Brain pathways of verbal working memory
A lesion–function correlation study

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doi:10.1016/j.neuroimage.2009.04.054

Abstract
Working memory relies on information processing by several well-identified gray matter regions. However, the white matter regions and pathways involved in this cognitive process remain unknown. An attractive and underexplored approach to study white matter connectivity in cognitive functions is through the use of non-aprioristic models, which specifically search disrupted white matter pathways. For this purpose, we used voxel-based lesion–function mapping to correlate white matter lesions on the magnetic resonance images of 54 multiple sclerosis patients with their performance on a verbal working memory task. With this approach, we have identified critical white matter regions involved in verbal working memory in humans. They are located in the cingulum, parieto-frontal pathways and thalamo-cortical projections, with a left-sided predominance, as well as the right cerebellar white matter. Our study provides direct evidence on the white matter pathways subserving verbal working memory in the human brain.

Keywords:
Verbal working memory
White matter pathways
White matter lesions
Gray matter atrophy
Multiple sclerosis
Voxel-based morphometry

Introduction
Working memory is a process in brain systems that provides temporary storage and manipulation of the information necessary not only for some memory process but also for such complex cognitive tasks as language, learning, and reasoning (Baddeley, 2003). Lesion and neuroimaging studies, mainly involving functional magnetic resonance imaging and positron emission tomography, have contributed to the identification of the cortical and other gray matter regions involved in working memory in humans; such as prefrontal cortex (Brodmann’s areas [BA] 6/9/44/45/46), parietal cortex (BA 40), anterior cingulate cortex (BA 24/32) and right cerebellum (Audoin et al., 2005b; Baddeley, 1992, 2003; Braver et al., 1997; Mazoyer et al., 2001; Smith and Jonides, 1998, 1999; Wager and Smith, 2003; Woodward et al., 2005). However, the lack of suitable models to study with imaging the structure–function correlation of the white matter has precluded a similar understanding of the corresponding brain circuits in working memory. Some previous studies on stroke and brain development using magnetic resonance tractography hint at the importance of some frontal and parietal white matter regions in spatial working memory (Klingberg, 2006; Malhotra et al., 2005), but a more detailed mapping of the white matter network directly implicated in this cognitive function continues to be elusive.

In this study, we use a recently developed approach to analyze in humans the contribution of white matter disrupted pathways to memory processes (Sepulcre et al., 2008b). Briefly, it is based on five principles: 1) in the model, the white matter needs to be primarily and preferentially affected; 2) in each subject, the white matter has to be particularly damaged in discrete areas; 3) these areas need to vary in location and extent among different subjects; 4) in the sample studied, the sum total of the lesions of all the subjects needs to cover most of the brain, so that there are no unaffected volumes about which no information exists; and 5) the location and extent of the lesions should be measured with a method that does not presuppose any prior knowledge about relevant or target location to account for a given cognitive impairment. Thus, unbiased lesion–function correlations can be obtained between a type of measured cognitive impairment and the corresponding white matter lesions associated with it (Sepulcre et al., 2008b). Taking into account these principles, we analyzed the relationship between verbal working memory and...
white matter disrupted locations caused by a paradigmatic white matter disease, multiple sclerosis.

Multiple sclerosis is predominantly a disease of white matter lesions (Comi et al., 2000; Compston and Coles, 2002), although currently we know that there is also a neurodegenerative component affecting the gray matter (Filippi, 2001; Hauser and Oksenberg, 2006; Rovaris et al., 2006; Sepulcre et al., 2006a). White matter lesions can disrupt brain connectivity anywhere in the CNS, leading to sensory-motor and cognitive impairment. Working memory is one of the cognitive domains more frequently impaired in multiple sclerosis (Benedict et al., 2002; Rao, 1995; Rao et al., 1991; Sepulcre et al., 2006b). Several functional magnetic resonance image (fMRI) studies have identified abnormalities in working memory in patients with multiple sclerosis (Au Duong et al., 2005; Audoin et al., 2005a; Chiaravalloti et al., 2005; Hillary et al., 2003; Sweet et al., 2006; Wishart et al., 2004). More recently, an elegant study using diffusion tensor imaging (DTI) has described white matter changes in tracts connecting gray matter regions of the known working memory system in patients with early multiple sclerosis (Audoin et al., 2007). However, it is also important to explore directly the white matter pathways related to working memory performance without aprioristic assumptions as to their location. In this study, we determine the correlation between white matter injury and verbal working memory without being constrained by any a priori gray matter region and with the particular aim to analyze the brain pathways involved in the structural working memory network.

