Brain T1 intensity changes after levodopa administration in healthy subjects: a voxel-based morphometry study

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Aim

To test T1 intensity variations induced by levodopa administration in the regional fixation area in the human brain.

Method

Using non-invasive magnetic resonance imaging (MRI) technique [T1-weighted sequence MPRAGE; TE/TR/TI = 5/25/800 ms; impulsion angle = 15°; field of view = 256 × 230 × 180 mm³; acquisition matrix = 256 × 192 × 104; reconstruction matrix = 256 × 256 × 128], we tested changes in the T1 MRI signal intensity resulting in changes in the grey matter automatic classification after administration of a single dose of 100 mg of levodopa by a voxel-based morphometry method (VBM) in 12 healthy subjects.

Results

The VBM analysis demonstrated an increased number of voxels attributed to grey matter after levodopa administration in an anatomical cluster which included substantia nigra, tegmental ventral area and subthalamic nucleus bilaterally, the principal origin and first relay nuclei of projections in brain dopaminergic systems ($t = 8.61$; corrected for all grey matter volume $P < 0.001$).

Conclusion

Our results suggest that levodopa administration could induce an MRI T1 signal intensity variation that is not evident to the naked eye, but is detectable by measuring local signal intensities. Possible clinical applications are discussed.

Introduction

Magnetic resonance (MR) neuroimaging methods have been extensively used in pharmaceutical research [1–4]. The primary role of MR methods in pharmacological research is to study drug effects on tissue morphology,

physiology and (endogenous) biochemistry. Anatomical MRI becomes indispensable when non-invasiveness is an essential requirement, e.g. in longitudinal studies of chronic disease. Moreover, due to its multiparametric signal dependence, MRI provides a comprehensive

characterization of soft tissue pathologies. The main advantage of MRI are its non-invasiveness and high spatial resolution.

For most drugs, the average tissue concentration is too low to be detected by MRI. Only nuclear medicine techniques, such as single-photon emission computed tomography (SPECT) or positron emission tomography (PET), possess the required sensitivity for exposure and pharmacokinetic studies. However, these methods are limited by relatively poor resolution (1 mm) and lack chemical specificity, i.e. the inability to distinguish whether the emitting radionuclide is bound to the parent drug molecule or to a metabolite thereof [5]. Moreover, neuroimaging techniques such as SPECT and PET involve the injection of a radiotracer, a radioactively labelled drug or agent.

Recently, a new application, derived from the functional magnetic resonance imaging (fMRI) approach for monitoring drug-induced changes in brain activity, has been developed. This method is called pharmacological magnetic resonance imaging (phMRI). It uses the BOLD phenomenon (blood-oxygen-level-dependent [6]) to test pharmacological manipulation by monitoring neural activation specifically caused by drug administration [7, 8]. To date, the central transmitter system that has been most extensively studied using fMRI is the dopaminergic system, of particular interest in psychiatric and neurological research since dysfunction of this system has long been associated with schizophrenia and is at the root of Parkinson's disease (PD) [9].

We used voxel-based morphometry (VBM) to conduct an exploratory study into the putative changes in MRI signal intensity of grey matter after levodopa administration (at the theoretical maximal peak plasma concentration of the drug). VBM is one such method developed for automated, unbiased analysis of structural MRI scans. It applies statistical models to test for significant differences between groups on a voxel-by-voxel basis throughout the whole brain. So one of its major advantages is the fact that data processing is almost completely user independent and inter- and intraobserver variations are avoided [10]. This method can detect structural anomalies not apparent on visual inspection of the scans [10–12]. The implicit purpose of the present study was to test the possible utility of a standard neuroimaging test in pharmacological studies, e.g. levodopa fixation in the human brain.

Our hypothesis was that, if a change in T1 MRI intensity occurs in the human brain after levodopa administration, it would induce an adjustment in the number of voxels attributed to grey matter in the base of their T1

intensity (and localization) and therefore would be detected by VBM analysis.

