with the classical "blind" method and the subsequent statistical analysis showed the same patterns of increased and decreased activation (data not shown).

Finally, both ICA and voxel based morphometry are highly exploratory analysis. As a consequence, the results provided in the present work should be interpreted as new hypothesis to be tested in future and more focused studies.

To conclude, although the present analysis should be seen as a preliminary and exploratory study for the assessment of the benefits of the drug directly on functional activity, we believe that the emerging evidences could add new insights and hypothesis for the understanding of the memantine action in the brain as well as the patho-physiological mechanisms of AD. New analysis of the effect of memantine on the others resting state networks and, more generally, on the global connectivity, are currently under study.

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Tables

Table 1. Socio demographic and neuropsychological test scores for Placebo and Memantine groups at baseline. p denotes significance of the differences between the two groups on standard t-test for continuous outcomes and non parametric Mann-Whitney test for categorical data. The two groups do not show significant differences among the different features.

	Baseline			**6 months follow-up**		
	Placebo N=8	**Memantine N=7**	**p**	**Placebo N=8**	**Memantine N=7**	**p**
Sociodemographic features						
Age, years	76 (±6)	77 (±8)	.68	---	---	---
Gender, women	6 (75%)	7 (100%)	.14	---	---	---
Education, years	4.6 (± .7)	4.4 (± .7)	.64	---	---	---
Cognitive Assessment						
Mini Mental State Examination	13.12 (±3.5)	15.57 (±4.9)	.29	14.13 (±4.96)	10.71 (±3.73)	.13
Non-Verbal Reasoning						
Raven Colored Progressive Matrices	8.75 (5.6)	6.57 (6.55)	.5	6.63 (±6.46)	5.71 (±8.83)	.82
Constructional Abilities						
Rey-Osterrieth complex	3.6 (±3.7)	3.7 (±8)	.96	2.31(±2.66)	10.85 (4.5)	.6

figure copy						
Attentional and Executive functions						
Trail Making Test A (seconds)	310.3 (±110.4)	353.5 (±156.6)	.54	287.75 (±128.24)	388.86 (±130.41)	.15
Trail Making Test B	506 (±220.9)	648 (±150.6)	.18	558.2 (±229.7)	683.43 (±140.17)	.23
Trail Making Test B-A	262.5 (±109)	297.5 (±18.6)	.42	276.75 (±117.5)	294.57 (±17.15)	.7
Language						
Fluency, phonemic	10.1 (±4.6)	11.2 (±7.2)	.71	9.5 (±6.19)	7.14 (±9.32)	.57
Fluency, semantic	11.7 (±4.8)	7.5 (±5.5)	.24	8.6 (±6.3)	6.14 (±6.52)	.47
Token test	21.9 (±4)	22.2 (±4)	.87	19.1 (±8.3)	17.6 (±9.22)	.75
Memory						
Story Recall (units/scoring)	.25 (±.37)	.35 (± .37)	.56	0.88 (±0.69)	1.29 (±2.58)	.67
Rey–Osterrieth complex figure–recall (units scoring)	0 (± 0)	.14 (± .37)	---	0.13 (±0.35)	0 (0)	---
Digit Span	3.5 (±1.69)	3.7 (± .75)	.76	2.88 (±2)	1.43 (±1.8)	.17
Spatial Span	2.62 (± 1.3)	3 (± 1)	.55	1.88 (±2)	1.43 (±1.5)	.64

Titles and legends to Figures

Figure 1. Average DMNs in the group of Memantine treated (left column) and Placebo (right column) at the different time points. First row: Average DMN at baseline. Middle row: Average DMN at the follow-up. Last row: DMN masks used for the statistical analysis computed from the baseline activations. Activations are overlaid on the stereotaxic MNI space (indices of the slices: coronal 161, sagittal 112, axial 204).

Figure 2. Differences in DMN activation between Placebo and Memantine groups at baseline. Blue colour denotes those areas where the activation is greater in the Memantine group compared to the Placebo, while red areas denote the opposite comparison. Analysis was conducted using SPM two sample t-test restricted in DMN ROI resulting from the union of the two groups DMN maps. Results are displayed with statistical threshold of 0.05 uncorrected for multiple comparison for illustrative purpose. The tables show location of maximally significant results at T0 for (a) Placebo Group greater DMN activation than Memantine and (b) Memantine Group greater DMN activation than Placebo, p-values denoted with * are significant after 0.05 FDR correction. Image and voxels locations given in the stereotaxic MNI space (indices of the axial slices :188,196,204,212).

Figure 3. Longitudinal DMN activation differences in the time x treatment model.

Table shows location of maximally significant results for the time x treatment interaction term, representing increased DMN activation in the memantine group compared to the placebo during the 6 months of the trial. The opposite comparison gave no significant results. p-values denoted with * are significant after 0.05 FDR correction. Color bars denote T-values. Image and voxels locations given in the stereotaxic MNI space (indices of the axial slices: 174,180,188,195,201,209).