Procedures and methods

Patients

We studied 54 subjects with relapsing-remitting multiple sclerosis (revised McDonald criteria; Polman et al., 2005). Subject demographics are listed in Table 1. The local Research Ethics Committee approved the study and all subjects gave their informed consent according to the Declaration of Helsinki. Included were only right-handed (70% Oldfield scale) (Oldfield, 1971) native Spanish-speakers with no history of psychiatric or neurological disease other than multiple sclerosis, as well as no history of visual or auditory deficits, alcohol or drug abuse, or any other major medical illness. Excluded were those who had an active relapse, were taking steroids or had suffered a clinical relapse within the previous 3 months. Excluded also were patients with psychiatric disturbances identified with the Cummings’ Neuropsychiatric Inventory (Cummings, 1997), the Hamilton’s Depression Rating Scale (Hamilton, 1960) (>8 points), or the Hamilton’s Anxiety Rating Scale (Hamilton, 1959) (>6 points) and those taking psychoactive drugs.

Table 1

| Demographic, clinical, MRI and neuropsychological data of patients. |
|---------------------------|---------------------------|
| N                         | 54                       |
| Age (years)a              | 35.4±8.7                 |
| Sex ratio (male/female)   | 18/36                    |
| Education (years)b        | 15 (8 to 28)             |
| Disease duration (years)c | 4.86±6.59                |
| PASAT score               | 39±14                    |
| EDSS score                | 2 (0 to 6.0)             |
| MSFS score                | 0.23 (1.42 to 1.51)       |
| Vol. T2 lesion load (cm³) | 33.91 (1,133 to 162.74)   |
| Vol. T1 lesion load (cm³) | 7.72 (0.76 to 96.35)      |
| Vol. GM (cm³)             | 523.66±51.64             |
| Vol. WM (excluding lesion volume (cm³)) | 499.24±53.54 |

EDSS: Expanded Disability Status Scale; MSFS: Multiple Sclerosis Functional Composite; Vol.: volume; GM: Gray Matter; WM: White Matter. The data are expressed in mean±standard deviation or median (min to max) depending on the parametric or non-parametric distribution of the variable. The max data is high because of one outlier with a long period of schooling.

Neuropsychological testing

Verbal working memory was evaluated with the Paced Auditory Serial Addition Task (PASAT) in 3 s (Au Duong et al., 2005; Audoin et al., 2005a,b; Gronwall, 1977; Rao et al., 1991; Sepulcre et al., 2006b) included in the Spanish translation of the validated Brief Repeatable Battery—Neuropsychology (Sepulcre et al., 2006b). The subject listens as a series of 61 single-digit numbers are delivered every 3 s. After each number, the subject is asked to vocalize overtly the result of the addition of the two last numbers heard before. To ensure that subjects understood the task, a 6 digits sequence training trial was performed. None of the subjects had performed this test previously and no one refused to perform it. As the test was administered only once, a training effect was avoided.

MRI acquisition

Subjects had a 3D T1-weighted MRI scan (1.5 T; TR 2140 ms; TE 5.04 ms, flip angle 15°; matrix size 256×256; 2-mm slice thickness; 88 contiguous axial slices; FOV 25 cm; in-plane resolution of 1.1 mm) of the whole head. In this study, we used T1-weighted images, rather than T2-weighted images, because they more accurately identify the existence of axonal damage (Comi et al., 2000), which has a stronger impact on the disruption of white matter pathways and brain function. The MRI assessment was performed within the month of the neuropsychological testing and blind to clinical and neuropsychological data. No subject suffered clinical reactivation of the disease between the period of the neuropsychological study and the MR assessment.