Materials and methods

Subjects

Twelve right-handed subjects of both sexes (eight females, four males) aged 46–73 years (mean age 55.6 ± 6.8 years) participated in the study. All subjects were in good health as assessed by a complete medical questionnaire including medical history, physical examination and psychiatric interview. Excluded from the study were subjects who presented contraindications for MRI (mainly claustrophobia, dental prosthesis), smoked more than 10 cigarettes per day or were not able to refrain from smoking on test days and those with a history of alcoholism or drug abuse. None of the subjects was taking central nervous system drugs. This study was conducted in accordance with the principles of the Declaration of Helsinki. Approval was obtained from the local Ethics Committee of La Timone Hospital (CCPPRB Marseille). Each participant was registered on the French National File and gave informed written consent before entering the study.

The study design was a randomized, double-blind, cross-over experiment consisting of two 1-day MRI sessions, separated by an interval of 5–7 days. In each session, the subjects received a single oral dose of either 100 mg of L-Dopa with 25 mg of benserazide or placebo. Because the use of decarboxylase inhibitors, such as benserazide, does not completely overcome all the peripheral side-effects of L-Dopa (notably, vomiting and orthostatic hypotension), subjects were treated with 20 mg domperidone three times daily for 3 days before and throughout each MRI session. The domperidone treatment was conducted for both conditions in order to maintain the double blind. Indeed, levodopa and placebo were supplied as indistinguishable capsules in order to maintain the double blind. Domperidone does not cross the blood–brain barrier at the usual dose and acts as a peripheral dopamine antagonist [13, 14]. One subject was excluded due to claustrophobia and another because of head movement artefacts. Therefore, the anatomical analysis was carried out on 10 MRI scans. After crossing the haemato–meningeal barrier, levodopa is transformed into dopamine by dopa-decarboxylase. The maximum blood levels were assessed between 1 h and 3 h. The theoretical peak plasma concentration of levodopa's effects in the human brain is considered to be 90 min [15]. For all subjects in our study, the treatment was administered at the precise time needed to reach the theoretical peak plasma concentration when the subject underwent the MRI acquisition. The mean of the time

between MRI acquisition and administration of the treatment (levodopa or placebo) was 105.21 min (SD = 10.10 min).

MRI acquisition and processing

All anatomical data acquisition for the subjects was performed on a 3-T MEDSPECT 30/80 AVANCE imager (Bruker, Ettlingen, Germany), equipped with a circular polarized head coil. A set of high-resolution T1-weighted images was acquired (Sequence MPRAGE; TE = 5 ms/TR = 25 ms/TI = 800 ms/Trecov = 2300 ms; bandwidth = 43 kHz/echo position = 30%; 4 segments/central phasing code 2D; impulsion angle = 15°/shape = Gaussian/duration = 1 ms/bandwidth = 8 kHz; field of view = 256 × 230 × 180 mm³/acquisition matrix = 256 × 192 × 104/reconstruction matrix = 256 × 256 × 128) The slices covered the whole brain and were acquired parallel to the anterior–posterior commissure (AC–PC) plane in an axial plane yielding contiguous slices thickness of 1.2 mm.

During the study, the subjects reclined in a supine position on the bed of the scanner and a radiofrequency coil was placed over the participant's head. Other precautions were taken to minimize subject motion by instructing subjects to remain still and packing foam packing around their heads. All scans were examined by an expert neuroradiologist for possibly unacceptable degrees of motion.

Data analysis

Voxel-based morphometry analysis Data were processed using SPM2 software (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, University College London, UK) implemented in Matlab 6.5 (Mathworks, Sherborn, MA, USA). A single investigator performed the manual steps in image preparation (determination of the anterior commissure and reorienting the images according to the anterior–posterior commissure line).