Figure 4. Differences in DMN activation between Placebo and Memantine groups at six months. Red colour denotes those areas where the activation is greater in the Memantine group compared to the Placebo one, while the opposite comparison didn't show significant results. Results are displayed with statistical threshold of 0.05 uncorrected for multiple comparison for illustrative purpose. The tables show location of maximally significant results at T6 for Memantine Group

greater DMN activation than Placebo, p-values denoted with * are significant after 0.05 FDR correction. Image and voxels locations given in the stereotaxic MNI space (indices of the axial slices :184,193,201,208,220,226).

Supplementary Figure 1. Longitudinal DMN activation differences in

1) Memantine Group between T0 and T6. Red areas denote topography of increased activation at T6. Results are obtained using SPM paired t-test restricted on Memantine Group DMN map at T0 and displayed with statistical threshold of 0.05 uncorrected for multiple comparison for illustrative purpose.

Table shows location of maximally significant results for Memantine representing increased DMN activation at T6 than T0. The opposite comparison gave no significant results, p-values denoted with * are significant after 0.05 FDR correction. Color bars denote T-values. Image and voxels locations given in the stereotaxic MNI space (indices of the axial slices: 100,104,108,112)

2) Placebo Group between T0 and T6. Red areas denote topography of increased activation at T0 while blue areas denote increased DMN activation at T6. Results are obtained using SPM paired t-test restricted on Placebo Group DMN map at T0. Statistical threshold has been set at 0.05 uncorrected for illustrative purpose. The tables show location of maximally significant results for Placebo representing (a) increased DMN activation at T0 compared to T6 and (b) increased activation at T6 compared to T0. p-values denoted with * are significant after 0.05 FDR correction. Color bars denote T-values. Image and voxels locations given in the stereotaxic MNI space (indices of the axial slices: 92,96,100,104)

Fig. 1

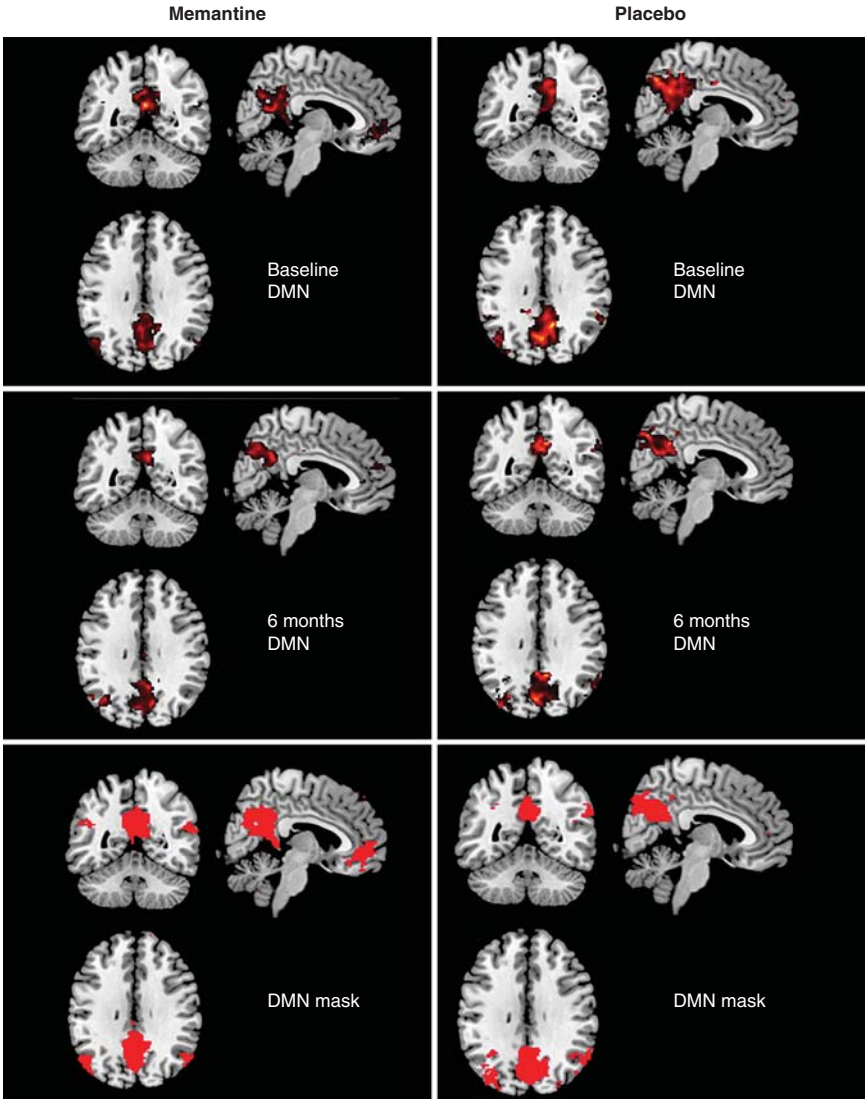
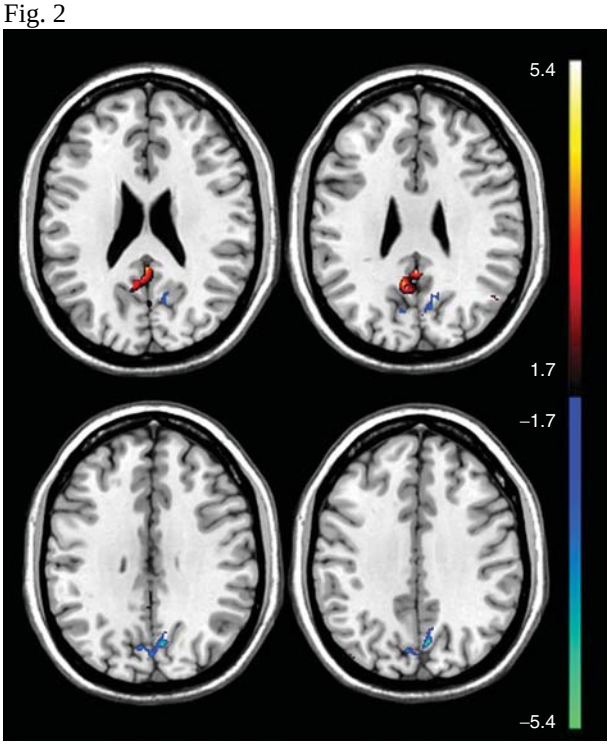