Lesion probability maps and gray matter maps

To obtain the 3D lesion probability maps (LPMs) we used a protocol described elsewhere (Sepulcre et al., 2008a,b). This method has an excellent inter-rater reliability (intraclass correlation coefficient = 0.892; p<0.001) (Esteban et al., 2007). Briefly, we outlined at a voxel level all the white matter lesions on the 3D T1-weighted MRI scan of each patient using MRicro software (Chris Rorden, University of Nottingham, Great Britain, UK). We then normalized original T1 scans from a native to a stereotactic space using SPM2 software (Wellcome Department of Cognitive Neurology, University College London, London, UK) and a voxel-based morphometry protocol (Sepulcre et al., 2006a) running under Matlab v. 6.5 (Mathworks Inc., Natick, MA). In this step, we used a modified version of the optimized voxel-based morphometry protocol of Good et al. (2001) to obtain the normalized and segmented gray matter probability maps from each subject MRI, but avoiding the bias introduced by white matter lesions in the normalization and segmentation procedures (Sepulcre et al., 2006a). Lesion masks of each patient were normalized applying the previously obtained parameters in the normalization of the T1 scans. Finally, lesion masks were smoothed and converted to LPMs by applying a 12 mm full-width at half maximum isotropic Gaussian kernel (Sepulcre et al., 2008b). To approximate the multiple sclerosis lesions to the real multiple sclerosis neuropathology, with more severe tissue damage in the centre of the lesions than in the periphery and greater peripheral extension than observed for each MRI T1 lesion (Comi et al., 2000), and also to statistically normalize the LPMs, we used a high Gaussian filter. Therefore, we obtained statistically and spatially normalized 3D LPMs where each voxel has a probability value of being classified as disrupted white matter pathway (ranging from 0 to 1), with a higher value at the centre of the lesion than in the periphery. By means of this voxel-by-voxel approach, we used all available information, avoiding reliance on cutoff scores or specified regions of interest (Bates et al., 2003). Finally, by comparing a normal segmented white matter template (VoiTool; SPM extension toolbox; Sergei Pakomov http://www.ihb.)
To correlate the LPMs and the PASAT score we applied a voxel-by-voxel multiple linear regression model, using SPM2 software. To ensure an unbiased voxel-by-voxel statistical estimation, we corrected by regional lesion frequency. This is important in multiple sclerosis because lesions are more frequent in the periventricular region. For this purpose we created a white matter lesion frequency mask using the sum of the individual 3D normalized-binary masks (Supplementary Figure). Then, each voxel value of this lesion frequency mask was introduced in the multiple linear regression models as a nuisance covariate to adjust the possible bias introduced by this factor at the final voxel-level result. In addition, an explicit binary white matter template (Voitool) was used in this process to ensure that only white matter voxels were included in the analysis. All correlations were adjusted by gender, age and years of education. The level of significance for the results was set at p < 0.05 after correction for multiple comparisons to minimize type I error (False Discovery Rate method; Genovese et al., 2002). To achieve consistent results only clusters with 100 or more voxels were retained (Grossman et al., 2004). The mean signal intensity values of each significant cluster were extracted with the VOI toolbox of SPM2 in order to show the r correlation coefficient and p values (Table 2). The locations of the relevant white matter clusters were compared with three white matter atlases (Dejerine, 1895; Mori et al., 2005; Schmahmann and Pandya, 2006), as well as with a source for the functional correlations of brain lesions (Brazis et al., 2006). These comparisons were made not only by visual inspection of the results but also using overlaps of the white matter bundles templates.

Finally, to assess the relationship between white matter lesion location and atrophy of target gray matter areas related to working memory processing we extracted the eigenvalues of working memory-related gray matter regions of interest (ROIs) and each significant white matter cluster previously identified. In this step, we only considered gray matter ROIs that according to previous research have shown clear evidence of participating in working memory processes (Baddeley, 1992, 2003; Smith and Jonides, 1998). To further prove the specificity of the results we also analyzed cortical areas unrelated to working memory, such as primary motor (BA 4) and somatosensory (BA 3, 1, 2) cortex. For this analysis, we used SPM2 software and the Marsbar automated parcellation toolbox (Tzourio-Mazoyer et al., 2002) to extract the data and SPSS software (SPSS 13.0; SPSS Inc., Chicago, Illinois) for the statistical analysis.

### Results

Lesions in eight white matter lesion clusters correlated with impaired verbal working memory performance (Table 2). Anatomically, they were located as follows (Fig. 1): (a, b and h) both cinguli and the more laterally located superior occipito-frontal and longitudinal fascicles, making up part of the fronto-parietal centrum semiovale of both hemispheres. On the right side, lesions in the paracentral region of these fascicles did not reach significance, probably because at that point they are crossed by the thalamo-cortical projection, which, on the right hemisphere, seems to be less involved with verbal working memory. In both hemispheres, these clusters included white matter underlying the prefrontal (BA 6/44/45/46), anterior cingulate (BA 24/32) and parietal (BA 40) cortex; (c and d) the left temporo-occipital white matter including the inferior occipito-frontal and longitudinal fascicles (underlying BA 19/20/21/37), and a much smaller, but partially symmetrical area of the right hemisphere; (e) the genu of the left internal capsule; (f) the right ponto-mesencephalic tegmentum; and (g) the right cerebellar peduncle.