First, a customized anatomical T1 template and prior probability images (for grey and white matter as well as for cerebrospinal fluid) were created from all T1 original images. The 10 brains in each sample were segmented and cleaned up by an affine transformation using the default SPM2–T1 brain template. The spatial normalization parameters were then estimated by matching the grey matter (GM) segments to the GM SPM2 template. Using these parameters, a spatially normalized version of the original brain images was created for each subject. These normalized images were then segmented. An average image was written

out for each partition as well for the T1 whole brain. Finally, these averaged images were smoothed with an 8-mm FWHM kernel.

The VBM protocol used these customized anatomical and prior images. The optimized VBM method is described exhaustively elsewhere [11, 12]. Briefly, the following steps were carried out:

- 1 Segmenting and cleaning (using a mathematical function to clean possible voxels occurring outside the brain from the SPM-segmented images) of the T1 original images.
- 2 Estimation of spatial normalization parameters by matching GM images to customized *a priori* GM.
- 3 Application of the normalization set of parameters to the original whole-brain structural image.
- 4 Segmenting and cleaning of normalized GM images.
- 5 Smoothing normalized GM images with a 10-mm FWHM kernel.

The processed GM images were analysed using an SPM2 group comparison. We performed a paired *t*-test to test for regional differences in concentration (density) of GM between the two conditions (levodopa or placebo administration). Results were thresholded at *P* (FDR-corrected) < 0.05; false discovery rate (FRD) is the proportion of false positives in the suprathreshold voxels. Only clusters of more than 20 contiguous voxels were considered in the analysis. The original optimized VBM approach has a double analysis option: tests for regional differences in GM volume (applying to the images modulated by the Jacobian determinants derived from the spatial normalization step); and tests for regional differences in the concentration of grey matter (using the unmodulated images). We have not used a posterior modulation process (of Jacobian determinants), because our goal was to test the difference in density, not in volume.

Results

VBM analysis

Only one regional GM difference between the two medication conditions was found. A cluster of 11 472 mm³ (Talairach coordinates of physical centre: −1, −24, −10; *t* = 8.61 and *P* (FDR corrected for the whole GM) < 0.001) in the midbrain showed an increased GM concentration in the L-dopa condition relative to placebo. This cluster included the substantia nigra, the tegmental ventral area and the subthalamic nucleus bilaterally (see Figure 1).

To focus the analysis on the resulting GM intensity difference, we used a region of interest (ROI) approach, using the Marsbar toolbox for SPM2 [16] which allows

Figure 1

Voxel-based morphometry results: grey matter density/intensity increment after levodopa administration

for computation of statistical differences (paired *t*-test) between conditions. We used the WFU-Pickatlas SPM tool to define this ROI which included the midbrain bilaterally. The ROI analysis revealed a significant effect at $t = 7.89$, $P = 0.000\ 012$, and with the adjusted intensity values showing an extremely close fit to those predicted by the model (see Figure 2).

Discussion

The VBM analysis demonstrated an increased number of voxels attributed to GM by their T1 intensity in the

Figure 2

Fitted and adjusted intensity signals from the bilateral midbrain region of interest (WFU-Picatlas' definition) in the paired *t*-test; subjects are represented consecutively in the two conditions. For all subjects 1 = placebo and 2 = levodopa. Adjusted data (—●—), fitted data (-----)

midbrain, for the L-dopa condition relative to placebo; this effect was probably transient but since we did not study the time-course effect, this point deserves further experiment. This cluster included the substantia nigra and the tegmental ventral area, the principal nuclei of origin for projections in brain dopaminergic systems. The subthalamic nucleus also seemed to be included bilaterally in this cluster. This small nucleus is physically placed next to the tegmental ventral area and it is possible that the inclusion of this nucleus in the cluster could be due simply to a smoothing phenomenon. The subthalamic nucleus is a target of the dopaminergic projection receiving input from the striatum. Thus, if a change in the subthalamic nucleus is possible, one may hypothesize that other target areas, such as the striatum, could also be affected at this point of dopamine distribution in the brain. L-Dopa is converted to dopamine by the L-aromatic amino acid decarboxylase. This enzyme turns over L-dopa so rapidly that L-dopa levels in the brain are negligible under normal conditions. Dopamine can be released not only from nerve terminals but also from dendrites of dopamine neurons that originate in the mesencephalon. The substantia nigra possesses a pool of dopamine that has a much faster turnover than the terminals in the striatum. In addition, in contrast to the striatum, it appears that a considerable proportion of released dopamine in the substantia nigra is taken up by nondopaminergic cells [17]. Five of the 12 subjects in this study verbally reported a marked somnolence and another experienced some sickness in the levodopa condition. No such effects were reported for the placebo condition. Two previous investigations have studied the sedative effects of L-dopa administration in healthy volunteers [13, 14]. Although we could consider this sub-

