Thalamic Involvement in Fluctuating Cognition in Dementia with Lewy Bodies: Magnetic Resonance Evidences

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Dementia with Lewy bodies (DLB) is characterized by fluctuation in cognition and attention. Thalamocortical connectivity and integrity of thalami are central to attentional function. We hypothesize that DLB patients with marked and frequent fluctuating cognition (fCog) have a loss of thalamocortical connectivity, an intrinsic disruption to thalamic structure and imbalances in thalamic neurotransmitter levels. To test this, magnetic resonance imaging (MRI), diffusion tensor imaging (DTI) and proton MR spectroscopy on thalami were performed on 16 DLB, 16 Alzheimer’s disease (AD) and 13 healthy subjects. MRI and DTI were combined to subdivide thalami according to their cortical connectivity and to investigate microstructural changes in connectivity-defined thalamic regions. Compared with controls, lower N-acetyl-aspartate/total creatine (NAA/tCr) and higher total choline/total creatine (tCho/tCr) values were observed within thalami of DLB patients. tCho/tCr increase was found within right thalami of DLB patients as compared with AD. This increase correlated with severity and frequency of fCog. As compared with controls, DLB patients showed bilateral damage within thalamic regions projecting to prefrontal and parieto-occipital cortices, whereas AD patients showed bilateral alteration within thalamic region projecting to temporal cortex. We posit that microstructural thalamic damage and cholinergic imbalance may be central to the etiology of fCog in DLB.

Keywords: attention, choline, dementia with Lewy bodies, fluctuating cognition, thalamus

Introduction

The thalamus plays a central role in altering and maintaining arousal (Steriade 2006; Ward 2011). Anatomically, its nuclei are topographically organized to modulate and synchronize distributed cortical networks supporting large-scale cerebral dynamics related to goal-directed behaviors and awareness (Schiff 2008). On this basis, it was suggested that phenomenal consciousness is generated by synchronized neural activity in thalamic neurons and that thalamic activity is driven by information arising from the cortical computation (Ward 2011).

Abnormal functional connectivity and microstructural damage within thalami have been previously reported in dementia with Lewy bodies (DLB) (Watson et al. 2012; Kenny et al. 2013). DLB patients were clinically characterized by spontaneous alteration in cognition, attention, and arousal (Lee et al. 2012). These symptoms were reported as fluctuating cognition (fCog), which represents, together with visual hallucinations and extrapyramidal signs, the core clinical features of DLB (McKeith et al. 2005).

Several neuroimaging studies have focused on the neural bases of fCog in DLB patients, suggesting a relation between the severity of fCog and covariant perfusional network changes (Taylor et al. 2013) or functional magnetic resonance imaging (fMRI) blood oxygen-dependent level (BOLD) (Franciotti et al. 2013; Peraza et al. 2014) alterations. In addition, increased thalamic perfusion on single photon emission computed tomography (SPECT) (O’Brien et al. 2005) and changes in dopaminergic and cholinergic systems within thalami have been linked to fCog in DLB (Pimlott et al. 2006; Piggott et al. 2007). Of note, the cholinergic system is widely distributed in the brain and it is more affected in DLB than in Alzheimer’s disease (AD) patients (Kotagal et al. 2012). This concept is amply supported by pharmacological evidences which suggest that (1) anticholinergic drugs can induce a symptom profile of altered arousal comparable to fCog in DLB (Perry et al. 1999) and (2) cholinesterase inhibitors can significantly improve fCog and attentional function in DLB (McKeith et al. 2000; Onofri et al. 2003; Wenes et al. 2005).

Based on these considerations and on the central role of thalamic neurons in regulating arousal and attention, we hypothesized that cholinergic imbalance within thalami and damage of the structural connectivity between thalami and cortical regions modulating alertness and attention are associated with fCog in DLB.