To support the notion that these white matter locations constitute part of the brain network subserving verbal working memory, we analyzed their relationship with cortical areas known to be involved in working memory (Baddeley, 1992, 2003; Smith and Jonides, 1998). We hypothesized that white matter lesions disrupting the verbal working memory network would induce atrophy in their target cortical areas. To test this hypothesis we correlated the eigenvalues of the white matter regions identified in our previous step with the regional volume of known working memory-cortical regions. Lesions in the main white matter areas described above correlated with reduced volume (or atrophy) of working memory cortical areas, such as prefrontal (BA 6/9/44/45/46) and parietal cortex (BA 40) (Fig. 1; Table 2). As a control, we found no correlations with cortical areas unrelated to working memory, such as the paracentral cortex (primary motor and somatosensory cortex).

### Discussion

For many decades, brain lesion–symptom studies have provided valuable insights into the relationship between brain areas and symptom studies have provided valuable insights into the relationship between brain areas and...
cognitive functions (Brazis et al., 2006). More recently, the development of voxel-based MRI analysis tools has offered a perfect framework to analyze more accurately the relationship between brain tissue damage and continuous behavioral data on a MRI voxel-by-voxel basis (Bates et al., 2003; Sepulcre et al., 2008b). These lesion–symptom mapping approaches improve over previous methods because they use all available neuropsychological and image information, avoiding reliance on cutoff scores or specified a priori regions of interest.

Current information indicates that working memory relies on processing by gray matter functional areas distributed throughout the brain. Little is known, however, about the white matter regions or pathways that are involved in this system to form a coherent network of information processing. In this sense, the study of the “human brain connectome”, sustaining cognition by enabling high interconnectivity and short conduction delays between areas (Wen and Chklovskii, 2005), has been proposed as one of the next frontiers in cognitive neuroscience (Catani, 2006; Mesulam, 2005). A better understanding of the contribution of the brain white matter pathways to working memory processes has been hindered by the lack of adequate models in humans. The extensive research on working memory has relied mostly on PET or fMRI techniques that depict much better gray matter than white matter functional changes (Baddeley, 2003), and previous evidence from lesional models in humans derives mainly from stroke or trauma patients, with a mixture of gray and white matter damage and therefore inadequate for the identification of the specific role of the white matter. In contrast, directed damage of distinct white matter

Fig. 1. White matter regions associated with verbal working memory impairment and their relation with decreased volume in specific working memory cortical regions (see text for details). The color bar – F statistic – indicates the correlation between the Paced Auditory Serial Addition Task performance and the white matter lesion probability maps. The blue color represents areas of decreased cortical volume.
regions in non-human primates has yielded important attentional and memory deficits (Gaffan, 2005). Parallel information in terms of white matter pathway disruption is not available in humans. However, the method we describe, with limited and variously located white matter lesions in different individuals, allows for a correlation of specific areas of cognitive dysfunction with the white matter regions affected. The methodology follows a non-aprioristic approach, such that the entire brain is explored and there is no bias as to the selection of any particular white matter region. Taking advantage of the existence of a human disease, multiple sclerosis, that has a predominant target in the white matter, we analyzed in this study the lesion–function correlation between brain white matter regions and working memory.

As expected, the working memory–white matter network has a very wide and extensive distribution involving cortico-cortical and cortico-subcortical pathways, paralleling the wide distribution of gray matter activation in fMRI studies (Audoin et al., 2005b; Baddeley, 2003; Braver et al., 1997; Smith and Jonides, 1998). The network identified is complex, including shorter fiber bundles underlying the working memory-related cortex, and longer association bundles, such as the cingulum, the occipito-frontal and longitudinal fascicles, as well as left-hemispheric thalamo-parietal and thalamo-frontal pathways. It makes anatomic sense that pathways connecting the parietal lobes with the lateral prefrontal regions and anterior cingulate cortex, as well as the thalamic radiations, be involved in working memory processes, as all these regions are known to mediate working memory (Audoin et al., 2005b; Baddeley, 2003; Braver et al., 1997; Smith and Jonides, 1998). As we explored verbal memory, we found left-hemispheric pathways critical for verbal processes to be more extensive than right-sided ones. Elegant model-driven studies using DTI have shown the left-sided lateralization of white matter pathways related to language cortex, particularly in males (Hagmann et al., 2006). Another group has also used an aprioristic approach and DTI to study, in a model of verbal working memory pathways, the differences between patients with multiple sclerosis and healthy controls (Audoin et al., 2007). This study is difficult to compare with ours, because the outcome measures were the diffusion parameters of white matter bundles in a predefined model for the executive system of working memory. Sources for the DTI study were the gray matter regions previously found to be activated during a functional MRI study of verbal working memory, using the PASAT (Audoin et al., 2005b). However, it is remarkable how the pathways we found non-aprioristically overlap to a great extent with the theoretical construct used for that study (Audoin et al., 2007).

