NeuroImage 52 (2010) 1175–1180

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Connectivity-based segmentation of the substantia nigra in human and its implications in Parkinson's disease

Ricarda A. Menke [a], Saad Jbabdi [a], Karla L. Miller [a], Paul M. Matthews [a,b], Mojtaba Zarei [a,b,*]

[a] FMRIB Centre, John Radcliffe Hospital, University of Oxford, Oxford, UK
[b] Department of Clinical Neuroscience and GSK Clinical Imaging Centre, Hammersmith Hospital, Imperial College London, UK

ARTICLE INFO

Article history:
Received 6 April 2010
Revised 27 May 2010
Accepted 31 May 2010
Available online 8 June 2010

Keywords:
Diffusion tensor imaging
Parkinson's disease
Substantia nigra

ABSTRACT

The aims of this study were to i) identify substantia nigra subregions i.e. pars reticulata (SNr) and pars compacta (SNc), in human, and ii) to assess volumetric changes in these subregions in the diagnosis of Parkinson's disease. Current MR imaging techniques are unable to distinguish SNr and SNc. Segmentation of these regions may be clinically useful in Parkinson's disease (PD) as substantia nigra is invariably affected in PD.

We acquired quantitative T1 as well as diffusion tensor imaging (DTI) data from ten healthy subjects and ten PD patients. For each subject, the left and right SN were manually outlined on T1 images and then classified into two discrete regions based on the characteristics of their connectivity with the rest of the brain using an automated clustering method on the DTI data.

We identified two regions in each subjects' SN: an internal region that is likely to correspond with SNc because it was mainly connected with posterior striatum, pallidum, anterior thalamus, and prefrontal cortex; and an external region that corresponds with SNr because it was chiefly connected with posterior thalamus, ventral thalamus, and motor cortex. Volumetric study of these regions in PD patients showed a general atrophy in PD particularly in the right SNr.

This pilot study showed that automated DTI-based parcellation of SN subregions may provide a useful tool for in-vivo identification of SNc and SNr and might therefore assist to detect changes that occur in patients with PD.

Introduction

The substantia nigra (SN) is divided into two functionally and anatomically distinct regions — pars compacta (SNc) and pars reticulata (SNr). The SNc neurones have reciprocal connections with the striatum, pallidum, anterior thalamic nuclei and the prefrontal cortex. The SNr neurones are predominantly connected to the ventral (sensorimotor) thalamic nuclei and motor cortex (Beckstead et al., 1979; Parent and Hazrati, 1994).

Several attempts have been made in improving visualization of the SN using MRI techniques (Adachi et al., 1999; Hutchinson and Raff, 2008; Minati et al., 2007; Oikawa et al., 2002; Pujol et al., 1992; Sasaki et al., 2006; Stern et al., 1989), but none provides a clear discrimination between SNc and SNr. An accurate method for in-vivo parcellation of the SN, however, could be useful for differential diagnosis and monitoring of Parkinson's disease (PD).

Diffusion-based tractography has been successfully applied in several studies to segment subcortical regions like the thalamus (Johansen-Berg et al., 2005) and cortical regions like lateral premotor cortex (Tomassini et al., 2007) based on their connections with other parts of the brain. The latter method is to merge areas with similar long-distance connectivity patterns into one region that can be distinguished from neighbouring regions with different connectivity characteristics.

We previously reported that T1 map can be used to delineate SN and that SN volume is smaller in PD patients than in controls (Menke et al., 2009). In this study, we take one step further and use DTI and T1 map to identify SNc and SNr in controls and PD patients using differential connectivity profile of SNc and SNr.

Materials and methods

The imaging and clinical data set that is used in this study was obtained from our previous study (Menke et al., 2009) but different hypothesis and analysis methods were used. Demographic and descriptive data are therefore similar to our previous study on these subjects.

Subjects

Ten PD patients (3 women and 7 men; mean ± SD age, 63.7 ± 6.7 years; range, 54–73 years) and ten age- and sex-matched healthy

* Corresponding author. FMRIB Centre, John Radcliffe Hospital, Oxford OX3 9DU, UK. Fax: +44 1865 222717.
E-mail address: mojtaba@fmrib.ox.ac.uk (M. Zarei).

1053-8119/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2010.05.086

subjects (3 women and 7 men; mean ± SD age, 64.4 ± 9.9 years; range, 48–78 years) were scanned. All subjects were right handed. Detailed demographic information for all subjects can be found in our previous publication (Menke et al., 2009). The study was conducted in compliance with the Declaration of Helsinki and with the approval from the local ethics committee. All subjects provided written consent to participate in this study. Patients were scanned whilst taking their medication as usual and were "on" period. All patients were assessed clinically and the Hoehn and Yahr score was calculated for PD patients.

Data acquisition

The driven equilibrium single pulse observation of T1 (DESPOT1)-HIFI quantitative imaging method (Deoni, 2007; Deoni et al., 2003) implemented on a 3 T Siemens (Erlangen, Germany) Trio MR scanner with a 12-channel head coil was used to generate high-resolution T1 maps. The respective DESPOT1-HIFI imaging protocol included two spoiled gradient recalled (SPGR) images (flip angles: 4° and 18°, 144 slices, $1.1 \times 1.1 \times 1.1$ mm^3, TE/TR = 4.8 ms/12 ms) and two IR-SPGR images (72 sagittal slices, $1.1 \times 1.1 \times 2.2$ mm^3, TE/TR = 4.8 ms/9.1 ms, inversion times TI = 450 ms and TI = 650 ms) per T1 map. To improve the signal-to-noise ratio (SNR), two whole-brain DESPOT1-HIFI data sets were acquired for each subject and the respective images were coregistered using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson and Smith, 2001) before the two T1 maps were calculated and averaged.