To test this, we carried out a study using multimodal techniques including structural magnetic resonance imaging (MRI), diffusion tensor imaging (DTI) and proton MR Spectroscopy (1H-MRS) in a cohort of DLB and AD patients as well as healthy controls. 1H-MRS technique assesses, in vivo, different metabolites such as N-acetyl-aspartate (NAA) and total choline (tCho) which are specifically related to decrease of neuronal integrity and to cholinergic deficits respectively. The combination of structural MRI and DTI allows parcellation of the thalami according to their cortical structural connectivity and to investigate microstructural integrity in each connectivity-defined region. The comparison between DLB and AD was performed to assess whether any possible structural and metabolic changes in the thalami were specific of a dementia characterized by the presence of fCog (DLB) or more as a result of a process of dementia per se. Furthermore, the variation of severity and frequency of fCog among DLB patients provides a means by which to assess whether the MR thalamic changes are associated with more overt fCog.

Material and Methods

Study Sample

This study was approved by Local Institutional Ethics Committee. All participants (or their caregivers) gave written informed consent.

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Sixteen AD and 16 DLB patients were recruited from our Memory Clinic and Movement Disorder Clinic. Thirteen age-matched volunteers come from our nondemented case register cohorts. The diagnosis of probable AD was made using National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer’s Disease and Related Disorders Association criteria (McKhann et al. 1984). The probable DBL diagnosis was based on consensus guidelines (McKeith et al. 2005) with a specific restriction: we included only patients with the presence of fICog, plus at least one additional core feature (visual hallucination or parkinsonism) or the presence of fICog plus one or more suggestive features (McKeith et al. 2005).

As part of their clinical work up within 6 months before the inclusion in the study, all patients underwent computerized tomography or MRI and dopaminergic presynaptic ligand ioflupane SPECT (DAT scan). In addition all patients were assessed with electroencephalography (EEG) recordings as abnormalities characterized by parieto-occipital dominant frequency alterations have previously been shown to reliably differentiate probable DBL from AD (Bonanni et al. 2008). DAT scans and EEG Compressed Spectral Array patterns (as defined in Bonanni et al. 2008), performed prior to entry into the study, were used to support the diagnosis of DBL.

Clinical Evaluation
Mini mental state examination (MMSE), clinical dementia rating (CDR) and Dementia rating scale-2 (DRS-2) (Jurica et al. 2001) were performed for global cognitive assessment. Frontal assessment battery (FAB) was performed to evaluate the severity of frontal dysfunction (Dubois et al. 2000). The frequency and duration of fICog were assessed by CAF questionnaire (Walker et al. 2000); this is a well established Parkinson’s disease (PD) screening tool. CAF score between 2 and 16 was considered sufficient to define a patient as affected by fICog (Bonanni et al. 2008).

MR Protocol
All MR data were acquired with a Philips Achieva 3 T scanner (Philips Medical System, Best, the Netherlands) equipped with 8-channel receiver coil. Three-dimensional $T_1$-weighted images were acquired by using Turbo Field-Echo sequence (TFE, TR/TE = 11/5 ms, slice thickness of 0.8 mm). Two $T_1$-MRV voxels of 1.5 x 1.0 x 1.5 mm$^3$ were accurately placed on right and left thalami, respectively (Fig. 1A). Point-resolved spectroscopy sequences (TR/TE = 2000/39 ms, 16-step phase-cycle and an average of 128 scan) with and without water suppression were performed by using chemically shift selective (CHESS) pulses. One thousand and twenty-four points were acquired with a spectral width of 2000 Hz. $T_2$-weighted fluid attenuation inversion recovery sequence [FLAIR, time precession (TR)/time echo (TE) = 11 000/ 125 ms, slice thickness of 4 mm, field of view (FOV) = 240 x 129 x 222 mm] was performed to exclude participants with concomitant vascular pathology or with white matter abnormalities outside the normal range.

1H-MRS Analysis
1H-MRS data analysis was performed by using JMRUI version 4.0 (Naressi et al. 2001). Spectra with water suppression were filtered for removal of residual water by using the Hankel Lanczos Singular Values Decomposition algorithm. After autophasing, baseline and frequency shifts correction, a priori knowledge database (NAA, 2.02 ppm; total creatine =tCr, 3.03 ppm; tCho, 3.22 ppm) was created to put constraints on the advanced magnetic resonance fitting algorithm within JMRUI package. Peak shifts were restricted to +5 ppm of the theoretical location. Spectra with artifact and metabolites fits with Cramer Rao Lower Bounds >20% were excluded.