In our study, other white matter regions such as the genu of the left internal capsule, the right ponto-mesencephalic tegmentum and the right cerebellar white matter were found to correlate with working memory scores. The participation of the left internal capsule probably reflects involvement of the thalamo-prefrontal pathways present in this structure, particularly arising in the dorsomedial and anterior nuclei of the thalamus (Chiha-Schmid and Bogousslavsky, 2000; Tatemichi et al., 1992). Previously, the right cerebellum has been discovered to participate in working memory processes (Baddeley, 2003; Smith and Jonides, 1998). The fibers at the right ponto-mesencephalic tegmentum and the right cerebellar white matter that we found are likely to represent the white matter component of this system. As the right cerebellum projects to the left hemisphere, again the lateralization of language representation to the left hemisphere becomes explicit in this study of right-handed subjects.

Our study has some limitations. Currently, it is very well known that MS is not only a white matter disease but also a neurodegenerative disorder (Filippi, 2001; Hauser and Oksenberg, 2006; Rovaris et al., 2006; Sepulcre et al., 2006a). In an attempt to analyze both features of the disease we also report in this study the relationship between white matter lesions and gray matter atrophy under the assumption that some gray matter changes could be secondary to the white matter lesions. However, we are aware that some other aspects like primary damage of gray matter are not addressed in the present study. Second, although T1 images provide the best correlation with axonal damage, they may not be as accurate as T2-weighted images on other pathological events, such as active inflammation that also contribute to nerve conduction impairment. The use of a high Gaussian filter allowed us to approach the neuropathology of multiple sclerosis lesions (Comi et al., 2000) but at the price of losing some specificity. Moreover, we studied working memory with a single cognitive test such as the PASAT. This has the advantage of using a single score from a test that it is very sensitive to working memory performance. However, verbal working memory is a complex process not fully captured by the PASAT. Additionally, this test also involves to a lesser degree other cognitive domains such as calculation, language or executive function and it is highly dependent on information processing speed. Output on other working memory tests should be analyzed using this methodology to try to capture the common findings and get more robust results for a core of working memory pathways independent of contamination from other cognitive domains. Another possible limitation is that some white matter pathways, mainly u-fiber or yuxtacortical pathways, may be under-represented in this study. However, the lesion sample included the major long-distance pathways (Supplementary Figure). Finally, our lesion–function correlation method is only able to analyze the linear relationship between white matter lesions and the final working memory score, acting as a black box for other possible intermediate steps and therefore it is totally insensitive to isolated different working memory components.

In conclusion, using a lesion–function correlation approach in a paradigmatic white matter disease, we have identified critical in vivo white matter pathways associated with verbal working memory in humans. This is an example of how lesion–function or lesion–symptom mapping can be useful tools to unveil white matter networks supporting cognitive functions in the human brain.

Acknowledgments

We wish to thank Prof. Joaquin Fuster (University of California in Los Angeles, USA), and Dra. Herminia Perañta (UNED, Spain), for their helpful comments. Prof. Alan J. Thompson and Dra. Mara Cercignani (Institute of Neurology, London, UK) for their help in the development of the VBM protocol for MS, and the Multiple Sclerosis Society of Navarra for their help with patient recruitment. The authors would like to acknowledge the support of the European Union (JM, FP6-project DIMI, LSFB-CT-2005-512146), the Spanish Ministry of Health (JM, CMº405/00222; JM, FISºPI052520; and PV, FISºPI052101), the Spanish Ministry of Education and Science (MAP, SAF200-07813), the Navarra Government (JC, MAP and JM), the Basque Country Government (NVM), the foundation “UTE project CIMA” (JM, MAP and PV) and the Fundación Uriach (PV).

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.neuroimage.2009.04.054.

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