jective measurement to be a marker of adequate levodopa fixation, it would be better to use an objective measurement and, undoubtedly, an optimized version of this study would take into account the individual point of maximum fixation. To our knowledge, there are no VBM studies comparing differences in volume of clusters considered as GM between controls and unmedicated PD patients in the first stage of the illness that could give us an idea of dopaminergic deficits in the substantia nigra, in terms of MRI signal.

These results suggest that one could use MRI to detect abnormal concentrations, or transitory fixations, of a molecule like the levodopa and/or dopamine. These changes could be measured by techniques that assess intensity changes, like the VBM method for the brain. These results could indicate that levodopa and/or dopamine molecules have intrinsic magnetic properties that could result in MRI T1 signal intensity variation and, thus, changes in the classification based in this factor of the voxels into grey and white matter. However, another explanation is possible. Further studies (i.e. *in vitro* and animal studies) should be developed to elucidate these hypotheses.

Although dopamine–iron interactions are known and well reported in the literature (for a review see [18, 19]), we have taken several precautions in the present study to avoid our results being contaminated by a potential interaction between dopamine and iron. First, the administration of levodopa was oral and the dose significantly smaller than that reported in studies of dopamine–iron interactions (our oral dose was about 6×10^{-4} μmol , compared with those previously reported, which vary from 5 to ≥ 100 μmol and were injected into the animal brain and/or cultured cells). Second, our patients were in good health and all were >45 years old to ensure that there was not an occulted haematomacrosis (excess iron levels in the blood). This avoids the risk that a latent and undiagnosed disease, with no visible symptoms, could affect the results of a study such as this. Third, we used a cross-over design and thus, even if a microlesion were present, the subjects that received the first MRI scan under levodopa and the second under placebo would not show changes between sessions since the brain damage was already present.

This finding may also have great clinical promise. To take our dopamine example, one could hypothesize that it would be possible to obtain a statistical distribution of ‘normal’ T1 signal intensity for the substantia nigra, by taking measurements of a large sample of the substantia nigra’s intensity (in relation to a control brain region in order to avoid session effects); and therefore establish a critical T1 signal threshold for the ‘normal’ level of

dopamine in this region. This may lead to the early diagnosis of deficits such as those which can occur in PD. The sensitivity manifested by this technique may make it a useful tool for prescreening of PD in the earlier stages. Since symptoms of PD do not seem to occur until roughly 80% of the dopaminergic neurons of the substantia nigra are destroyed [20], there is the possibility of MRI signal changes being sensitive to small changes in local dopamine in the order of $\geq 80\%$ loss. The possibility of using MRI longitudinally and repeatedly may enable one to study up- and downregulation of dopamine concentration over time.

In spite of the care taken with this study, the data and their interpretation are preliminary and must be treated with caution. The exact mechanism underlying our findings remains unclear. The present results would benefit greatly from further support, either direct (i.e. experimental data from high-resolution MR of tissue or cells) or indirect (i.e. studying the T1 signal changes over time). Nevertheless, the present results encourage us to investigate the possibility of using a non-invasive technique, such as magnetic resonance, in the study of abnormal concentrations of substances with magnetic properties. Further studies in this direction should be developed.

Competing interests: None to declare.

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