For applicability to the clinical practice and because several 1H-MRS studies report stable tCr levels in dementia (Valenzuela and Sachdev 2001; Dedegolu et al. 2004; Chao et al. 2005; Cantarci 2013), we expressed NAA and tCho relatively to tCr. By using spectra without water suppression, we calculated the area of the water peak and we used it as an internal reference standard for absolute tCr quantification (Christiansen et al. 1993; Delli Pizzi et al. 2012, 2013).

Parcellation of Thalami
Functional MRI of the Brain (FMRIB) Software Library (FSL, version 5.0; http://www.fmrib.ox.ac.uk/fsl, Smith et al. 2004) was used to perform structural connectivity-based parcellation of thalami and to investigate microstructural changes in each thalamic region. For each subject, mean diffusivity (MD) maps were generated from a tensor model fit in FSL (FDT, FMRIB’s Diffusion Toolbox). Thalami parcellation was performed according to methods described by Behrens et al. (2003). Four cortical masks including prefrontal, sensorimotor, temporal, and parieto-occipital regions were defined using the Harvard Oxford Cortical Atlas (implemented in FSL) (Fig. 2A). The composition of each target region was reported in Supplementary Table 1. FMRIB’s Integrated Registration and Segmentation Tool (FIRST) was used to automatically segment thalami (Patenaude et al. 2011) and its outputs were binarized to obtain masks. All cortical and subcortical masks were in MNI space (1 x 1 x 1 mm). After Bayesian estimation of diffusion parameters obtained using sampling techniques, the DTI maps were registered to MNI standard space using: (1) FLIRT to register each subject’s b0 image to its native structural image, and (2) FMRIB’s non-linear registration tools to register the structural and diffusion images to MNI space (1 x 1 x 1 mm). All masks were then propagated onto each individual’s DTI scalar maps using the inverse of the above transformations. To exclude thalamic voxels that contained cerebrospinal fluid (CSF), the b0 images were segmented using FMRIB’s Automated Segmentation Tool (FAST) and CSF binarized to be used as exclusion mask. To exclude voxels out of the thalamic range, manual editing was applied where needed. Next, probabilistic tracking was carried out by probabilistic tracking (PROBTRACKX) tool. “Find the biggest” command line was used to define the thalamic connectivity-defined subregions according to their highest probability of connection with cortical regions (Fig. 2B). Two experienced operators visually inspected the “find the biggest” outputs, verifying the anatomic correspondence of each thalamic connectivity-defined subregion among subjects and their accordance with Oxford Thalamic Connectivity Atlas (integrated in FSL, Johansen-Berg et al. 2005; Fig. 2C).

Finally, MD values were calculated in each connectivity-defined subregion.

Given that some cortical regions in DBL and/or AD could be characterized by changes in regional cerebral blood flow (Colloby et al. 2013) and the diffusion indices could be biased by different location and volume of thalamic connectivity-defined regions determined by the tracking algorithm, we adopted 3 different strategies. Firstly, we verified whether the spatial distribution and the connection probability within each thalamic connectivity-defined region were altered by the pathological underlying condition (Nair et al. 2013). Specifically, by using “randomize” command line, we carried out comparisons within and between groups on the maps of connection probability generated by PROBTRACKX (Fig. 2D). Family-wise error (FEW) correction was applied to obtain the significant voxels. Secondly, we verified whether the volumes of each thalamic region defined by “find the biggest” were significantly different among groups. Specifically, for each subject, the
Figure 1. Proton magnetic resonance spectroscopy (^1H-MRS). (A) Two voxels of 1.5 × 1.0 × 1.5 mm^3 were, respectively, placed on right and left thalami by using T1-weighted image as anatomical reference. (B) Representative spectra for DLB, AD, and controls. Estimated signals (violet) were reported on original signals (red). NAA, N-acetyl-aspartate (2.02 ppm); tCr, total creatine (3.03 ppm); tCho, total choline (3.22 ppm). (C) Scatterplot expresses the linear regression between CAF scores and tCho/tCr values in the right thalamus. AD, Alzheimer’s disease; DLB, dementia with Lewy bodies. Values marked with an asterisk are overlapped.