Diffusion data were obtained using an echo planar imaging sequence (60 directions, b-value = 1000 s/mm^2, TE/TR = 94 ms/9300 ms, $2 \times 2 \times 2$ mm^3 voxel size, 65 slices). In addition, we acquired three images without diffusion weighting. Furthermore, a field map was acquired using a gradient echo imaging sequence ($2 \times 2 \times 2$ mm^3 voxel size, 65 slices, TE1/TE2/TR = 5.19 ms/7.65 ms/655 ms). The entire image acquisition took 25 min (12 min for T1 Map and 13 min for DTI).

Structural T1 map processing

Analysis of images was performed using tools from the FMRIB Software Library (FSL) (Smith et al., 2004) and in-house software for processing the quantitative T1 maps. All SPGR images were skull-stripped (Smith, 2002) and aligned to the first acquired SPGR images (fa18 = flip angle 18°) using affine registration. Subsequently, two quantitative T1 maps were calculated from the two separate sets of four SPGR images each and averaged afterwards to improve the signal-to-noise ratio. To enable a standardized identification of the SN for all subjects we registered the T1 map into MNI space using linear registration with six degrees of freedom. This allows transformation of images with no impact on the volume of the brain. The respective affine transformation matrix was derived from registering the fa18 image to the MNI template.

The left and right SN were manually segmented by one rater (RM) and verified by another (MZ) as described previously (Menke et al., 2009). Both raters were blind to the subjects' diagnosis and age at the time of the tracing and verification.

DTI preprocessing and tractography

Diffusion data were unwarped based on field map data with FUGUE, skull-stripped (Smith, 2002) and then registered to a reference volume using affine registration (Jenkinson and Smith, 2001) to correct for eddy currents and head motion.

Voxelwise estimates of fibre orientations and their uncertainty were calculated using FMRIB's Diffusion Toolbox (part of FSL4.1), using a model that accounts for the possibility of crossing fibres within each voxel (Behrens et al., 2007). Subsequently, for each voxel in the SN, we estimated the spatial probability distribution for the *location* of fibre connections passing through that voxel. This results in a spatial histogram that represents the likelihood of the location of connections from the SN within each brain voxel — i.e. a *connectivity profile*. Then, for each pair of voxels in the SN, we calculated the correlation between their connectivity profiles, resulting in a cross-correlation matrix, which entries determine *similarities* between the spatial arrangements of connections emanating from the corresponding voxels. This method has been described in detail previously (Johansen-Berg et al., 2004).

Creation of map of SN subregions

The cross-correlation matrices were used as an input to a *k*-means clustering algorithm with the constraint of identifying two clusters, based on the understanding that SN has two distinct anatomical regions. These clusters consist of voxels in the SN that have similar connectivity profiles (Klein et al., 2007), which we hypothesize to represent the SNr (external part) and SNc (internal part) respectively. To examine the between-subject reproducibility of the SN parcellation, we binarized the SN subregions for each subject and registered them into MNI space with FMRIB's Non-linear Image Registration Tool (FNIRT). The affine transformation and the warpfield used for non-linear transformation were both derived by using the fa18 image that defined the seed space as source image. The MNI template with $1 \times 1 \times 1$ mm^3 voxel size served as reference image. We then added all subjects' segments in MNI space together to create a probabilistic group map of the two different SN subregions.

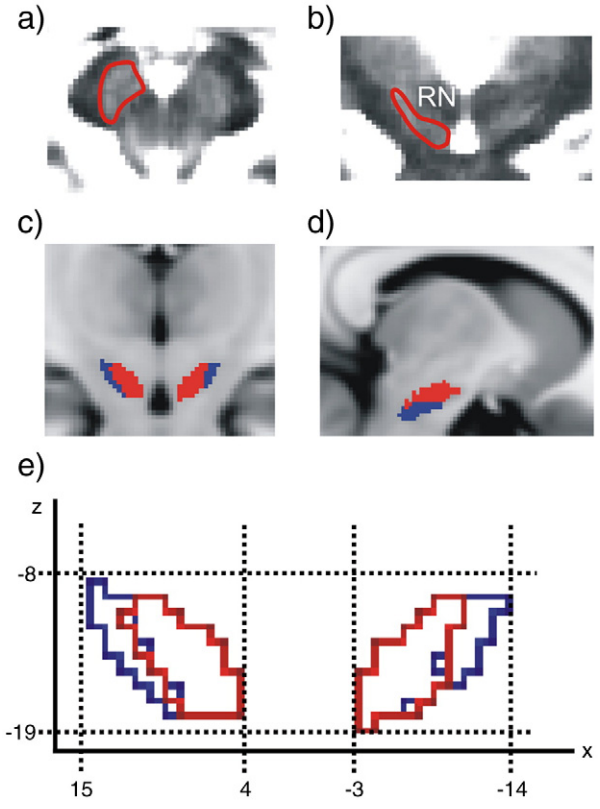

Fig. 1. Segmentation of substantia nigra. Top row: the SN visible as a hyperintense region (see red outline) in the T1 map in seed space in a) axial and b) coronal views for a representative subject (NC06). Middle row: group map for the *k*-means segmentation of the SN with a predetermined number of two clusters in a group of ten healthy elderly subjects overlaid onto the MNI template (thresholded to include 60% of the population) in c) coronal and d) sagittal views. e) Group map illustrating the overlap between internal (red) and external (blue) parts of the SN. Coordinates are given in MNI space (y = −18). RN = red nucleus.