Memantine
Placebo
Baseline
DMN
Baseline
DMN
6 months
DMN
6 months
DMN
DMN mask
DMN mask

Fig. 2



a Placebo > memantine

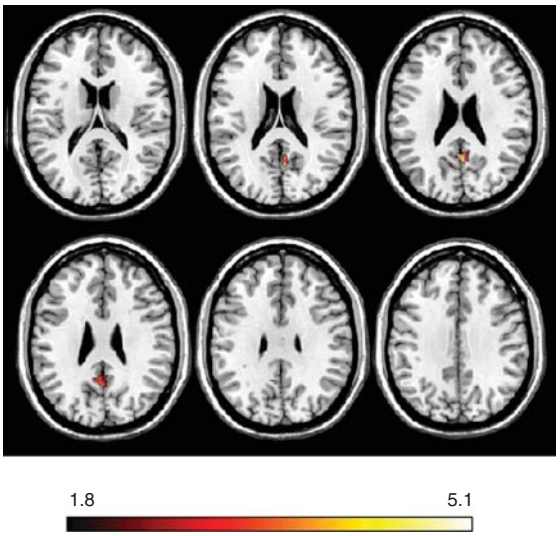
Cluster size	p-Value	Voxel (x,y,z)	Region
9	<0.001	0, –46, 28	Post cing
	<0.001	–2, –50, 20	Post cing
4	0.001	–8, –54, 28	Precuneus

b Memantine > placebo

Cluster size	p-Value	Voxel (x,y,z)	Region
15	<0.001*	6, –72, 36	Precuneus
3	<0.001	0, –80, 34	Cuneus
1	0.001	–8, –74, 32	Cuneus

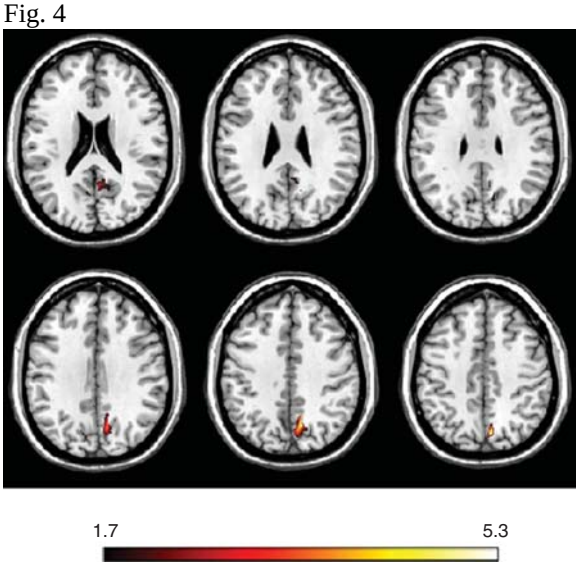
Fig. 3

Time x treatment interaction



Cluster size	p-Value	Voxel (x,y,z)	Region
14	<0.001*	0, –56, 24	Precuneus
5	<0.001*	4, –62, 20	Calcarine

Fig. 4



a Placebo > memantine

No significant results

b Memantine > placebo

Cluster size	p-Value	Voxel (x,y,z)	Region
25	<0.001*	10, −64, 40	Right precuneus
10	<0.001	8, −72, 44	