Figure 2. Structural connectivity. (A) Cortical rendering of target regions used for thalami parcellation (colors were in agreement with thalamic connectivity). (B) Connectivity-based subdivision of thalami for controls (CON), dementia with Lewy bodies (DLB), and Alzheimer’s disease (AD). Thalamic voxels are classified and colored according to the highest probability of connection to specific cortical regions. Red, connectivity-defined subregion (CDR) that projects from thalamus to prefrontal cortex; blue, CDR that projects from thalamus to sensorimotor cortex; yellow, CDR that projects from thalamus to parieto-occipital cortex; green, CDR that projects from thalamus to temporal cortex. (C) Thalami regions defined by Oxford Thalamic Connectivity Atlas. (D) Within-group probabilistic tractography maps for each cortical target region. The significant results are shown by voxels rating from red to yellow (P < 0.05, FWE-corrected). No significant differences were found among groups. PFC, prefrontal; SM, sensorimotor; PO, parieto-occipital; TMP, temporal.
volumes of thalamic regions were calculated by "fslstats" command line and, next, they were normalized for ipsilateral thalamic volume. Third, we verified whether the MD values in each thalamic region were related to differences found by tracking probability. Specifically, we defined the thalamic regions from Oxford thalamic connectivity atlas and we extracted MD values from them. Finally, we performed multivariate analysis of variance (MANOVA) on MD values to test whether the 2 methods (probabilistic parcellation and atlas) provided different results for thalamic regions.

Statistical Analysis

Analysis of variance (ANOVA) among groups was carried out on demographic and clinical data. \( x^2 \) test was carried out for gender.

We performed multivariate analysis of covariance (MANCOVA) to exclude the possible effect of cognitive impairment on different imaging findings obtained from demented patients and controls. Next, MANOVA was carried out to test the differences among groups (AD, DLB, and controls). Tukey’s HSD post hoc test was performed for assessing pair-wise differences between groups. For all comparisons, significance level was set at \( P < 0.05 \).

Linear regression was performed to assess the relationship between MR outcomes in DLB patients (MD within right and left connectivity-defined subregions and metabolites/tCr within left and right thalami) and CAF scores (our primary clinical measure). Age, MMSE, NPI hallucinations, UPDRS scores, on global test of cognition and on severity of frontal dysfunction were reported between AD and DLB patients. Fourteen DLB patients had RBD. Dopamine-transporter hypocapitation in the caudate nuclei at SPECT-DAT scan was observed in all DLB patients, and it was bilateral in 12 patients. SPECT-DAT scan abnormalities were not observed in AD patients or control subjects.

The patients were on a range of medications including L-Dopa (all DLB patients), rivastigmine or donepezil (all AD and DLB patients with no differences in daily dosages between the 2 groups of patients), quetiapine (8 DLB and 6 AD), clozapine (4 DLB), and risperidone (4 AD) and clonazepam (the 14 DLB patients with RBD).

1H-MRS Findings

Table 2 summarizes 1H-MRS results. Figure 1B shows representative spectra for each group. Supplementary Table 2 shows the effects among groups from MANOVA and MANOVA analyses. Lower NAA/tCr and higher tCho/tCr values were bilaterally observed in the thalami of DLB patients respect to controls. No changes were found in the thalami of AD patients compared with controls. The comparison between DLB and AD demonstrated tCho/tCr increase in the right thalamus of DLB patients. No tCr changes were found among groups. Assuming as nuisance factors age, MMSE, NPI hallucinations, UPDRS scores, on linear regression a significant relationship between tCho/tCr and CAF values within right thalamus (\( R = 0.86, t = 5.391, P < 0.001 \)) (Fig 1C) was observed.

Thalamocortical Structural Connectivity

Figure 2B shows the thalamic connectivity-defined subregions projecting to:
1. prefrontal cortex including the ventro-anterior and dorso-medial parts of the thalami;
2. sensorimotor cortex including the ventrolateral part of the thalami;
3. parieto-occipital cortex including the ventro-posterior part of the thalami and the superior portion of pulvinar;
4. temporal cortex including the dorso-anterior part of the thalami and the inferior portion of pulvinar.

Figure 2D shows the maps of connection probability of each thalamic subregion to specific cortical regions, for each group. When comparisons between groups were performed, no significant differences were observed on connection probability. As verified by visual inspection of “find the biggest” outputs, the location of each connectivity-defined thalamic region showed good correspondence among groups and it was in agreement with Oxford Thalamic Connectivity Atlas (Fig. 2C). Furthermore, the volumes of each thalamic region defined by “find the biggest” were not different among groups (see Supplementary Table 3). Hence, the anatomy for each thalamic connectivity-defined region did not significantly change in pathological conditions with controls.

Mean Diffusivity Changes Within Thalamic Regions

Table 3 shows grand mean MD values for each connectivity-defined subregion obtained from tractography-based subdivision
of thalami. Supplementary Table 4 shows the effects among groups from MANCOVA and MANOVA analyses. Supplementary Table 5 shows grand mean MD values for each connectivity-defined subregion obtained from Oxford thalamic connectivity atlas. No differences were found between MD values obtained with probabilistic parcellation and MD values obtained with Oxford Thalamic Connectivity Atlas (see Supplementary Table 6).

In comparison to controls, DLB patients showed bilateral increases in MD in the connectivity-defined subregions projecting to the prefrontal and parieto-occipital cortices. As compared with controls, AD patients showed increase of MD in left connectivity-defined subregion projecting to the temporal cortex. No significant differences were found in the comparison between DLB and AD. Within the DLB group MD increases were not correlated with our primary measure of fLCog (CAF score) or other clinical variables (e.g. FAB).

**Discussion**

In the current study, we demonstrated that DLB patients with fLCog were affected by neurochemical imbalance in the thalami and by microstructural changes in the connectivity-defined regions projecting from thalami to the frontal and parieto-occipital cortices. Specifically, we found reduced NAA/tCr and increased tCho/tCr in the thalami of DLB with respect to AD (Wurtman et al. 1985; MacKay et al. 1996; Tiraboschi et al. 2002; Kantarci 2013). In this context, recent 1H-MRS studies have suggested that the increase in tCho levels in DLB could be linked to the increase of membrane turnover due to dying of the neuropil and to down-regulation of choline acetyltransferase activity that it is reduced more severely in DLB compared to AD pathology (Kantarci 2013; Graff-Radford et al. 2014). Furthermore, consistently with our findings on thalami, Kotagal et al. (2012) observed thalamic cholinergic denervation in DLB but not in AD, suggesting a relationship between neurodegenerative involvement of thalamic cholinergic afferent projections and cognitive alteration in DLB.

TCho provides a marker of the contribution of cytosolic glycerolphosphocholine and phosphocholine which are the products of membrane phosphatidyl choline breakdown and the precursors of choline and acetylcholine synthesis, respectively (Klein 2000). It was hypothesized that higher tCho levels in DLB could be linked to the increase of membrane turnover due to dying of the neuropil and to down-regulation of choline acetyltransferase activity that it is reduced more severely in DLB with respect to AD (Wurtman et al. 1985; MacKay et al. 1996; Tiraboschi et al. 2002; Kantarci 2013). In this context, recent 1H-MRS studies have suggested that the increase in tCho could be a characteristic feature of DLB and independent from AD pathology (Kantarci 2013; Graff-Radford et al. 2014). Furthermore, consistently with our findings on thalami, Kotagal et al. (2012) observed thalamic cholinergic denervation in DLB but not in AD, suggesting a relationship between neurodegenerative involvement of thalamic cholinergic afferent projections and cognitive alteration in DLB.

NAA is primarily located in neuron bodies, axons, and dendrites and it is a sensitive marker for neuronal density or viability (Kantarci 2013). Therefore, NAA reduction suggested that DLB pathology could be associated to neurodegenerative processes in the thalami. Notably, this finding is consistent with a recent DTI study describing thalamic white matter disruption in DLB patients (Watson et al. 2012).

**Table 2**

| Metabolites       | Thalamus | Controls | AD    | DLB   | AD versus controls | DLB versus controls | AD versus DLB |
|-------------------|----------|----------|-------|-------|--------------------|---------------------|---------------|
| NAA/tCr           | Right    | 1.84 ± 0.12 | 1.73 ± 0.15 | 1.65 ± 0.2 | P = 0.160          | P = 0.008           | P = 0.363     |
|                   | Left     | 1.82 ± 0.15 | 1.78 ± 0.19 | 1.68 ± 0.14 | P = 0.738          | P = 0.045           | P = 0.173     |
| tCho/tCr          | Right    | 0.72 ± 0.04 | 0.72 ± 0.07 | 0.68 ± 0.09 | P = 0.896          | P = 0.014           | P = 0.011     |
|                   | Left     | 0.72 ± 0.05 | 0.76 ± 0.08 | 0.8 ± 0.11  | P = 0.315          | P = 0.034           | P = 0.465     |
| tCr/water         | Right    | 5.2 ± 0.2  | 5.1 ± 0.4  | 4.9 ± 0.9  | P = 0.958          | P = 0.014           | P = 0.546     |
|                   | Left     | 5.2 ± 0.6  | 5.0 ± 0.7  | 5.0 ± 0.7  | P = 0.733          | P = 0.774           | P = 0.997     |

AD, Alzheimer’s disease; DLB, dementia with Lewy bodies; NAA, N-acetyl-aspartate; tCr, total creatine; tCho, total choline.

*Tukey’s HSD post hoc test was performed for assessing pair-wise differences between groups.

**Table 3**

| Connectivity-defined region | Thalamus | Mean diffusivity (MD) | Statistical comparison |
|-----------------------------|----------|-----------------------|-----------------------|
|                             |          | AD        | DLB       | AD versus controls | DLB versus controls | AD versus DLB |
| Prefrontal cortex Right     | 752 ± 15 | 785 ± 67  | 809 ± 52  | P = 0.211          | P = 0.014           | P = 0.395     |
|                           | Left     | 752 ± 16  | 784 ± 72  | 815 ± 52  | P = 0.257          | P = 0.008           | P = 0.243     |
| Sensorimotor cortex Right   | 750 ± 19 | 757 ± 71  | 797 ± 60  | P = 0.933          | P = 0.079           | P = 0.131     |
|                           | Left     | 750 ± 37  | 760 ± 80  | 801 ± 47  | P = 0.876          | P = 0.061           | P = 0.136     |
| Parieto-occipital cortex Right | 759 ± 24 | 793 ± 88  | 843 ± 61  | P = 0.385          | P = 0.004           | P = 0.085     |
|                           | Left     | 759 ± 28  | 798 ± 93  | 837 ± 59  | P = 0.289          | P = 0.010           | P = 0.224     |
| Temporal cortex Right       | 779 ± 20 | 828 ± 70  | 818 ± 52  | P = 0.046          | P = 0.134           | P = 0.856     |
|                           | Left     | 782 ± 24  | 830 ± 59  | 818 ± 47  | P = 0.024          | P = 0.111           | P = 0.757     |

Note: MD values (×10−3mm²/s) are expressed as mean ± standard deviation. Bold characters indicate statistically significant results. Significant mean differences were found among groups by MANOVA (F(12,74) = 3.216, P = 0.001).
cholinergic alterations related to fflCog in the thalami of DLB patients (Perry et al. 1998; O’Brien et al. 2005; Pimlott et al. 2006). Thus, this strongly favors the role of cholinergic imbalance within thalami in the etiology of flfCog in DLB.

In agreement with previous functional and structural studies by our groups (Franciotti et al. 2006, 2013; Delli Pizzi et al. 2014), we also observed predominant right lateralization of neurochemical alterations in DLB. These findings could be linked to the dominant role of the right hemisphere in attention (Thiebaut de Schotten et al. 2011). Further studies integrating structural and functional MRI techniques could clarify whether predominant right hemispheric dysfunction is truly a specific feature of DLB.

We observed that structural thalamocortical connectivity was affected in DLB patients with fflCog. In this context, MD was specifically assessed because it is an index for both grey and white matters damage and its high values are associated to reduction in membrane density and cell loss of both neurons and glia (Canu et al. 2010).

Thalamic neurons regulate the cortico-cortical control acting as neuronal hub to synchronize oscillations between cortical areas (Sherman 2007). The modulation of thalamic activity can shift cortical activity by desynchronizing activated states (depolarized tonic firing mode) associated with arousal and/or by synchronizing deactivated states (burst firing mode) indicative of drowsiness (Llinás and Steriade 2006; Hirata and Castro-Alamancos 2010). In this context, impaired thalamic activity has been associated with decreased levels of arousal (Volkow et al. 1995; Fiset et al. 1999).

We found that MD was bilaterally increased in the connectivity-defined subregion projecting from thalamus to frontal cortex in DLB as compared with controls. The thalamic nuclei serving prefrontal cortex play a relevant role in consciousness (Foucher et al. 2004; Ward 2011) and alertness (Tomasi et al. 2009). In particular, deficits in concentration can be observed when thalamic region projecting to prefrontal cortical network is damaged (Van Der Werf et al. 1999). Moreover, in agreement with our findings, a recent study showed that functional connectivity within thalamic region projecting to frontal lobe was altered in DLB patients with respect to controls (Kenny et al. 2013). However, we did not observe a significant relationship between MD and CAF or FAB scores. However, in some respects this is not unexpected as across different neuroimaging modalities there has been an emphasis on symptom in DLB being more reliant upon posterior cortical function (Imamura et al. 1999; Lobotesis et al. 2001; Pasquier et al. 2002; Kemp et al. 2007; Taylor et al. 2012; Delli Pizzi et al. 2014). Indeed, in support of this we found that MD was bilaterally increased in the connectivity-defined subregion projecting from thalamus to parieto-occipital cortex in DLB as compared with controls although again there was no relationship between MD and CAF for these projections. Therefore, while the integrity of thalamocortical projections may be disrupted in DLB, this may be less relevant to the pathophysiology of fflCog; the 1H-MRS findings noted above support an argument that intrinsic neurochemical changes in the thalami are more important, at least for fflCog.

The ventrolateral region of thalamus and anterior portion of pulvinar synchronize the activity across posterior cortical regions, filtering distracters (Fischer and Whitney 2012) and regulating visuospatial attention (Saalmann et al. 2012). Particularly, pulvinar is ideally situated to integrate bottom-up orienting, driven either by sensory and subcortical inputs, with top-down orienting, driven by goals and context (Ward 2011). Notably, it was observed close relationship between pulvinar lesion and visuo-attentional deficits (Arend et al. 2008). On these bases, we propose that the deregulation of parieto-occipito-thalamic connectivity could be related to alteration in visual attention and visual dysfunction characterizing DLB patients.

Furthermore, we observed that AD patients were affected by bilateral microstructural damage in the connectivity-defined subregion projecting from thalamus to temporal cortex. As assessed by structural connectivity analysis, this connectivity-defined subregion included the antero-ventral nuclei of thalamus and the posterior pulvinar nuclei, which are directly connected to hippocampus via the fornix and via the temporopulvinar tract. The integrity of these connections is essential for episodic memory (Aggleton and Brown 1999), which is specifically affected in AD (Di Paola et al. 2007). In this context, Zarei et al. (2010) reported that AD patients were affected by structural alteration of the antero-dorsal thalamic nuclei. Therefore, we suggest that the microstructural alteration of connectivity-defined subregion projecting from thalamus to temporal cortex could be linked to great memory impairment affecting AD patients.

In conclusion, our findings highlight the critical role of thalami in DLB pathology. By multimodal techniques including structural MRI, DTI, and 1H-MRS, we have shown, firstly, microstructural damage in thalamic regions related to alertness and attention and, secondly, metabolic alterations related to neuronal damage and cholinergic dysfunction in the thalami. Furthermore, our results suggest that cholinergic imbalance within thalami is closely linked to frequency and duration of fflCog occurring in DLB.

A possible limitation of our study is that we focused our analyses on DLB patients with fflCog. Therefore our conclusions may be less applicable to DLB without fflCog. However, we underline that in the present study a CAF score ≥2 was considered sufficient to define a patient as affected by fflCog (Bonanni et al. 2008), at difference with the study in which this scale was originally proposed, where a higher cut-off of 5 at the CAF questionnaire was shown to have high sensitivity and specificity to DLB.

**Supplementary Material**

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

**Funding**

Funding to pay the Open Access publication charges for this article was provided by Wellcome Trust.

**Notes**

Conflict of Interest: None declared.

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